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081-PT

PROFICIENCY TESTING 2018

SALMONELLA (SAL)

Isolation of Salmonella spp. from organs

SCIENTIFIC DIRECTORATE INFECTIOUS DISEASES IN ANIMALS
SCIENSANO

DATE BEGIN PT: 10 DECEMBER 2018

DATE REPORT: 28 FEBRUARY 2019

I. Introduction

Details relevant to the proficiency test (PT) are available in the procedure SOP 25/01 'Beheer van de proficiency testen georganiseerd door de Wetenschappelijke Directie Infectieziekten Dier/Gestion des essais d'aptitude organisés par la Direction Scientifique Maladies Infectieuses Animales', 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 eight aliquots were analyzed on the 25th of September 2018 for the presence of *Salmonella* spp. by the *Salmonella* Gallinarum/Pullorum reference laboratory of the Scientific directorate Infectious diseases in animals of Sciensano, 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 the 10th of December 2018 (start date of the PT), 70 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* Gallinarum/Pullorum reference laboratory of the Scientific directorate Infectious diseases in animals of Sciensano in parallel with the PT (30 aliquots):

- 22 aliquots (16 for the PT and 6 for the verification tests) were used as such and considered as negative liver samples ('PT2018SALBACN01')
- 14 aliquots (8 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 ('PT2018SALBACP01').
- 10 aliquots (4 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 ('PT2018SALBACP02').
- 14 aliquots (8 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 ('PT2018SALBACP03').
- 10 aliquots (4 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 ('PT2018SALBACP04').

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 liver sample PT2018SALBACN01, 1 aliquot of the weak positive liver samples PT2018SALBACP02 (*Salmonella* Pullorum) and PT2018SALBACP04 (*Salmonella* Gallinarum) and 2 aliquots of the strong positive liver samples PT2018SALBACP01 (*Salmonella* Pullorum) and PT2018SALBACP03 (*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* Gallinarum/Pullorum reference laboratory of the Scientific directorate Infectious diseases in animals of Sciensano tested 3 aliquots of each sample (PT2018SALBACN01, PT2018SALBACP01, PT2018SALBACP02, PT2018SALBACP03 and PT2018SALBACP04) for the presence of *Salmonella* on both 10th (day 1) and 11th (day 2) of December 2018, 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 (PT2018SALBACP01, PT2018SALBACP03). For the negative (PT2018SALBACN01) and weak positive samples (PT2018SALBACP02, PT2018SALBACP04) 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 Scientific Directorate Infectious Diseases in Animals of Sciensano.

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 10th of December 2018 (40 aliquots in total). All laboratories acknowledged receipt of the samples on the same day. Analyses were started on 10th (LAB2, LAB3 and LAB4) and 11th (LAB1) of December 2018 (Table 1).

IV.2. Dates at which results were returned to the Scientific Directorate Infectious Diseases in Animals of Sciensano

Results were submitted to the Scientific Directorate Infectious Diseases in Animals of Sciensano between 14th and 20th of December 2018 (Table 1).

Table 1. Overview of the dates on which (i) the reference samples were received and analyzed by the participating laboratories, and (ii) the obtained results were submitted to the Scientific Directorate Infectious Diseases in Animals of Sciensano.

Participating laboratory	Reference samples received	Start of analysis	Submission of the results (Excel file)
LAB1	10/12/2018	11/12/2018	17/12/2019
LAB2	10/12/2018	10/12/2018	19/12/2018
LAB3	10/12/2018	10/12/2018	20/12/2018
LAB4	10/12/2018	10/12/2018	14/12/2018

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

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. Three of the 4 participating laboratories reached 100% of agreement, LAB1 reached 90 % of agreement with 1 misclassification for the weak positive sample PT2018SALBACP04. (Table 2).

Table 2. Agreement between the results obtained by the participating laboratories (LABNR) and the status of the liver samples assigned by the *Salmonella* Gallinarum/Pullorum reference laboratory of the Scientific Directorate Infectious Diseases in Animals of Sciensano. 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	**1 (10)**	0 (0)	0 (0)	0 (0)
success	**9 (90)**	10 (100)	10 (100)	10 (100)

IV.4.2. Variability among participating laboratories

No variability in qualitative laboratory results could be observed between LAB2, LAB3 and LAB4 since these participants correctly identified all reference liver samples. In contrast, LAB1 misclassified the weak positive reference liver sample PT2018SALBACP04 (NEG instead of POS).

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* Gallinarum/Pullorum reference laboratory of the Scientific Directorate Infectious Diseases in Animals of Sciensano (STATUS). NEG: negative; POS: positive

	LABNR	LABPOSIT	SAMPLE	STATUS	RESULT	SUCCESS
1	1	1	PT2018SALBACP02	POS	POS	1
2	1	2	PT2018SALBACP04	**POS**	**NEG**	**0**
3	1	3	PT2018SALBACN01	NEG	NEG	1
4	1	4	PT2018SALBACP01	POS	POS	1
5	1	5	PT2018SALBACN01	NEG	NEG	1
6	1	6	PT2018SALBACP03	POS	POS	1
7	1	7	PT2018SALBACP01	POS	POS	1
8	1	8	PT2018SALBACN01	NEG	NEG	1
9	1	9	PT2018SALBACP03	POS	POS	1
10	1	10	PT2018SALBACN01	NEG	NEG	1
11	2	1	PT2018SALBACP03	POS	POS	1
12	2	2	PT2018SALBACN01	NEG	NEG	1
13	2	3	PT2018SALBACP03	POS	POS	1
14	2	4	PT2018SALBACP01	POS	POS	1
15	2	5	PT2018SALBACP02	POS	POS	1
16	2	6	PT2018SALBACN01	NEG	NEG	1
17	2	7	PT2018SALBACN01	NEG	NEG	1
18	2	8	PT2018SALBACP01	POS	POS	1
19	2	9	PT2018SALBACN01	NEG	NEG	1
20	2	10	PT2018SALBACP04	POS	POS	1
21	3	1	PT2018SALBACP02	POS	POS	1
22	3	2	PT2018SALBACP04	POS	POS	1
23	3	3	PT2018SALBACN01	NEG	NEG	1
24	3	4	PT2018SALBACP01	POS	POS	1
25	3	5	PT2018SALBACN01	NEG	NEG	1
26	3	6	PT2018SALBACP03	POS	POS	1
27	3	7	PT2018SALBACP01	POS	POS	1
28	3	8	PT2018SALBACN01	NEG	NEG	1
29	3	9	PT2018SALBACP03	POS	POS	1
30	3	10	PT2018SALBACN01	NEG	NEG	1
31	4	1	PT2018SALBACP03	POS	POS	1
32	4	2	PT2018SALBACN01	NEG	NEG	1
33	4	3	PT2018SALBACP03	POS	POS	1
34	4	4	PT2018SALBACP01	POS	POS	1
35	4	5	PT2018SALBACP02	POS	POS	1
36	4	6	PT2018SALBACN01	NEG	NEG	1
37	4	7	PT2018SALBACN01	NEG	NEG	1
38	4	8	PT2018SALBACP01	POS	POS	1
39	4	9	PT2018SALBACN01	NEG	NEG	1
40	4	10	PT2018SALBACP04	POS	POS	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.

The 4 participating laboratories reached the required level of agreement. LAB2, LAB3 and LAB4 correctly identified all liver samples (100% of agreement), LAB1 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 (Table 2 and Table 3).

Two differences were observed in the reagents used by the laboratories: (i) LAB2, LAB3 and LAB4 used the liquid enrichment medium RVS from the same producer Bio-Rad, while LAB1 used the one from Biomérieux, and (ii) LAB2, LAB3 and LAB4 used BGA plates, while LAB1 used XLD in place of BGA plates. During the course of the PT, it was noticed that the laboratory instructions followed by the *Salmonella* Gallinarum/Pullorum reference laboratory of the Scientific Directorate Infectious Diseases in Animals of Sciensano and described in the internal procedure SOP/BAC/ANA/23 do not follow the ISO6579 annex D nor the ISO6579-1 norms while the instructions used are well adapted for the isolation of *Salmonella* Pullorum and *Salmonella* Gallinarum. This will be clarified for the next PT in the instructions for participants. Nevertheless, all the participating laboratories reached the required level of agreement.

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* Gallinarum/Pullorum reference laboratory of the Scientific Directorate Infectious Diseases in Animals of Sciensano, no mistakes were made for the strong positive samples and maximum 1 mistake for both the weak positive and the negative samples (see III.3.3.). Consequently, all participants in the PT 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)

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

Sciensano (Ukkel, Belgium)