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172-PT

## **PROFICIENCY TESTING 2013**

***SALMONELLA (SAL)***

***Isolation of Salmonella sp. from faeces***

**OPERATIONAL UNIT  
COORDINATION OF VETERINARY DIAGNOSIS  
EPIDEMIOLOGY AND RISK ASSESSMENT  
(CVD-ERA)**

**DATE BEGIN PT: 07 OCTOBER 2013**

**DATE REPORT: 03 FEBRUARY 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* sp. 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* sp. by the *Salmonella* reference laboratory of CODA-CERVA, hereby following method ISO 6579 annex D. Since all tested aliquots were found negative for *Salmonella* sp., the collected faeces were considered as *Salmonella* negative and hence the remaining aliquots as suitable for the PT.

On 7<sup>th</sup> of October 2013 (start date of the PT), 230 aliquots of faecal samples were prepared and randomized, either for the PT (200 aliquots) or the verification tests that had to be performed by the *Salmonella* reference laboratory of CODA-CERVA in parallel with the PT (30 aliquots):

- 130 aliquots (120 for the PT and 10 for the verification tests) were used as such and considered as negative faecal samples ('PT2013SALBACNF1')
- 60 aliquots (50 for the PT and 10 for the verification tests) were inoculated with 171 cfu *Salmonella* Typhimurium (antigenic formula O4,5,12:i:1,2) and were considered as strong positive faecal samples ('PT2013SALBACPF1').
- 40 aliquots (30 for the PT and 10 for the verification tests) were inoculated with 17 cfu *Salmonella* Typhimurium (antigenic formula O4,5,12:i:1,2) and were considered as weak positive faecal samples ('PT2013SALBACPF2').

In total, 200 aliquots of faecal samples were distributed to 10 participating laboratories. All participants were given 20 aliquots of faecal samples: 12 aliquots of the negative faecal sample PT2013SALBACNF1, 5 aliquots of the strong positive faecal sample PT2013SALBACPF1 and 3 aliquots of the weak positive faecal sample PT2013SALBACPF2.

For most PTs organized by the operational unit CVD-ERA of CODA-CERVA, 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 tested 5 aliquots of each category of faecal samples for the presence of *Salmonella* on both 7<sup>th</sup> (day 1) and 8<sup>th</sup> (day 2) of October 2013, 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 20 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 20 aliquots of faecal samples is at least 90% and if no mistakes were made for the strong positive faecal samples and maximum 1 mistake for both the weak positive and the negative faecal samples.

## 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 20 aliquots of faecal samples were sent at  $5\pm 3^{\circ}\text{C}$  to each of the 10 participating laboratories by national courier on 7<sup>th</sup> of October 2013 (200 aliquots in total). All laboratories acknowledged receipt of the samples on the same day. Analyses were started on 7<sup>th</sup>, 8<sup>th</sup> and 9<sup>th</sup> of October 2013. Hereby, LAB7 did not respect the deadline of 8<sup>th</sup> of October 2013 for analysis of the faecal samples, as indicated in the instructions provided by the PT provider (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 14<sup>th</sup> and 31<sup>st</sup> of October 2013. LAB3 hereby exceeded the deadline of 18<sup>th</sup> of October 2013 for submission of the results (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 operational unit CVD-ERA of CODA-CERVA.

Laboratory	Reference samples received	Start of analysis	Submission of the results (Excel file)	Dated & signed hard copy of the results received
LAB1	07/10/2013	07/10/2013	16/10/2013	YES
LAB2	07/10/2013	08/10/2013	17/10/2013	<b>NO (only questionnaire)</b>
LAB3	07/10/2013	07/10/2013	<b>31/10/2013</b>	<b>NO (only questionnaire)</b>
LAB4	07/10/2013	08/10/2013	15/10/2013	YES
LAB5	07/10/2013	07/10/2013	15/10/2013	YES
LAB6	07/10/2013	08/10/2013	18/10/2013	YES
LAB7	07/10/2013	<b>09/10/2013</b>	16/10/2013	YES
LAB8	07/10/2013	08/10/2013	14/10/2013	YES
LAB9	07/10/2013	07/10/2013	17/10/2013	YES (only Excel)
LAB10	07/10/2013	08/10/2013	15/10/2013	YES

### IV.3. Compliance with the procedure

Except LAB2 and 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 faecal samples and hence reached 100% 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. All participating laboratories received 20 aliquots of faecal samples. Results are presented as absolute values and percentages (in parentheses).

	LABNR									
	1	2	3	4	5	6	7	8	9	10
<b>failure</b>	0 (0.0)	0 (0.0)	0 (0.0)	0 (0.0)	0 (0.0)	0 (0.0)	0 (0.0)	0 (0.0)	0 (0.0)	0 (0.0)
<b>success</b>	20 (100.0)	20 (100.0)	20 (100.0)	20 (100.0)	20 (100.0)	20 (100.0)	20 (100.0)	20 (100.0)	20 (100.0)	20 (100.0)

### IV.4.2. Variability among participating laboratories

At the qualitative data level, no variability between the participating laboratories could be observed since all participants correctly identified all faecal samples.

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 (STATUS). NEG: negative; POS: positive.

	LABNR	LABPOSIT	SAMPLE	STATUS	RESULT	SUCCESS
1	1	1	PT2013SALBACNF1	NEG	NEG	1
2	1	2	PT2013SALBACNF1	NEG	NEG	1
3	1	3	PT2013SALBACPF1	POS	POS	1
4	1	4	PT2013SALBACNF1	NEG	NEG	1
5	1	5	PT2013SALBACPF2	POS	POS	1
6	1	6	PT2013SALBACNF1	NEG	NEG	1
7	1	7	PT2013SALBACPF2	POS	POS	1
8	1	8	PT2013SALBACPF1	POS	POS	1
9	1	9	PT2013SALBACNF1	NEG	NEG	1
10	1	10	PT2013SALBACNF1	NEG	NEG	1
11	1	11	PT2013SALBACPF2	POS	POS	1
12	1	12	PT2013SALBACNF1	NEG	NEG	1
13	1	13	PT2013SALBACNF1	NEG	NEG	1
14	1	14	PT2013SALBACPF1	POS	POS	1
15	1	15	PT2013SALBACNF1	NEG	NEG	1
16	1	16	PT2013SALBACNF1	NEG	NEG	1
17	1	17	PT2013SALBACPF1	POS	POS	1
18	1	18	PT2013SALBACNF1	NEG	NEG	1
19	1	19	PT2013SALBACPF1	POS	POS	1
20	1	20	PT2013SALBACNF1	NEG	NEG	1
21	2	1	PT2013SALBACPF2	POS	pos	1
22	2	2	PT2013SALBACNF1	NEG	neg	1
23	2	3	PT2013SALBACPF2	POS	pos	1
24	2	4	PT2013SALBACPF1	POS	pos	1
25	2	5	PT2013SALBACNF1	NEG	neg	1
26	2	6	PT2013SALBACNF1	NEG	neg	1
27	2	7	PT2013SALBACPF2	POS	pos	1
28	2	8	PT2013SALBACNF1	NEG	neg	1
29	2	9	PT2013SALBACNF1	NEG	neg	1
30	2	10	PT2013SALBACPF1	POS	pos	1
31	2	11	PT2013SALBACNF1	NEG	neg	1
32	2	12	PT2013SALBACNF1	NEG	neg	1
33	2	13	PT2013SALBACPF1	POS	pos	1
34	2	14	PT2013SALBACNF1	NEG	neg	1
35	2	15	PT2013SALBACPF1	POS	pos	1
36	2	16	PT2013SALBACNF1	NEG	neg	1
37	2	17	PT2013SALBACNF1	NEG	neg	1
38	2	18	PT2013SALBACNF1	NEG	neg	1
39	2	19	PT2013SALBACPF1	POS	pos	1
40	2	20	PT2013SALBACNF1	NEG	neg	1



(Table 3 - CONTINUED)

	LABNR	LABPOSIT	SAMPLE	STATUS	RESULT	SUCCESS
41	3	1	PT2013SALBACNF1	NEG	NEG	1
42	3	2	PT2013SALBACNF1	NEG	NEG	1
43	3	3	PT2013SALBACPF1	POS	POS	1
44	3	4	PT2013SALBACNF1	NEG	NEG	1
45	3	5	PT2013SALBACPF2	POS	POS	1
46	3	6	PT2013SALBACNF1	NEG	NEG	1
47	3	7	PT2013SALBACPF2	POS	POS	1
48	3	8	PT2013SALBACPF1	POS	POS	1
49	3	9	PT2013SALBACNF1	NEG	NEG	1
50	3	10	PT2013SALBACNF1	NEG	NEG	1
51	3	11	PT2013SALBACPF2	POS	POS	1
52	3	12	PT2013SALBACNF1	NEG	NEG	1
53	3	13	PT2013SALBACNF1	NEG	NEG	1
54	3	14	PT2013SALBACPF1	POS	POS	1
55	3	15	PT2013SALBACNF1	NEG	NEG	1
56	3	16	PT2013SALBACNF1	NEG	NEG	1
57	3	17	PT2013SALBACPF1	POS	POS	1
58	3	18	PT2013SALBACNF1	NEG	NEG	1
59	3	19	PT2013SALBACPF1	POS	POS	1
60	3	20	PT2013SALBACNF1	NEG	NEG	1
61	4	1	PT2013SALBACPF2	POS	POS	1
62	4	2	PT2013SALBACNF1	NEG	NEG	1
63	4	3	PT2013SALBACPF2	POS	POS	1
64	4	4	PT2013SALBACPF1	POS	POS	1
65	4	5	PT2013SALBACNF1	NEG	NEG	1
66	4	6	PT2013SALBACNF1	NEG	NEG	1
67	4	7	PT2013SALBACPF2	POS	POS	1
68	4	8	PT2013SALBACNF1	NEG	NEG	1
69	4	9	PT2013SALBACNF1	NEG	NEG	1
70	4	10	PT2013SALBACPF1	POS	POS	1
71	4	11	PT2013SALBACNF1	NEG	NEG	1
72	4	12	PT2013SALBACNF1	NEG	NEG	1
73	4	13	PT2013SALBACPF1	POS	POS	1
74	4	14	PT2013SALBACNF1	NEG	NEG	1
75	4	15	PT2013SALBACPF1	POS	POS	1
76	4	16	PT2013SALBACNF1	NEG	NEG	1
77	4	17	PT2013SALBACNF1	NEG	NEG	1
78	4	18	PT2013SALBACNF1	NEG	NEG	1
79	4	19	PT2013SALBACPF1	POS	POS	1
80	4	20	PT2013SALBACNF1	NEG	NEG	1



(Table 3 - CONTINUED)

	LABNR	LABPOSIT	SAMPLE	STATUS	RESULT	SUCCESS
81	5	1	PT2013SALBACNF1	NEG	NEG	1
82	5	2	PT2013SALBACNF1	NEG	NEG	1
83	5	3	PT2013SALBACPF1	POS	POS	1
84	5	4	PT2013SALBACNF1	NEG	NEG	1
85	5	5	PT2013SALBACPF2	POS	POS	1
86	5	6	PT2013SALBACNF1	NEG	NEG	1
87	5	7	PT2013SALBACPF2	POS	POS	1
88	5	8	PT2013SALBACPF1	POS	POS	1
89	5	9	PT2013SALBACNF1	NEG	NEG	1
90	5	10	PT2013SALBACNF1	NEG	NEG	1
91	5	11	PT2013SALBACPF2	POS	POS	1
92	5	12	PT2013SALBACNF1	NEG	NEG	1
93	5	13	PT2013SALBACNF1	NEG	NEG	1
94	5	14	PT2013SALBACPF1	POS	POS	1
95	5	15	PT2013SALBACNF1	NEG	NEG	1
96	5	16	PT2013SALBACNF1	NEG	NEG	1
97	5	17	PT2013SALBACPF1	POS	POS	1
98	5	18	PT2013SALBACNF1	NEG	NEG	1
99	5	19	PT2013SALBACPF1	POS	POS	1
100	5	20	PT2013SALBACNF1	NEG	NEG	1
101	6	1	PT2013SALBACPF2	POS	POS	1
102	6	2	PT2013SALBACNF1	NEG	NEG	1
103	6	3	PT2013SALBACPF2	POS	POS	1
104	6	4	PT2013SALBACPF1	POS	POS	1
105	6	5	PT2013SALBACNF1	NEG	NEG	1
106	6	6	PT2013SALBACNF1	NEG	NEG	1
107	6	7	PT2013SALBACPF2	POS	POS	1
108	6	8	PT2013SALBACNF1	NEG	NEG	1
109	6	9	PT2013SALBACNF1	NEG	NEG	1
110	6	10	PT2013SALBACPF1	POS	POS	1
111	6	11	PT2013SALBACNF1	NEG	NEG	1
112	6	12	PT2013SALBACNF1	NEG	NEG	1
113	6	13	PT2013SALBACPF1	POS	POS	1
114	6	14	PT2013SALBACNF1	NEG	NEG	1
115	6	15	PT2013SALBACPF1	POS	POS	1
116	6	16	PT2013SALBACNF1	NEG	NEG	1
117	6	17	PT2013SALBACNF1	NEG	NEG	1
118	6	18	PT2013SALBACNF1	NEG	NEG	1
119	6	19	PT2013SALBACPF1	POS	POS	1
120	6	20	PT2013SALBACNF1	NEG	NEG	1



(Table 3 - CONTINUED)

	LABNR	LABPOSIT	SAMPLE	STATUS	RESULT	SUCCESS
121	7	1	PT2013SALBACNF1	NEG	NEG	1
122	7	2	PT2013SALBACNF1	NEG	NEG	1
123	7	3	PT2013SALBACPF1	POS	POS	1
124	7	4	PT2013SALBACNF1	NEG	NEG	1
125	7	5	PT2013SALBACPF2	POS	POS	1
126	7	6	PT2013SALBACNF1	NEG	NEG	1
127	7	7	PT2013SALBACPF2	POS	POS	1
128	7	8	PT2013SALBACPF1	POS	POS	1
129	7	9	PT2013SALBACNF1	NEG	NEG	1
130	7	10	PT2013SALBACNF1	NEG	NEG	1
131	7	11	PT2013SALBACPF2	POS	POS	1
132	7	12	PT2013SALBACNF1	NEG	NEG	1
133	7	13	PT2013SALBACNF1	NEG	NEG	1
134	7	14	PT2013SALBACPF1	POS	POS	1
135	7	15	PT2013SALBACNF1	NEG	NEG	1
136	7	16	PT2013SALBACNF1	NEG	NEG	1
137	7	17	PT2013SALBACPF1	POS	POS	1
138	7	18	PT2013SALBACNF1	NEG	NEG	1
139	7	19	PT2013SALBACPF1	POS	POS	1
140	7	20	PT2013SALBACNF1	NEG	NEG	1
141	8	1	PT2013SALBACPF2	POS	POS	1
142	8	2	PT2013SALBACNF1	NEG	NEG	1
143	8	3	PT2013SALBACPF2	POS	POS	1
144	8	4	PT2013SALBACPF1	POS	POS	1
145	8	5	PT2013SALBACNF1	NEG	NEG	1
146	8	6	PT2013SALBACNF1	NEG	NEG	1
147	8	7	PT2013SALBACPF2	POS	POS	1
148	8	8	PT2013SALBACNF1	NEG	NEG	1
149	8	9	PT2013SALBACNF1	NEG	NEG	1
150	8	10	PT2013SALBACPF1	POS	POS	1
151	8	11	PT2013SALBACNF1	NEG	NEG	1
152	8	12	PT2013SALBACNF1	NEG	NEG	1
153	8	13	PT2013SALBACPF1	POS	POS	1
154	8	14	PT2013SALBACNF1	NEG	NEG	1
155	8	15	PT2013SALBACPF1	POS	POS	1
156	8	16	PT2013SALBACNF1	NEG	NEG	1
157	8	17	PT2013SALBACNF1	NEG	NEG	1
158	8	18	PT2013SALBACNF1	NEG	NEG	1
159	8	19	PT2013SALBACPF1	POS	POS	1
160	8	20	PT2013SALBACNF1	NEG	NEG	1





(Table 3 - CONTINUED)

	LABNR	LABPOSIT	SAMPLE	STATUS	RESULT	SUCCESS
161	9	1	PT2013SALBACNF1	NEG	NEG	1
162	9	2	PT2013SALBACNF1	NEG	NEG	1
163	9	3	PT2013SALBACPF1	POS	POS	1
164	9	4	PT2013SALBACNF1	NEG	NEG	1
165	9	5	PT2013SALBACPF2	POS	POS	1
166	9	6	PT2013SALBACNF1	NEG	NEG	1
167	9	7	PT2013SALBACPF2	POS	POS	1
168	9	8	PT2013SALBACPF1	POS	POS	1
169	9	9	PT2013SALBACNF1	NEG	NEG	1
170	9	10	PT2013SALBACNF1	NEG	NEG	1
171	9	11	PT2013SALBACPF2	POS	POS	1
172	9	12	PT2013SALBACNF1	NEG	NEG	1
173	9	13	PT2013SALBACNF1	NEG	NEG	1
174	9	14	PT2013SALBACPF1	POS	POS	1
175	9	15	PT2013SALBACNF1	NEG	NEG	1
176	9	16	PT2013SALBACNF1	NEG	NEG	1
177	9	17	PT2013SALBACPF1	POS	POS	1
178	9	18	PT2013SALBACNF1	NEG	NEG	1
179	9	19	PT2013SALBACPF1	POS	POS	1
180	9	20	PT2013SALBACNF1	NEG	NEG	1
181	10	1	PT2013SALBACPF2	POS	POS	1
182	10	2	PT2013SALBACNF1	NEG	NEG	1
183	10	3	PT2013SALBACPF2	POS	POS	1
184	10	4	PT2013SALBACPF1	POS	POS	1
185	10	5	PT2013SALBACNF1	NEG	NEG	1
186	10	6	PT2013SALBACNF1	NEG	NEG	1
187	10	7	PT2013SALBACPF2	POS	POS	1
188	10	8	PT2013SALBACNF1	NEG	NEG	1
189	10	9	PT2013SALBACNF1	NEG	NEG	1
190	10	10	PT2013SALBACPF1	POS	POS	1
191	10	11	PT2013SALBACNF1	NEG	NEG	1
192	10	12	PT2013SALBACNF1	NEG	NEG	1
193	10	13	PT2013SALBACPF1	POS	POS	1
194	10	14	PT2013SALBACNF1	NEG	NEG	1
195	10	15	PT2013SALBACPF1	POS	POS	1
196	10	16	PT2013SALBACNF1	NEG	NEG	1
197	10	17	PT2013SALBACNF1	NEG	NEG	1
198	10	18	PT2013SALBACNF1	NEG	NEG	1
199	10	19	PT2013SALBACPF1	POS	POS	1
200	10	20	PT2013SALBACNF1	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.

All participating laboratories correctly identified all faecal samples (100% of agreement) (Table 2 and Table 3). Hereby, culture media from different producers were used (Bio-Rad, Oxoid, Biomérieux, Biokar, Beckton-Dickinson).

## 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 faecal samples assigned by the *Salmonella* reference laboratory of CODA-CERVA, and if 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, all participants achieved a satisfactory performance for the isolation of *Salmonella* sp. from faeces.

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)

FLVVM (Melle, Belgium)

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

Lavetan NV (Turnhout, Belgium)

LFSAGx (Gembloux, Belgium)

MicroBioMetrix bvba (Sint-Katelijne-Waver, Belgium)

Plukon Food Laboratory (Wezep, The Netherlands)

Servaco Food Control NV (Wetteren, Belgium)

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