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172-TEST

PROFICIENCY TESTING 2010

SALMONELLA PULLORUM (PUL) - MYCOPLASMA GALLISEPTICUM (CRD)

***Detection of antibodies in serum by
rapid plate agglutination test***

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

DATE BEGIN PT: 21 JUNE 2010

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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 specific serum antibodies against *Salmonella enterica* subspecies *enterica* serotype Pullorum (*Salmonella* Pullorum) and *Mycoplasma gallisepticum*, 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 a rapid plate agglutination test. The procedures for the rapid plate agglutination test must be fully described in the SOPs of the participating laboratories.

III.2. Reference samples

III.2.1. *Salmonella* Pullorum reference serum samples

Replicates of six reference serum samples either free from detectable *Salmonella* Pullorum-specific antibodies ($n = 3$; coded 'PT2010PULSERNS1', 'PT2010PULSERNS2', and 'PT2010PULSERNS3') or containing detectable *Salmonella* Pullorum-specific antibodies ($n = 3$; coded 'PT2010PULSERPS1', 'PT2010PULSERPS2', and 'PT2010PULSERPS3') were used. In total 90 aliquots were sent to the participating laboratories. Each reference serum sample was accompanied with a certificate containing the assigned value (status of the sample = 'golden standard'). The assigned value for each reference serum sample was obtained by the reference laboratory of the Veterinary and Agrochemical Research Center (CODA-CERVA) by testing each reference serum sample 10 times. Each reference serum sample was also tested once after the proficiency test (post verification) to confirm the stability and status of the samples. Consequently, these reference serum samples were considered as reliable samples to evaluate the ability to identify the absence or presence of *Salmonella* Pullorum-specific antibodies in serum of poultry origin.

III.2.2. *Mycoplasma gallisepticum* reference serum samples

Replicates of six reference serum samples either free from detectable *Mycoplasma gallisepticum*-specific antibodies ($n = 3$; coded 'PT2010CRDSERNS1', 'PT2010CRDSERNS2', and 'PT2010CRDSERNS3') or containing detectable *Mycoplasma gallisepticum*-specific antibodies ($n = 3$; coded 'PT2010CRDSERPS1', 'PT2010CRDSERPS2', and 'PT2010CRDSERPS4') were used. In total 90 aliquots were sent to the participating laboratories. Each reference serum sample was accompanied with a certificate containing the assigned value (status of the sample = 'golden standard'). The assigned value for each reference serum sample was obtained by the reference laboratory of CODA-CERVA by testing each reference serum sample 10 times. Each reference serum sample was also tested once after the proficiency test (post verification) to confirm the status and stability of the samples. Consequently, these reference serum samples were considered as reliable samples to evaluate the ability to identify the absence or presence of *Mycoplasma gallisepticum*-specific antibodies in serum of poultry origin.

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

III.3.2. Level of agreement

The level of agreement achieved by each participating laboratory is expressed as the percentage of success for all 30 samples (aliquots) for *Salmonella Pullorum* and 30 samples (aliquots) for *Mycoplasma gallisepticum* 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 serum samples to participating laboratories

All participating laboratories were given:

- i. 15 aliquots of reference serum samples free from detectable *Salmonella Pullorum*-specific antibodies: PT2010PULSERNS1 samples (n = 5), PT2010PULSERNS2 samples (n = 5), and PT2010PULSERNS3 samples (n = 5);
- ii. 15 aliquots of reference serum samples containing detectable *Salmonella Pullorum*-specific antibodies: PT2010PULSERPS1 samples (n = 5), PT2010PULSERPS2 samples (n = 5), and PT2010PULSERPS3 samples (n = 5).
- iii. 15 aliquots of reference serum samples free from detectable *Mycoplasma gallisepticum*-specific antibodies: PT2010CRDSERNS1 samples (n = 5), PT2010CRDSERNS2 samples (n = 5), and PT2010CRDSERNS3 samples (n = 5);
- iv. 15 aliquots of reference serum samples containing detectable *Mycoplasma gallisepticum*-specific antibodies: PT2010CRDSERPS1 samples (n = 5), PT2010CRDSERPS2 samples (n = 5), and PT2010CRDSERPS3 samples (n = 5).

IV.1.2. Transfer and start of the analyses

The 60 aliquots (30 for *Salmonella Pullorum* and 30 for *Mycoplasma gallisepticum*) of reference serum samples were sent on 21 June 2010 to each of the three participating laboratories (180 aliquots in total). The three laboratories acknowledged receipt of the samples on the same day. The analyses were carried out on 21 (LAB1) and 22 (LAB3) June 2010. No information about the start of the analysis was provided by LAB2.

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

Results from participating laboratories have been received on 23 (LAB3), 24 (LAB1), and 28 (LAB2) June 2010.

IV.3. Compliance with the procedure

Two participating laboratories (LAB1 and LAB3) have provided a duly dated and signed copy of the results.

IV.4. Level of agreement

Rapid plate agglutination test for *Salmonella* Pullorum: all participating laboratories reached 100% of agreement for the detection of *Salmonella* Pullorum-specific antibodies (Table 1).

Rapid plate agglutination test for *Mycoplasma gallisepticum*: all participating laboratories reached 100% of agreement for the detection of *Mycoplasma gallisepticum*-specific antibodies (Table 2).

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* Pullorum-specific antibodies in reference serum samples by a rapid plate agglutination test.

Success while screening the samples (0 = Failure, 1 = Success)			
Variable	Laboratories		
	1 (N=30)	2 (N=30)	3 (N=30)
	N (%)	N (%)	N (%)
0	0 (0.0)	0 (0.0)	0 (0.0)
1	30 (100.0)	30 (100.0)	30 (100.0)

Table 2. 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 *Mycoplasma gallisepticum*-specific antibodies in reference serum samples by a rapid plate agglutination test.

Success while screening the samples (0 = Failure, 1 = Success)			
Variable	Laboratories		
	1 (N=30)	2 (N=30)	3 (N=30)
	N (%)	N (%)	N (%)
0	0 (0.0)	0 (0.0)	0 (0.0)
1	30 (100.0)	30 (100.0)	30 (100.0)

IV.5. Variability among participating laboratories

The responses of the three participating laboratories that provided their results for the reference serum samples are displayed in Table 3 and 4.

Table 3. *Salmonella Pullorum* rapid plate agglutination test: The 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 CODA-CERVA.

	LABNR	LABPOSIT	SAMPLE	STATUS	RESULT	SUCCESS
1	1	1	PT2010PULSERPS2	POS	POS	1
2	1	2	PT2010PULSERPS3	POS	POS	1
3	1	3	PT2010PULSERPS3	POS	POS	1
4	1	4	PT2010PULSERNS1	NEG	NEG	1
5	1	5	PT2010PULSERNS2	NEG	NEG	1
6	1	6	PT2010PULSERNS3	NEG	NEG	1
7	1	7	PT2010PULSERPS1	POS	POS	1
8	1	8	PT2010PULSERPS2	POS	POS	1
9	1	9	PT2010PULSERPS3	POS	POS	1
10	1	10	PT2010PULSERNS1	NEG	NEG	1
11	1	11	PT2010PULSERNS2	NEG	NEG	1
12	1	12	PT2010PULSERNS3	NEG	NEG	1
13	1	13	PT2010PULSERPS1	POS	POS	1
14	1	14	PT2010PULSERNS1	NEG	NEG	1
15	1	15	PT2010PULSERNS2	NEG	NEG	1
16	1	16	PT2010PULSERNS1	NEG	NEG	1
17	1	17	PT2010PULSERNS2	NEG	NEG	1
18	1	18	PT2010PULSERNS3	NEG	NEG	1
19	1	19	PT2010PULSERPS1	POS	POS	1
20	1	20	PT2010PULSERNS1	NEG	NEG	1
21	1	21	PT2010PULSERNS2	NEG	NEG	1
22	1	22	PT2010PULSERNS3	NEG	NEG	1
23	1	23	PT2010PULSERPS1	POS	POS	1
24	1	24	PT2010PULSERPS2	POS	POS	1
25	1	25	PT2010PULSERPS3	POS	POS	1
26	1	26	PT2010PULSERPS2	POS	POS	1
27	1	27	PT2010PULSERNS3	NEG	NEG	1
28	1	28	PT2010PULSERPS1	POS	POS	1
29	1	29	PT2010PULSERPS2	POS	POS	1
30	1	30	PT2010PULSERPS3	POS	POS	1



(CONTINUED)

	LABNR	LABPOSIT	SAMPLE	STATUS	RESULT	SUCCESS
31	2	1	PT2010PULSERPS2	POS	POS	1
32	2	2	PT2010PULSERNS3	NEG	NEG	1
33	2	3	PT2010PULSERPS1	POS	POS	1
34	2	4	PT2010PULSERPS2	POS	POS	1
35	2	5	PT2010PULSERPS3	POS	POS	1
36	2	6	PT2010PULSERPS2	POS	POS	1
37	2	7	PT2010PULSERPS3	POS	POS	1
38	2	8	PT2010PULSERPS3	POS	POS	1
39	2	9	PT2010PULSERNS1	NEG	NEG	1
40	2	10	PT2010PULSERNS2	NEG	NEG	1
41	2	11	PT2010PULSERNS3	NEG	NEG	1
42	2	12	PT2010PULSERPS1	POS	POS	1
43	2	13	PT2010PULSERPS2	POS	POS	1
44	2	14	PT2010PULSERPS3	POS	POS	1
45	2	15	PT2010PULSERNS1	NEG	NEG	1
46	2	16	PT2010PULSERNS2	NEG	NEG	1
47	2	17	PT2010PULSERNS3	NEG	NEG	1
48	2	18	PT2010PULSERPS1	POS	POS	1
49	2	19	PT2010PULSERNS1	NEG	NEG	1
50	2	20	PT2010PULSERNS2	NEG	NEG	1
51	2	21	PT2010PULSERNS1	NEG	NEG	1
52	2	22	PT2010PULSERNS2	NEG	NEG	1
53	2	23	PT2010PULSERNS3	NEG	NEG	1
54	2	24	PT2010PULSERPS1	POS	POS	1
55	2	25	PT2010PULSERNS1	NEG	NEG	1
56	2	26	PT2010PULSERNS2	NEG	NEG	1
57	2	27	PT2010PULSERNS3	NEG	NEG	1
58	2	28	PT2010PULSERPS1	POS	POS	1
59	2	29	PT2010PULSERPS2	POS	POS	1
60	2	30	PT2010PULSERPS3	POS	POS	1



(CONTINUED)

	LABNR	LABPOSIT	SAMPLE	STATUS	RESULT	SUCCESS
61	3	1	PT2010PULSERNS2	NEG	NEG	1
62	3	2	PT2010PULSERNS3	NEG	NEG	1
63	3	3	PT2010PULSERPS1	POS	POS	1
64	3	4	PT2010PULSERPS2	POS	POS	1
65	3	5	PT2010PULSERPS3	POS	POS	1
66	3	6	PT2010PULSERPS2	POS	POS	1
67	3	7	PT2010PULSERNS3	NEG	NEG	1
68	3	8	PT2010PULSERPS1	POS	POS	1
69	3	9	PT2010PULSERPS2	POS	POS	1
70	3	10	PT2010PULSERPS3	POS	POS	1
71	3	11	PT2010PULSERPS2	POS	POS	1
72	3	12	PT2010PULSERPS3	POS	POS	1
73	3	13	PT2010PULSERPS3	POS	POS	1
74	3	14	PT2010PULSERNS1	NEG	NEG	1
75	3	15	PT2010PULSERNS2	NEG	NEG	1
76	3	16	PT2010PULSERNS3	NEG	NEG	1
77	3	17	PT2010PULSERPS1	POS	POS	1
78	3	18	PT2010PULSERPS2	POS	POS	1
79	3	19	PT2010PULSERPS3	POS	POS	1
80	3	20	PT2010PULSERNS1	NEG	NEG	1
81	3	21	PT2010PULSERNS2	NEG	NEG	1
82	3	22	PT2010PULSERNS3	NEG	NEG	1
83	3	23	PT2010PULSERPS1	POS	POS	1
84	3	24	PT2010PULSERNS1	NEG	NEG	1
85	3	25	PT2010PULSERNS2	NEG	NEG	1
86	3	26	PT2010PULSERNS1	NEG	NEG	1
87	3	27	PT2010PULSERNS2	NEG	NEG	1
88	3	28	PT2010PULSERNS3	NEG	NEG	1
89	3	29	PT2010PULSERPS1	POS	POS	1
90	3	30	PT2010PULSERNS1	NEG	NEG	1

Table 4. Mycoplasma gallisepticum rapid plate agglutination test: The 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 CODA-CERVA.

	LABNR	LABPOSIT	SAMPLE	STATUS	RESULT	SUCCESS
1	1	1	PT2010CRDSERNS2	NEG	NEG	1
2	1	2	PT2010CRDSERNS1	NEG	NEG	1
3	1	3	PT2010CRDSERNS2	NEG	NEG	1
4	1	4	PT2010CRDSERPS3	POS	POS	1
5	1	5	PT2010CRDSERNS3	NEG	NEG	1
6	1	6	PT2010CRDSERPS1	POS	POS	1
7	1	7	PT2010CRDSERPS2	POS	POS	1
8	1	8	PT2010CRDSERPS3	POS	POS	1
9	1	9	PT2010CRDSERNS1	NEG	NEG	1
10	1	10	PT2010CRDSERNS2	NEG	NEG	1
11	1	11	PT2010CRDSERNS3	NEG	NEG	1
12	1	12	PT2010CRDSERPS1	POS	POS	1
13	1	13	PT2010CRDSERNS3	NEG	NEG	1
14	1	14	PT2010CRDSERPS1	POS	POS	1
15	1	15	PT2010CRDSERPS2	POS	POS	1
16	1	16	PT2010CRDSERNS1	NEG	NEG	1
17	1	17	PT2010CRDSERNS2	NEG	NEG	1
18	1	18	PT2010CRDSERNS3	NEG	NEG	1
19	1	19	PT2010CRDSERPS1	POS	POS	1
20	1	20	PT2010CRDSERPS2	POS	POS	1
21	1	21	PT2010CRDSERPS3	POS	POS	1
22	1	22	PT2010CRDSERPS3	POS	POS	1
23	1	23	PT2010CRDSERPS2	POS	POS	1
24	1	24	PT2010CRDSERNS1	NEG	NEG	1
25	1	25	PT2010CRDSERNS2	NEG	NEG	1
26	1	26	PT2010CRDSERNS3	NEG	NEG	1
27	1	27	PT2010CRDSERPS1	POS	POS	1
28	1	28	PT2010CRDSERPS2	POS	POS	1
29	1	29	PT2010CRDSERPS3	POS	POS	1
30	1	30	PT2010CRDSERNS1	NEG	NEG	1



(CONTINUED)

	LABNR	LABPOSIT	SAMPLE	STATUS	RESULT	SUCCESS
31	2	1	PT2010CRDSERNS3	NEG	NEG	1
32	2	2	PT2010CRDSERPS1	POS	POS	1
33	2	3	PT2010CRDSERPS2	POS	POS	1
34	2	4	PT2010CRDSERPS3	POS	POS	1
35	2	5	PT2010CRDSERNS1	NEG	NEG	1
36	2	6	PT2010CRDSERNS2	NEG	NEG	1
37	2	7	PT2010CRDSERNS1	NEG	NEG	1
38	2	8	PT2010CRDSERNS2	NEG	NEG	1
39	2	9	PT2010CRDSERPS3	POS	POS	1
40	2	10	PT2010CRDSERNS3	NEG	NEG	1
41	2	11	PT2010CRDSERPS1	POS	POS	1
42	2	12	PT2010CRDSERPS2	POS	POS	1
43	2	13	PT2010CRDSERPS3	POS	POS	1
44	2	14	PT2010CRDSERNS1	NEG	NEG	1
45	2	15	PT2010CRDSERNS2	NEG	NEG	1
46	2	16	PT2010CRDSERNS3	NEG	NEG	1
47	2	17	PT2010CRDSERPS1	POS	POS	1
48	2	18	PT2010CRDSERNS3	NEG	NEG	1
49	2	19	PT2010CRDSERPS1	POS	POS	1
50	2	20	PT2010CRDSERPS2	POS	POS	1
51	2	21	PT2010CRDSERNS1	NEG	NEG	1
52	2	22	PT2010CRDSERNS2	NEG	NEG	1
53	2	23	PT2010CRDSERNS3	NEG	NEG	1
54	2	24	PT2010CRDSERPS1	POS	POS	1
55	2	25	PT2010CRDSERPS2	POS	POS	1
56	2	26	PT2010CRDSERPS3	POS	POS	1
57	2	27	PT2010CRDSERPS3	POS	POS	1
58	2	28	PT2010CRDSERPS2	POS	POS	1
59	2	29	PT2010CRDSERNS1	NEG	NEG	1
60	2	30	PT2010CRDSERNS2	NEG	NEG	1



(CONTINUED)

	LABNR	LABPOSIT	SAMPLE	STATUS	RESULT	SUCCESS
61	3	1	PT2010CRDSERPS3	POS	POS	1
62	3	2	PT2010CRDSERPS3	POS	POS	1
63	3	3	PT2010CRDSERPS2	POS	POS	1
64	3	4	PT2010CRDSERNS1	NEG	NEG	1
65	3	5	PT2010CRDSERNS2	NEG	NEG	1
66	3	6	PT2010CRDSERNS3	NEG	NEG	1
67	3	7	PT2010CRDSERPS1	POS	POS	1
68	3	8	PT2010CRDSERPS2	POS	POS	1
69	3	9	PT2010CRDSERPS3	POS	POS	1
70	3	10	PT2010CRDSERNS1	NEG	NEG	1
71	3	11	PT2010CRDSERNS2	NEG	NEG	1
72	3	12	PT2010CRDSERNS1	NEG	NEG	1
73	3	13	PT2010CRDSERNS2	NEG	NEG	1
74	3	14	PT2010CRDSERPS3	POS	POS	1
75	3	15	PT2010CRDSERNS3	NEG	NEG	1
76	3	16	PT2010CRDSERPS1	POS	POS	1
77	3	17	PT2010CRDSERPS2	POS	POS	1
78	3	18	PT2010CRDSERPS3	POS	POS	1
79	3	19	PT2010CRDSERNS1	NEG	NEG	1
80	3	20	PT2010CRDSERNS2	NEG	NEG	1
81	3	21	PT2010CRDSERNS3	NEG	NEG	1
82	3	22	PT2010CRDSERPS1	POS	POS	1
83	3	23	PT2010CRDSERNS3	NEG	NEG	1
84	3	24	PT2010CRDSERPS1	POS	POS	1
85	3	25	PT2010CRDSERPS2	POS	POS	1
86	3	26	PT2010CRDSERNS1	NEG	NEG	1
87	3	27	PT2010CRDSERNS2	NEG	NEG	1
88	3	28	PT2010CRDSERNS3	NEG	NEG	1
89	3	29	PT2010CRDSERPS1	POS	POS	1
90	3	30	PT2010CRDSERPS2	POS	POS	1

V. Discussion

The purpose of this proficiency test is to assess performances of participating laboratories when analyzing reference serum samples of poultry origin for the detection of *Salmonella* Pullorum-specific antibodies and *Mycoplasma gallisepticum*-specific antibodies by a rapid plate agglutination test.

For the rapid plate agglutination test for *Salmonella* Pullorum and *Mycoplasma gallisepticum*, all participating laboratories provided responses that were in full agreement with the true status of the reference serum samples for respectively *Salmonella* Pullorum and *Mycoplasma gallisepticum*.

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, all participating laboratories achieved a satisfactory performance.

Head CVD-ERA
Yves Van der Stede



Appendix:

Name of the participating Laboratories

ARSIA (Ciney)
CODA-CERVA
DGZ (Torhout)