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172-PT

PROFICIENCY TESTING 2014

SALMONELLA (SAL)

Isolation of Salmonella sp. from organs

OPERATIONAL UNIT

COORDINATION OF VETERINARY DIAGNOSIS

EPIDEMIOLOGY AND RISK ASSESSMENT

(CVD-ERA)

DATE BEGIN PT: 13 OCTOBER 2014

DATE REPORT: 18 NOVEMBER 2014

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 (Carrefour) and were homogenized, aliquoted per 10 – 15g and stored in the freezer. Twelve aliquots were analyzed on the 23th of July for the presence of *Salmonella* spp. by the *Salmonella* reference laboratory of CODA-CERVA, hereby following the methods, described in the laboratory SOP. 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 the13th of October 2014 (start date of the PT), 90 aliquots of liver samples were prepared and randomized, either for the PT (40 aliquots) or the verification tests that had to be performed by the *Salmonella* reference laboratory of CODA-CERVA in parallel with the PT (50 aliquots):

- 26 aliquots (16 for the PT and 10 for the verification tests) were used as such and considered as negative liver samples ('PT2014SALBACNO1')
- 18 aliquots (8 for the PT and 10 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 ('PT2014SALBACPO1').
- 14 aliquots (4 for the PT and 10 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 ('PT2014SALBACPO2').
- 18 aliquots (8 for the PT and 10 for the verification tests) were inoculated with a 10^{-3} dilution of *Salmonella* Pullorum and were considered as weak positive liver samples for *Salmonella* Pullorum ('PT2014SALBACPO3').
- 14 aliquots (4 for the PT and 10 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 ('PT2014SALBACPO4').

In total, 40 aliquots of liver samples were distributed to 4 participating laboratories. All participants were given 10 aliquots of liver samples: 4 aliquots of the negative faecal sample PT2014SALBACNO1, 2 aliquots of the weak positive liver samples PT2014SALBACPO1 (*Salmonella* Gallinarum) and PT2014SALBACPO3 (*Salmonella* Pullorum); and 1 aliquot of the strong positive liver samples PT2014SALBACPO2 (*Salmonella* Gallinarum) and PT2014SALBACPO4 (*Salmonella* Pullorum).
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 tested 5 aliquots of each sample (PT2014SALBACNO1, PT2014SALBACPO1, PT2014SALBACPO2, PT2014SALBACPO3 and PT2014SALBACPO4) for the presence of *Salmonella* on both 13th (day 1) and 14th (day 2) of October 2014, 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 (PT2014SALBACPO2, PT2014SALBACPO4). For the negative (PT2014SALBACNO1) and weak positive samples (PT2014SALBACPO1, PT2014SALBACPO3) 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 operational unit CVD-ERA of CODA-CERVA.

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 4 participating laboratories by national courier on 13th of October 2014 (40 aliquots in total). All laboratories acknowledged receipt of the samples on the same day. Analyses were started on 13th and 14th of October 2014 (Table 1).

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

Results from the participating laboratories have been received between 20th and 27th of October 2014. LAB3 hereby exceeded the deadline of 24th of October 2014 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 operational unit CVD-ERA of CODA-CERVA.

Laboratory	Reference samples received	Start of analysis	Submission of the results (Excel file)
LAB1	13/10/2014	13/10/2014	24/10/2014
LAB2	13/10/2014	14/10/2014	21-22/10/2014
LAB3	13/10/2014	13/10/2014	**27/10/2014**
LAB4	13/10/2014	13/10/2014	20/10/2014

IV.3. Compliance with the procedure

Except LAB1, 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 3 of the 4 participating laboratories provided qualitative results that were in full agreement with the assigned status of the liver samples. Two of the 4 participating laboratories reached 100% of agreement, LAB 2 reached 90 % of agreement with 1 misclassification for a weak positive sample PT2014SALBACPO3. LAB 3 did not reach the required level 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. All participating laboratories received 10 aliquots of liver samples. Results are presented as absolute values and percentages (in parentheses).

	LABNR			
	1	**2**	**3**	**4**
failure	0 (0.0)	1 (10.0)	6 (60.0)	0 (0.0)
success	10 (100.0)	9 (90.0)	4 (40.0)	10 (100.0)

IV.4.2. Variability among participating laboratories

No variability in qualitative laboratory results could be observed between LAB1 and LAB4 since these participants correctly identified all reference liver samples. In contrast, LAB2 misclassified 1 out of 2 aliquots of the weak positive reference liver sample PT2014SALBACPO3 (NEG instead of POS). LAB3 misclassified all positive reference liver samples (PT2014SALBACPO1, PT2014SALBACPO2, PT2014SALBACPO3 and PT2014SALBACPO4) and reported negative for all these 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 (STATUS). NEG: negative; POS: positive.

	LABNR	LABPOSIT	SAMPLE	STATUS	RESULT	SUCCESS
1	1	1	PT2014SALBACNO1	NEG	NEG	1
2	1	2	PT2014SALBACPO2	POS	POS	1
3	1	3	PT2014SALBACPO1	POS	POS	1
4	1	4	PT2014SALBACNO1	NEG	NEG	1
5	1	5	PT2014SALBACPO3	POS	POS	1
6	1	6	PT2014SALBACNO1	NEG	NEG	1
7	1	7	PT2014SALBACPO4	POS	POS	1
8	1	8	PT2014SALBACPO3	POS	POS	1
9	1	9	PT2014SALBACNO1	NEG	NEG	1
10	1	10	PT2014SALBACPO1	POS	POS	1
11	2	1	PT2014SALBACNO1	NEG	NEG	1
12	2	2	PT2014SALBACPO3	**POS**	**NEG**	**0**
13	2	3	PT2014SALBACNO1	NEG	NEG	1
14	2	4	PT2014SALBACPO4	POS	POS	1
15	2	5	PT2014SALBACPO3	POS	POS	1
16	2	6	PT2014SALBACNO1	NEG	NEG	1
17	2	7	PT2014SALBACPO1	POS	POS	1
18	2	8	PT2014SALBACNO1	NEG	NEG	1
19	2	9	PT2014SALBACPO2	POS	POS	1
20	2	10	PT2014SALBACPO1	POS	POS	1
21	3	1	PT2014SALBACPO4	**POS**	**NEG**	**0**
22	3	2	PT2014SALBACPO3	**POS**	**NEG**	**0**
23	3	3	PT2014SALBACNO1	NEG	NEG	1
24	3	4	PT2014SALBACPO1	**POS**	**NEG**	**0**
25	3	5	PT2014SALBACNO1	NEG	NEG	1
26	3	6	PT2014SALBACPO2	**POS**	**NEG**	**0**
27	3	7	PT2014SALBACPO1	**POS**	**NEG**	**0**
28	3	8	PT2014SALBACNO1	NEG	NEG	1
29	3	9	PT2014SALBACPO3	**POS**	**NEG**	**0**
30	3	10	PT2014SALBACNO1	NEG	NEG	1
31	4	1	PT2014SALBACPO1	POS	POS	1
32	4	2	PT2014SALBACNO1	NEG	NEG	1
33	4	3	PT2014SALBACPO2	POS	POS	1
34	4	4	PT2014SALBACPO1	POS	POS	1
35	4	5	PT2014SALBACNO1	NEG	NEG	1
36	4	6	PT2014SALBACPO3	POS	POS	1
37	4	7	PT2014SALBACNO1	NEG	NEG	1
38	4	8	PT2014SALBACPO4	POS	POS	1
39	4	9	PT2014SALBACPO3	POS	POS	1
40	4	10	PT2014SALBACNO1	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* sp. by bacteriological isolation.

Three of the 4 participating laboratories reached the required level of agreement. LAB1 and LAB4 correctly identified all liver samples (100% of agreement), LAB2 correctly identified 90% of all liver samples (90% of agreement with one mistake for a weak positive liver sample) and reached the required level of agreement. LAB 3 misclassified all positive liver samples (Table 2 and Table 3). Hereby, LAB1,LAB2 and LAB4 used the liquid enrichment medium RSV from the same producer Bio-Rad. In contrast, LAB3 used a semi-solid enrichment medium (MRSV) as foreseen in ISO6579 Annex D. The semi-solid MRSV enrichment medium is however not not suitable for the isolation/detection of immobile *Salmonella* spp., including the *Salmonella* spp. considered in this PT: *Salmonella* Pullorum and *Salmonella* Gallinarum. The reason why LAB3 was using the ISO 6579 Annex D method for the isolation of *Salmonella* Pullorum and –Gallinarum in organs remains unclear. The instructions, given by the PT provider, might have been misinterpreted by LAB3 and the reason for these misclassifications. The instructions did not clearly mention that MSRV could not be used in order to detect non-mobile *Salmonella* spp.

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, no mistakes were made for the strong positive faecal samples and maximum 1 mistake for both the weak positive and the negative faecal samples (see III.3.3.). Consequently, LAB1,LAB2 and LAB4 achieved a satisfactory performance for the isolation of *Salmonella* spp. from liver. LAB3 did not achieve 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) (Ciney, Belgium)

Dierengezondheidszorg Vlaanderen (DGZ) (Torhout, Belgium)

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

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