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172-TEST

PROFICIENCY TESTING 2010

SALMONELLA (SAL)

***Detection of Salmonella-specific antibodies in pig serum by
Enzyme Linked Immunosorbent Assay (ELISA)***

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

DATE BEGIN PT: 6 SEPTEMBER 2010

DATE REPORT: 27 OCTOBER 2010

I. Introduction

Details relevant to the proficiency test are available in the Procedure PRO/2.5/01 'Beheer van de proficiency testen/Gestion des essais d'aptitude'.

II. Aim

This proficiency test, focusing on the detection of *Salmonella*-specific antibodies in pig serum, aims to assess the analytical accuracy of tests conducted by participants.

III. Materials and methods

III.1. Conduct of diagnostic tests

In the framework of this proficiency test, predefined reference serum samples must be tested by means of an ELISA test. The procedures for the ELISA tests must be fully described in the SOPs of the participating laboratories.

III.2. Reference samples

III.2.1. *Salmonella*-specific antibody reference serum samples

Replicates of six reference serum samples either free from detectable *Salmonella*-specific antibodies ($n = 2$; coded 'PT2010SALSERNS1' and 'PT2010SALSERNS2') or containing detectable *Salmonella*-specific antibodies ($n = 4$; coded 'PT2010SALSERPS1', 'PT2010SALSERPS2', 'PT2010SALSERPS3', and 'PT2010SALSERPS4') were used. In total 180 aliquots, prepared by the *Salmonella* reference laboratory of the Veterinary and Agrochemical Research Center (CODA-CERVA), were distributed to the participating laboratories. For each reference serum sample a certificate containing the assigned value (status of the sample = 'golden standard') was made. The assigned value was obtained by testing each reference serum at least 10 times and for which each time the same qualitative result was obtained. Consequently, these reference serum samples were considered as reliable samples to evaluate the ability to identify the absence or presence of *Salmonella*-specific antibodies in pig serum.

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* (positive result when the reference sample is truly positive, negative result when the reference sample is truly negative) or *failure* (positive result when the reference sample is truly negative, negative result when the reference sample is truly positive).

III.3.2. Level of agreement

The level of agreement achieved by the participating laboratories is expressed as the percentage of success for all 30 samples (aliquots) for *Salmonella*-specific antibodies carried out for this proficiency test.

III.3.3. Threshold for qualification

Following the procedure, a participating laboratory is only qualified if the level of agreement for all reference serum samples is at least 90%.

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. Reference samples

IV.1.1. Allocation of reference serum samples to the participating laboratories

All participating laboratories were given:

- i. 10 aliquots of reference serum samples free from detectable *Salmonella*-specific antibodies: PT2010SALSERNS1 samples (n = 5) and PT2010SALSERNS2 samples (n = 5);
- ii. 20 aliquots of reference serum samples containing detectable *Salmonella*-specific antibodies: PT2010SALSERPS1 samples (n = 5), PT2010SALSERPS2 samples (n = 5), PT2010SALSERPS3 samples (n = 5), and PT2010SALSERPS4 samples (n = 5).

IV.1.2. Transfer and start of the analyses

The 30 aliquots of reference serum samples were sent by national or international courier on 6 September 2010 to each of the six participating laboratories (180 aliquots in total). Four laboratories (LAB1, LAB3, LAB4, and LAB5) acknowledged receipt of the samples on the same day. LAB6 received the samples on 7 September 2010 and LAB2 received the samples on 8 September 2010. The analyses were carried out on 7 (LAB3 and LAB4) and 9 (LAB2 and LAB5) September 2010. No information about the start of the analysis was provided by LAB1 and LAB6.

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

Results from the participating laboratories have been received on 7 (LAB4), 10 (LAB2), 13 (LAB3 and LAB5), 15 (LAB1), and 16 (LAB6) September 2010.

IV.3. Compliance with the procedure

Two (LAB3 and LAB5) out of six participating laboratories have provided a duly dated and signed copy of the results.

IV.4. Level of agreement

Three participating laboratories reached 100% of agreement for the detection of *Salmonella*-specific antibodies in the reference serum samples (Table 1).

Box plots of the calculated results (S/P ratio) per sample and per participating laboratory are attached in Annex 1.



Table 1. Agreement between results generated by the participating laboratories (LABNR) and the status of reference serum samples. The purpose of the proficiency test is to detect *Salmonella*-specific antibodies in reference serum samples by ELISA.

Success while screening the samples (0 = Failure, 1 = Success)						
Variable	LABNR					
	1 (N=30)	2 (N=30)	3 (N=30)	4 (N=30)	5 (N=30)	6 (N=30)
	N (%)	N (%)	N (%)	N (%)	N (%)	N (%)
0	0 (0.0)	0 (0.0)	0 (0.0)	5 (16.7)	1 (3.3)	10 (33.3)
1	30 (100.0)	30 (100.0)	30 (100.0)	25 (83.3)	29 (96.7)	20 (66.7)

IV.5. Variability among participating laboratories

The responses of the six participating laboratories that provided their results for the reference serum samples are displayed in Table 2.



Table 2. Responses (RESULT) of the participating laboratories (LABNR) with the identification (SAMPLE) of the reference serum samples, the position (LABPOSIT) of the reference serum samples as placed in the block, and the results (STATUS) obtained by repeated screening by the CODA-CERVA.

	LABNR	LABPOSIT	SAMPLE	STATUS	RESULT	SUCCESS
1	1	1	PT2010SALSERN1	NEG	NEG	1
2	1	2	PT2010SALSERPS2	POS	POS	1
3	1	3	PT2010SALSERPS3	POS	POS	1
4	1	4	PT2010SALSERN2	NEG	NEG	1
5	1	5	PT2010SALSERPS2	POS	POS	1
6	1	6	PT2010SALSERPS4	POS	POS	1
7	1	7	PT2010SALSERN1	NEG	NEG	1
8	1	8	PT2010SALSERPS2	POS	POS	1
9	1	9	PT2010SALSERN2	NEG	NEG	1
10	1	10	PT2010SALSERPS3	POS	POS	1
11	1	11	PT2010SALSERPS1	POS	POS	1
12	1	12	PT2010SALSERPS4	POS	POS	1
13	1	13	PT2010SALSERPS2	POS	POS	1
14	1	14	PT2010SALSERN1	NEG	NEG	1
15	1	15	PT2010SALSERPS4	POS	POS	1
16	1	16	PT2010SALSERPS3	POS	POS	1
17	1	17	PT2010SALSERN2	NEG	NEG	1
18	1	18	PT2010SALSERPS4	POS	POS	1
19	1	19	PT2010SALSERPS1	POS	POS	1
20	1	20	PT2010SALSERN1	NEG	NEG	1
21	1	21	PT2010SALSERPS2	POS	POS	1
22	1	22	PT2010SALSERPS1	POS	POS	1
23	1	23	PT2010SALSERPS3	POS	POS	1
24	1	24	PT2010SALSERN2	NEG	NEG	1
25	1	25	PT2010SALSERPS4	POS	POS	1
26	1	26	PT2010SALSERPS1	POS	POS	1
27	1	27	PT2010SALSERN1	NEG	NEG	1
28	1	28	PT2010SALSERPS3	POS	POS	1
29	1	29	PT2010SALSERPS1	POS	POS	1
30	1	30	PT2010SALSERN2	NEG	NEG	1



(CONTINUED)

	LABNR	LABPOSIT	SAMPLE	STATUS	RESULT	SUCCESS
31	2	1	PT2010SALSERPS4	POS	POS	1
32	2	2	PT2010SALSERNS1	NEG	NEG	1
33	2	3	PT2010SALSERPS2	POS	POS	1
34	2	4	PT2010SALSERNS2	NEG	NEG	1
35	2	5	PT2010SALSERPS3	POS	POS	1
36	2	6	PT2010SALSERPS1	POS	POS	1
37	2	7	PT2010SALSERPS4	POS	POS	1
38	2	8	PT2010SALSERPS2	POS	POS	1
39	2	9	PT2010SALSERNS1	NEG	NEG	1
40	2	10	PT2010SALSERPS4	POS	POS	1
41	2	11	PT2010SALSERPS3	POS	POS	1
42	2	12	PT2010SALSERNS2	NEG	NEG	1
43	2	13	PT2010SALSERPS4	POS	POS	1
44	2	14	PT2010SALSERPS1	POS	POS	1
45	2	15	PT2010SALSERNS1	NEG	NEG	1
46	2	16	PT2010SALSERPS2	POS	POS	1
47	2	17	PT2010SALSERPS1	POS	POS	1
48	2	18	PT2010SALSERPS3	POS	POS	1
49	2	19	PT2010SALSERNS2	NEG	NEG	1
50	2	20	PT2010SALSERPS4	POS	POS	1
51	2	21	PT2010SALSERPS1	POS	POS	1
52	2	22	PT2010SALSERNS1	NEG	NEG	1
53	2	23	PT2010SALSERPS3	POS	POS	1
54	2	24	PT2010SALSERPS1	POS	POS	1
55	2	25	PT2010SALSERNS2	NEG	NEG	1
56	2	26	PT2010SALSERNS1	NEG	NEG	1
57	2	27	PT2010SALSERPS2	POS	POS	1
58	2	28	PT2010SALSERPS3	POS	POS	1
59	2	29	PT2010SALSERNS2	NEG	NEG	1
60	2	30	PT2010SALSERPS2	POS	POS	1



(CONTINUED)

	LABNR	LABPOSIT	SAMPLE	STATUS	RESULT	SUCCESS
61	3	1	PT2010SALSERPS1	POS	POS	1
62	3	2	PT2010SALSERPS4	POS	POS	1
63	3	3	PT2010SALSERPS2	POS	POS	1
64	3	4	PT2010SALSERNS1	NEG	NEG	1
65	3	5	PT2010SALSERPS4	POS	POS	1
66	3	6	PT2010SALSERPS3	POS	POS	1
67	3	7	PT2010SALSERNS2	NEG	NEG	1
68	3	8	PT2010SALSERPS4	POS	POS	1
69	3	9	PT2010SALSERPS1	POS	POS	1
70	3	10	PT2010SALSERNS1	NEG	NEG	1
71	3	11	PT2010SALSERPS2	POS	POS	1
72	3	12	PT2010SALSERPS1	POS	POS	1
73	3	13	PT2010SALSERPS3	POS	POS	1
74	3	14	PT2010SALSERNS2	NEG	NEG	1
75	3	15	PT2010SALSERPS4	POS	POS	1
76	3	16	PT2010SALSERPS1	POS	POS	1
77	3	17	PT2010SALSERNS1	NEG	NEG	1
78	3	18	PT2010SALSERPS3	POS	POS	1
79	3	19	PT2010SALSERPS1	POS	POS	1
80	3	20	PT2010SALSERNS2	NEG	NEG	1
81	3	21	PT2010SALSERNS1	NEG	NEG	1
82	3	22	PT2010SALSERPS2	POS	POS	1
83	3	23	PT2010SALSERPS3	POS	POS	1
84	3	24	PT2010SALSERNS2	NEG	NEG	1
85	3	25	PT2010SALSERPS2	POS	POS	1
86	3	26	PT2010SALSERPS4	POS	POS	1
87	3	27	PT2010SALSERNS1	NEG	NEG	1
88	3	28	PT2010SALSERPS2	POS	POS	1
89	3	29	PT2010SALSERNS2	NEG	NEG	1
90	3	30	PT2010SALSERPS3	POS	POS	1



(CONTINUED)

	LABNR	LABPOSIT	SAMPLE	STATUS	RESULT	SUCCESS
91	4	1	PT2010SALSERPS3	POS	POS	1
92	4	2	PT2010SALSERNS2	NEG	NEG	1
93	4	3	PT2010SALSERPS4	POS	POS	1
94	4	4	PT2010SALSERPS1	POS	POS	1
95	4	5	PT2010SALSERNS1	NEG	POS	0
96	4	6	PT2010SALSERPS2	POS	POS	1
97	4	7	PT2010SALSERPS1	POS	POS	1
98	4	8	PT2010SALSERPS3	POS	POS	1
99	4	9	PT2010SALSERNS2	NEG	NEG	1
100	4	10	PT2010SALSERPS4	POS	POS	1
101	4	11	PT2010SALSERPS1	POS	POS	1
102	4	12	PT2010SALSERNS1	NEG	POS	0
103	4	13	PT2010SALSERPS3	POS	POS	1
104	4	14	PT2010SALSERPS1	POS	POS	1
105	4	15	PT2010SALSERNS2	NEG	NEG	1
106	4	16	PT2010SALSERNS1	NEG	POS	0
107	4	17	PT2010SALSERPS2	POS	POS	1
108	4	18	PT2010SALSERPS3	POS	POS	1
109	4	19	PT2010SALSERNS2	NEG	NEG	1
110	4	20	PT2010SALSERPS2	POS	POS	1
111	4	21	PT2010SALSERPS4	POS	POS	1
112	4	22	PT2010SALSERNS1	NEG	POS	0
113	4	23	PT2010SALSERPS2	POS	POS	1
114	4	24	PT2010SALSERNS2	NEG	NEG	1
115	4	25	PT2010SALSERPS3	POS	POS	1
116	4	26	PT2010SALSERPS1	POS	POS	1
117	4	27	PT2010SALSERPS4	POS	POS	1
118	4	28	PT2010SALSERPS2	POS	POS	1
119	4	29	PT2010SALSERNS1	NEG	POS	0
120	4	30	PT2010SALSERPS4	POS	POS	1



(CONTINUED)

	LABNR	LABPOSIT	SAMPLE	STATUS	RESULT	SUCCESS
121	5	1	PT2010SALSERPS2	POS	POS	1
122	5	2	PT2010SALSERPS1	POS	POS	1
123	5	3	PT2010SALSERPS3	POS	POS	1
124	5	4	PT2010SALSERNS2	NEG	NEG	1
125	5	5	PT2010SALSERPS4	POS	POS	1
126	5	6	PT2010SALSERPS1	POS	POS	1
127	5	7	PT2010SALSERNS1	NEG	NEG	1
128	5	8	PT2010SALSERPS3	POS	POS	1
129	5	9	PT2010SALSERPS1	POS	POS	1
130	5	10	PT2010SALSERNS2	NEG	NEG	1
131	5	11	PT2010SALSERNS1	NEG	NEG	1
132	5	12	PT2010SALSERPS2	POS	POS	1
133	5	13	PT2010SALSERPS3	POS	POS	1
134	5	14	PT2010SALSERNS2	NEG	NEG	1
135	5	15	PT2010SALSERPS2	POS	POS	1
136	5	16	PT2010SALSERPS4	POS	POS	1
137	5	17	PT2010SALSERNS1	NEG	NEG	1
138	5	18	PT2010SALSERPS2	POS	POS	1
139	5	19	PT2010SALSERNS2	NEG	NEG	1
140	5	20	PT2010SALSERPS3	POS	POS	1
141	5	21	PT2010SALSERPS1	POS	POS	1
142	5	22	PT2010SALSERPS4	POS	POS	1
143	5	23	PT2010SALSERPS2	POS	POS	1
144	5	24	PT2010SALSERNS1	NEG	NEG	1
145	5	25	PT2010SALSERPS4	POS	POS	1
146	5	26	PT2010SALSERPS3	POS	NEG	0
147	5	27	PT2010SALSERNS2	NEG	NEG	1
148	5	28	PT2010SALSERPS4	POS	POS	1
149	5	29	PT2010SALSERPS1	POS	POS	1
150	5	30	PT2010SALSERNS1	NEG	NEG	1



(CONTINUED)

	LABNR	LABPOSIT	SAMPLE	STATUS	RESULT	SUCCESS
151	6	1	PT2010SALSERPS1	POS	POS	1
152	6	2	PT2010SALSERNS1	NEG	NEG	1
153	6	3	PT2010SALSERPS3	<u>POS</u>	<u>NEG</u>	<u>0</u>
154	6	4	PT2010SALSERPS1	POS	POS	1
155	6	5	PT2010SALSERNS2	NEG	NEG	1
156	6	6	PT2010SALSERNS1	NEG	NEG	1
157	6	7	PT2010SALSERPS2	<u>POS</u>	<u>NEG</u>	<u>0</u>
158	6	8	PT2010SALSERPS3	<u>POS</u>	<u>NEG</u>	<u>0</u>
159	6	9	PT2010SALSERNS2	NEG	NEG	1
160	6	10	PT2010SALSERPS2	<u>POS</u>	<u>NEG</u>	<u>0</u>
161	6	11	PT2010SALSERPS4	POS	POS	1
162	6	12	PT2010SALSERNS1	NEG	NEG	1
163	6	13	PT2010SALSERPS2	<u>POS</u>	<u>NEG</u>	<u>0</u>
164	6	14	PT2010SALSERNS2	NEG	NEG	1
165	6	15	PT2010SALSERPS3	<u>POS</u>	<u>NEG</u>	<u>0</u>
166	6	16	PT2010SALSERPS1	POS	POS	1
167	6	17	PT2010SALSERPS4	POS	POS	1
168	6	18	PT2010SALSERPS2	<u>POS</u>	<u>NEG</u>	<u>0</u>
169	6	19	PT2010SALSERNS1	NEG	NEG	1
170	6	20	PT2010SALSERPS4	POS	POS	1
171	6	21	PT2010SALSERPS3	<u>POS</u>	<u>NEG</u>	<u>0</u>
172	6	22	PT2010SALSERNS2	NEG	NEG	1
173	6	23	PT2010SALSERPS4	POS	POS	1
174	6	24	PT2010SALSERPS1	POS	POS	1
175	6	25	PT2010SALSERNS1	NEG	NEG	1
176	6	26	PT2010SALSERPS2	<u>POS</u>	<u>NEG</u>	<u>0</u>
177	6	27	PT2010SALSERPS1	POS	POS	1
178	6	28	PT2010SALSERPS3	<u>POS</u>	<u>NEG</u>	<u>0</u>
179	6	29	PT2010SALSERNS2	NEG	NEG	1
180	6	30	PT2010SALSERPS4	POS	POS	1

V. Discussion

The purpose of this proficiency test is to assess performances of participating laboratories when analyzing reference serum samples of pig origin for the detection of *Salmonella*-specific antibodies by ELISA test.

Three participating laboratories (LAB1, LAB2, and LAB3) provided qualitative results that were in full agreement with the true status of the reference serum samples.

One participating laboratory (LAB5) reached 96.7% of agreement for the detection of *Salmonella*-specific antibodies. LAB5 misclassified one aliquot of PT2010SALSERPS3 which is considered as a weak positive reference serum sample.

One participating laboratory (LAB4) reached 83.3% of agreement for the detection of *Salmonella*-specific antibodies. This lab misclassified all five aliquots of PT2010SALSERNS1 (negative reference serum sample).

One participating laboratory (LAB6) reached 66.7% of agreement for the detection of *Salmonella*-specific antibodies. LAB6 misclassified the five aliquots of PT2010SALSERPS2 (strong positive reference serum sample) and those aliquots of the PT2010SALSERPS3 serum (weak positive reference serum sample). Applying the cut off of the ELISA kit producer and not the PT provider cut off LAB6 would have reached 76.7% of agreement for the detection of *Salmonella*-specific antibodies (misclassification of two aliquots of PT2010SALSERPS2 and five aliquots of PT2010SALSERPS3).

VI. Conclusions

According to the procedure currently in force, the performances of a participating laboratory is satisfactory if at least 90% of the results provided by this laboratory are in agreement with the status of the reference serum samples (Section III.3.3. of this Report). Consequently, four participating laboratories achieved a satisfactory performance for the detection of *Salmonella*-specific antibodies by ELISA test.

Head CVD-ERA
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Appendix:

Name of the participating Laboratories

AIControl
ARSIA (Loncin)
CODA-CERVA
DGZ (Torhout)
IDEXX (Switzerland)
LMVE (Laboratoire de Médecine Vétérinaire de l'Etat)

ANNEX 1:

Box plots of the S/P ratio per sample and per participating laboratory. Box plots represent the minimum value, the maximum value, the median, the lower and upper quartile and possible outliers per sample. A cut off of 0.5 S/P ratio was used to classify the samples as positive (if S/P ratio ≥ 0.5) and negative (if S/P ratio < 0.5).

