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172-TEST

(ISO 43-1)

## **PROFICIENCY-TESTING 2007**

***Salmonella Pullorum (PUL) and Mycoplasma gallisepticum (CRD)***

***Detection of antibodies in serum***

**COORDINATION CENTRE FOR VETERINARY DIAGNOSTICS**

**DATE ILT: 7- 8 MAY 2007**

**DATE REPORT: 24 MAY 2007**

## I. Introduction

Details relevant to the proficiency-test are available in the Procedure PRO/6.5/01 'Beheer van de interlaboratoriumproeven / Gestion des essais interlaboratoires'.

## II. Aim

This proficiency-test, which is 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. Material and methods

### III.1. Conduct of diagnostic tests

In the framework of this proficiency-test, reference serum samples free or containing *Salmonella* Pullorum-specific antibodies must be tested by means of an agglutination test and reference serum samples free or containing *Mycoplasma gallisepticum*-specific antibodies must be tested by means of agglutination and/or ELISA tests. The procedure for these tests must be fully described in the SOPs of the participating laboratories.

### III.2. Reference samples

#### III.2.1. *Salmonella* Pullorum reference samples

Replicates of eight reference serums either free from *Salmonella* Pullorum-specific antibodies (n = 4; coded 'ILT07-PULNS1', 'ILT07-PULNS2', 'ILT07-PULNS3', and 'ILT07-PULNS4') or containing *Salmonella* Pullorum-specific antibodies (n = 4; coded 'ILT07-PULPS1', 'ILT07-PULPS2', 'ILT07-PULPS3', and 'ILT07-PULPS4') were used. In total 120 aliquots were sent to the participating laboratories. Before the start of the proficiency-test these eight reference serums were tested 10 times by the reference laboratory of the Veterinary and Agrochemical Research Centre (VAR) and the validated results were issued between 11 April 2007 and 4 May 2007, i.e., before the start of the proficiency-test. On all occasions, the reference serums encoded 'ILT07-PULNS1', 'ILT07-PULNS2', 'ILT07-PULNS3', and 'ILT07-PULNS4' scored 'Negative' (absence of *Salmonella* Pullorum-specific antibodies) and the reference serums encoded 'ILT07-PULPS1', 'ILT07-PULPS2', 'ILT07-PULPS3', and 'ILT07-PULPS4' scored 'Positive' (presence of *Salmonella* Pullorum-specific antibodies). Consequently, these reference serums were considered as reliable samples to evaluate the absence or presence of *Salmonella* Pullorum-specific antibodies in serum of poultry origin.

#### III.2.2. *Mycoplasma gallisepticum* reference samples

Replicates of six reference serums either free from *Mycoplasma gallisepticum*-specific antibodies (n = 3; coded 'ILT07-CRDNS1', 'ILT07-CRDNS2', and 'ILT07-CRDNS3') or containing *Mycoplasma gallisepticum*-specific antibodies (n = 3; coded 'ILT07-CRDPS1', 'ILT07-CRDPS2', and 'ILT07-CRDPS3') were used. In total 120 aliquots were sent to the participating laboratories. Before the start of the proficiency-test these six reference serums were tested 10 times, by agglutination and ELISA test, by the reference laboratory of the Veterinary and Agrochemical Research Centre (VAR) and the validated results were issued between 11 April 2007 and 4 May 2007, i.e., before the start of the proficiency-test. On all occasions, the reference serums encoded 'ILT07-CRDNS1', 'ILT07-CRDNS2', and 'ILT07-CRDNS3' scored 'Negative' (absence of *Mycoplasma gallisepticum*-specific antibodies) and the reference serums encoded 'ILT07-CRDPS1', 'ILT07-CRDPS2', and 'ILT07-CRDPS3' scored 'Positive' (presence of *Mycoplasma gallisepticum*-specific antibodies). Consequently, these reference serums were

considered as reliable samples to evaluate the absence or presence of *Mycoplasma gallisepticum*-specific antibodies in serum of poultry origin.

### **III.3. Classification of results, levels 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. Levels of agreement*

The levels of agreement achieved by the participating laboratories are expressed as the percentages of successes for all 30 samples 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 is at least 90%.

## **IV. Results**

For confidentiality reasons, the participating laboratories are quoted anonymously and the concordance table is safely kept at the Coordination Centre for Veterinary Diagnostics (CCVD) of the Veterinary and Agrochemical Research Centre (VAR).

### **IV.1. Reference samples**

#### *IV.1.1. Allocation of samples to participating laboratories*

All participating laboratories were given:

- i. 14 reference serum samples free from *Salmonella* Pullorum-specific antibodies: ILT07-PULNS1 samples (n = 3), ILT07-PULNS2 samples (n = 3), ILT07-PULNS3 samples (n = 4), and ILT07-PULNS4 samples (n = 4)
- ii. 16 reference serum samples containing *Salmonella* Pullorum-specific antibodies: ILT07-PULPS1 samples (n = 4), ILT07-PULPS2 samples (n = 4), ILT07-PULPS3 samples (n = 4), and ILT07-PULPS4 samples (n = 4)
- iii. 15 reference serum samples free from *Mycoplasma gallisepticum*-specific antibodies: ILT07-CRDNS1 samples (n = 5), ILT07-CRDNS2 samples (n = 5), and ILT07-CRDNS3 samples (n = 5)
- iv. 15 reference serum samples containing *Mycoplasma gallisepticum*-specific antibodies: ILT07-CRDPS1 samples (n = 5), ILT07-CRDPS2 samples (n = 5), and ILT07-CRDPS3 samples (n = 3)

#### 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 7 May 2007 to each of the four participating laboratories (240 aliquots in total). The four laboratories acknowledged receipt of the samples on the same day. The analyses were carried out on 7 (LAB2), 8 (LAB3 and LAB4 Agglutination test) and 9 (LAB4 ELISA test) May 2007. The date for LAB1 was not reported by the laboratory.

#### IV.2. Dates at which results were returned to the CCVD

Results from participating laboratories have been received on 9 (LAB1 and LAB2) and 11 (LAB3 and LAB4) May 2007.

#### IV.3. Compliance with the procedure

All participating laboratories have provided a duly dated and signed copy of the results to the VAR.

#### IV.4. Levels of agreement

Agglutination test for *Salmonella* Pullorum: three participating laboratories reached 100% of agreement for the detection of *Salmonella* Pullorum-specific antibodies and one participating laboratory (LAB3) reached 90% of agreement for the detection of *Salmonella* Pullorum-specific antibodies (Table 1).

Agglutination test for *Mycoplasma gallisepticum*: three participating laboratories reached 100% of agreement for the detection of *Mycoplasma gallisepticum*-specific antibodies and one participating laboratory (LAB1) reached 96.7% of agreement for the detection of *Mycoplasma gallisepticum*-specific antibodies (Table 2).

ELISA test for *Mycoplasma gallisepticum*: The two participating laboratories reached 96.7% of agreement for the detection of *Mycoplasma gallisepticum*-specific antibodies (Table 3).

**Table 1.** Outcome of the assessment of *Salmonella* Pullorum reference serum samples:

Agreement between results generated by the participating laboratories (LABNR) and the status of reference samples as determined by repeated screening of samples free from *Salmonella* Pullorum-specific antibodies ('ILT07-PULNS1', 'ILT07-PULNS2', 'ILT07-PULNS3', and 'ILT07-PULNS4') or containing *Salmonella* Pullorum-specific antibodies ('ILT07-PULPS1', 'ILT07-PULPS2', 'ILT07-PULPS3', and 'ILT07-PULPS4'). The purpose of the proficiency-test is to determine the presence/absence of *Salmonella* Pullorum-specific antibodies in reference serums.

Success while screening the samples (0 = Failure, 1 = Success) – Agglutination Test PUL				
Variable	LABNR			
	1 (N=30)	2 (N=30)	3 (N=30)	4 (N=30)
	N (%)	N (%)	N (%)	N (%)
0	0 (0.0)	0 (0.0)	3 (10.0)	0 (0.0)
1	30 (100.)	30 (100.)	27 (90.0)	30 (100.)

**Table 2.** Outcome of the assessment of *Mycoplasma gallisepticum* reference serum samples:

Agreement between results generated by the participating laboratories for agglutination test (LABNR) and the status of reference samples as determined by repeated screening of samples free from *Mycoplasma gallisepticum*-specific antibodies ('ILT07-CRDNS1', 'ILT07-CRDNS2', and 'ILT07-CRDNS3') or containing *Mycoplasma gallisepticum*-specific antibodies ('ILT07-CRDPS1', 'ILT07-CRDPS2', and 'ILT07-CRDPS3'). The purpose of the proficiency-test is to determine the presence/absence of *Mycoplasma gallisepticum*-specific antibodies in reference serums.

Success while screening the samples (0 = Failure, 1 = Success) – Agglutination Test CRD				
Variable	LABNR			
	1 (N=30)	2 (N=30)	3 (N=30)	4 (N=30)
	N (%)	N (%)	N (%)	N (%)
0	1 ( 3.3)	0 ( 0.0)	0 ( 0.0)	0 ( 0.0)
1	29 (96.7)	30 (100.)	30 (100.)	30 (100.)

**Table 3.** Outcome of the assessment of *Mycoplasma gallisepticum* reference serum samples:

Agreement between results generated by the participating laboratories for ELISA test (LABNR) and the status of reference samples as determined by repeated screening of samples free from *Mycoplasma gallisepticum*-specific antibodies ('ILT07-CRDNS1', 'ILT07-CRDNS2', and 'ILT07-CRDNS3') or containing *Mycoplasma gallisepticum*-specific antibodies ('ILT07-CRDPS1', 'ILT07-CRDPS2', and 'ILT07-CRDPS3'). The purpose of the proficiency-test is to determine the presence/absence of *Mycoplasma gallisepticum*-specific antibodies in reference serums.

Success while screening the samples (0 = Failure, 1 = Success) – ELISA Test CRD		
Variable	LABNR	
	1 (N=30)	4 (N=30)
	N (%)	N (%)
0	1 ( 3.3)	1 ( 3.3)
1	29 (96.7)	29 (96.7)

#### IV.5. Variability among participating laboratories

The responses of the four participating laboratories that provided their results for *Salmonella Pullorum* and *Mycoplasma gallisepticum* reference serums are displayed in Table 4, 5, and 6.

**Table 4.** 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 in the block, and the results (STATUS) obtained by repeated screening. Reference samples include samples free from *Salmonella* Pullorum-specific antibodies ('ILT07-PULNS1', 'ILT07-PULNS2', 'ILT07-PULNS3', and 'ILT07-PULNS4') or containing *Salmonella* Pullorum-specific antibodies ('ILT07-PULPS1', 'ILT07-PULPS2', 'ILT07-PULPS3', and 'ILT07-PULPS4').

	LABNR	LABPOSIT	SAMPLE	STATUS	RESULT	SUCCESS
1	1	1	ILT07-PULPS2	POS	POS	1
2	1	2	ILT07-PULNS1	NEG	NEG	1
3	1	3	ILT07-PULPS1	POS	POS	1
4	1	4	ILT07-PULNS4	NEG	NEG	1
5	1	5	ILT07-PULPS4	POS	POS	1
6	1	6	ILT07-PULNS4	NEG	NEG	1
7	1	7	ILT07-PULPS1	POS	POS	1
8	1	8	ILT07-PULNS3	NEG	NEG	1
9	1	9	ILT07-PULPS3	POS	POS	1
10	1	10	ILT07-PULNS1	NEG	NEG	1
11	1	11	ILT07-PULNS2	NEG	NEG	1
12	1	12	ILT07-PULNS3	NEG	NEG	1
13	1	13	ILT07-PULPS2	POS	POS	1
14	1	14	ILT07-PULNS4	NEG	NEG	1
15	1	15	ILT07-PULPS1	POS	POS	1
16	1	16	ILT07-PULPS4	POS	POS	1
17	1	17	ILT07-PULPS3	POS	POS	1
18	1	18	ILT07-PULNS3	NEG	NEG	1
19	1	19	ILT07-PULNS1	NEG	NEG	1
20	1	20	ILT07-PULPS2	POS	POS	1
21	1	21	ILT07-PULPS4	POS	POS	1
22	1	22	ILT07-PULNS2	NEG	NEG	1
23	1	23	ILT07-PULNS4	NEG	NEG	1
24	1	24	ILT07-PULNS3	NEG	NEG	1
25	1	25	ILT07-PULPS1	POS	POS	1
26	1	26	ILT07-PULNS2	NEG	NEG	1
27	1	27	ILT07-PULPS4	POS	POS	1
28	1	28	ILT07-PULPS3	POS	POS	1
29	1	29	ILT07-PULPS2	POS	POS	1
30	1	30	ILT07-PULPS3	POS	POS	1

(CONTINUED)

	LABNR	LABPOSIT	SAMPLE	STATUS	RESULT	SUCCESS
31	2	1	ILT07-PULNS2	NEG	NEG	1
32	2	2	ILT07-PULPS4	POS	POS	1
33	2	3	ILT07-PULPS3	POS	POS	1
34	2	4	ILT07-PULPS2	POS	POS	1
35	2	5	ILT07-PULPS3	POS	POS	1
36	2	6	ILT07-PULPS2	POS	POS	1
37	2	7	ILT07-PULNS1	NEG	NEG	1
38	2	8	ILT07-PULPS1	POS	POS	1
39	2	9	ILT07-PULNS4	NEG	NEG	1
40	2	10	ILT07-PULPS4	POS	POS	1
41	2	11	ILT07-PULNS4	NEG	NEG	1
42	2	12	ILT07-PULPS1	POS	POS	1
43	2	13	ILT07-PULNS3	NEG	NEG	1
44	2	14	ILT07-PULPS3	POS	POS	1
45	2	15	ILT07-PULNS1	NEG	NEG	1
46	2	16	ILT07-PULNS2	NEG	NEG	1
47	2	17	ILT07-PULNS3	NEG	NEG	1
48	2	18	ILT07-PULPS2	POS	POS	1
49	2	19	ILT07-PULNS4	NEG	NEG	1
50	2	20	ILT07-PULPS1	POS	POS	1
51	2	21	ILT07-PULPS4	POS	POS	1
52	2	22	ILT07-PULPS3	POS	POS	1
53	2	23	ILT07-PULNS3	NEG	NEG	1
54	2	24	ILT07-PULNS1	NEG	NEG	1
55	2	25	ILT07-PULPS2	POS	POS	1
56	2	26	ILT07-PULPS4	POS	POS	1
57	2	27	ILT07-PULNS2	NEG	NEG	1
58	2	28	ILT07-PULNS4	NEG	NEG	1
59	2	29	ILT07-PULNS3	NEG	NEG	1
60	2	30	ILT07-PULPS1	POS	POS	1

(CONTINUED)

	LABNR	LABPOSIT	SAMPLE	STATUS	RESULT	SUCCESS
61	3	1	ILT07-PULPS4	POS	POS	1
62	3	2	ILT07-PULNS2	NEG	NEG	1
63	3	3	ILT07-PULNS4	NEG	NEG	1
64	3	4	ILT07-PULNS3	NEG	NEG	1
65	3	5	ILT07-PULPS1	POS	POS	1
66	3	6	ILT07-PULNS2	NEG	NEG	1
67	3	7	ILT07-PULPS4	POS	POS	1
68	3	8	ILT07-PULPS3	POS	POS	1
69	3	9	ILT07-PULPS2	POS	POS	1
70	3	10	ILT07-PULPS3	POS	POS	1
71	3	11	ILT07-PULPS2	POS	POS	1
72	3	12	ILT07-PULNS1	NEG	NEG	1
73	3	13	ILT07-PULPS1	POS	POS	1
74	3	14	ILT07-PULNS4	NEG	NEG	1
75	3	15	ILT07-PULPS4	POS	POS	1
76	3	16	ILT07-PULNS4	<b>NEG</b>	<b>POS</b>	<b>0</b>
77	3	17	ILT07-PULPS1	POS	POS	1
78	3	18	ILT07-PULNS3	<b>NEG</b>	<b>POS</b>	<b>0</b>
79	3	19	ILT07-PULPS3	POS	POS	1
80	3	20	ILT07-PULNS1	NEG	NEG	1
81	3	21	ILT07-PULNS2	NEG	NEG	1
82	3	22	ILT07-PULNS3	<b>NEG</b>	<b>POS</b>	<b>0</b>
83	3	23	ILT07-PULPS2	POS	POS	1
84	3	24	ILT07-PULNS4	NEG	NEG	1
85	3	25	ILT07-PULPS1	POS	POS	1
86	3	26	ILT07-PULPS4	POS	POS	1
87	3	27	ILT07-PULPS3	POS	POS	1
88	3	28	ILT07-PULNS3	NEG	NEG	1
89	3	29	ILT07-PULNS1	NEG	NEG	1
90	3	30	ILT07-PULPS2	POS	POS	1

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	LABNR	LABPOSIT	SAMPLE	STATUS	RESULT	SUCCESS
91	4	1	ILT07-PULPS4	POS	POS	1
92	4	2	ILT07-PULPS3	POS	POS	1
93	4	3	ILT07-PULNS3	NEG	NEG	1
94	4	4	ILT07-PULNS1	NEG	NEG	1
95	4	5	ILT07-PULPS2	POS	POS	1
96	4	6	ILT07-PULPS4	POS	POS	1
97	4	7	ILT07-PULNS2	NEG	NEG	1
98	4	8	ILT07-PULNS4	NEG	NEG	1
99	4	9	ILT07-PULNS3	NEG	NEG	1
100	4	10	ILT07-PULPS1	POS	POS	1
101	4	11	ILT07-PULNS2	NEG	NEG	1
102	4	12	ILT07-PULPS4	POS	POS	1
103	4	13	ILT07-PULPS3	POS	POS	1
104	4	14	ILT07-PULPS2	POS	POS	1
105	4	15	ILT07-PULPS3	POS	POS	1
106	4	16	ILT07-PULPS2	POS	POS	1
107	4	17	ILT07-PULNS1	NEG	NEG	1
108	4	18	ILT07-PULPS1	POS	POS	1
109	4	19	ILT07-PULNS4	NEG	NEG	1
110	4	20	ILT07-PULPS4	POS	POS	1
111	4	21	ILT07-PULNS4	NEG	NEG	1
112	4	22	ILT07-PULPS1	POS	POS	1
113	4	23	ILT07-PULNS3	NEG	NEG	1
114	4	24	ILT07-PULPS3	POS	POS	1
115	4	25	ILT07-PULNS1	NEG	NEG	1
116	4	26	ILT07-PULNS2	NEG	NEG	1
117	4	27	ILT07-PULNS3	NEG	NEG	1
118	4	28	ILT07-PULPS2	POS	POS	1
119	4	29	ILT07-PULNS4	NEG	NEG	1
120	4	30	ILT07-PULPS1	POS	POS	1

**Table 5.** The responses (RESULT), for the agglutination tests, of the participating laboratories (LABNR) with the identification (SAMPLE) of the reference serum samples, the position (LABPOSIT) of the reference serum samples in the block, and the results (STATUS) obtained by repeated screening. Reference samples include samples free from *Mycoplasma gallisepticum*-specific antibodies ('ILT07-CRDNS1', 'ILT07-CRDNS2', and 'ILT07-CRDNS3') or containing *Mycoplasma gallisepticum*-specific antibodies ('ILT07-CRDPS1', 'ILT07-CRDPS2', and 'ILT07-CRDPS3').

	LABNR	LABPOSIT	SAMPLE	STATUS	RESULT	SUCCESS
1	1	1	ILT07-CRDNS3	NEG	NEG	1
2	1	2	ILT07-CRDPS3	POS	POS	1
3	1	3	ILT07-CRDPS3	POS	POS	1
4	1	4	ILT07-CRDPS1	POS	POS	1
5	1	5	ILT07-CRDNS1	NEG	NEG	1
6	1	6	ILT07-CRDNS2	NEG	NEG	1
7	1	7	ILT07-CRDPS1	POS	POS	1
8	1	8	ILT07-CRDNS3	NEG	NEG	1
9	1	9	ILT07-CRDPS2	POS	POS	1
10	1	10	ILT07-CRDNS1	NEG	NEG	1
11	1	11	ILT07-CRDPS3	POS	POS	1
12	1	12	ILT07-CRDPS3	POS	POS	1
13	1	13	ILT07-CRDNS1	NEG	NEG	1
14	1	14	ILT07-CRDPS1	POS	POS	1
15	1	15	ILT07-CRDNS2	NEG	NEG	1
16	1	16	ILT07-CRDNS3	NEG	NEG	1
17	1	17	ILT07-CRDPS2	POS	POS	1
18	1	18	ILT07-CRDNS1	NEG	NEG	1
19	1	19	ILT07-CRDPS1	POS	POS	1
20	1	20	ILT07-CRDPS2	POS	POS	1
21	1	21	ILT07-CRDNS2	NEG	NEG	1
22	1	22	ILT07-CRDPS1	POS	POS	1
23	1	23	ILT07-CRDNS3	NEG	NEG	1
24	1	24	ILT07-CRDNS2	NEG	NEG	1
25	1	25	ILT07-CRDPS3	<b>POS</b>	<b>NEG</b>	<b>0</b>
26	1	26	ILT07-CRDNS1	NEG	NEG	1
27	1	27	ILT07-CRDNS3	NEG	NEG	1
28	1	28	ILT07-CRDPS2	POS	POS	1
29	1	29	ILT07-CRDNS2	NEG	NEG	1
30	1	30	ILT07-CRDPS2	POS	POS	1

(CONTINUED)

	LABNR	LABPOSIT	SAMPLE	STATUS	RESULT	SUCCESS
31	2	1	ILT07-CRDNS1	NEG	NEG	1
32	2	2	ILT07-CRDNS3	NEG	NEG	1
33	2	3	ILT07-CRDPS2	POS	POS	1
34	2	4	ILT07-CRDNS2	NEG	NEG	1
35	2	5	ILT07-CRDPS2	POS	POS	1
36	2	6	ILT07-CRDNS3	NEG	NEG	1
37	2	7	ILT07-CRDPS3	POS	POS	1
38	2	8	ILT07-CRDPS3	POS	POS	1
39	2	9	ILT07-CRDPS1	POS	POS	1
40	2	10	ILT07-CRDNS1	NEG	NEG	1
41	2	11	ILT07-CRDNS2	NEG	NEG	1
42	2	12	ILT07-CRDPS1	POS	POS	1
43	2	13	ILT07-CRDNS3	NEG	NEG	1
44	2	14	ILT07-CRDPS2	POS	POS	1
45	2	15	ILT07-CRDNS1	NEG	NEG	1
46	2	16	ILT07-CRDPS3	POS	POS	1
47	2	17	ILT07-CRDPS3	POS	POS	1
48	2	18	ILT07-CRDNS1	NEG	NEG	1
49	2	19	ILT07-CRDPS1	POS	POS	1
50	2	20	ILT07-CRDNS2	NEG	NEG	1
51	2	21	ILT07-CRDNS3	NEG	NEG	1
52	2	22	ILT07-CRDPS2	POS	POS	1
53	2	23	ILT07-CRDNS1	NEG	NEG	1
54	2	24	ILT07-CRDPS1	POS	POS	1
55	2	25	ILT07-CRDPS2	POS	POS	1
56	2	26	ILT07-CRDNS2	NEG	NEG	1
57	2	27	ILT07-CRDPS1	POS	POS	1
58	2	28	ILT07-CRDNS3	NEG	NEG	1
59	2	29	ILT07-CRDNS2	NEG	NEG	1
60	2	30	ILT07-CRDPS3	POS	POS	1

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	LABNR	LABPOSIT	SAMPLE	STATUS	RESULT	SUCCESS
61	3	1	ILT07-CRDNS2	NEG	NEG	1
62	3	2	ILT07-CRDPS1	POS	POS	1
63	3	3	ILT07-CRDNS3	NEG	NEG	1
64	3	4	ILT07-CRDNS2	NEG	NEG	1
65	3	5	ILT07-CRDPS3	POS	POS	1
66	3	6	ILT07-CRDNS1	NEG	NEG	1
67	3	7	ILT07-CRDNS3	NEG	NEG	1
68	3	8	ILT07-CRDPS2	POS	POS	1
69	3	9	ILT07-CRDNS2	NEG	NEG	1
70	3	10	ILT07-CRDPS2	POS	POS	1
71	3	11	ILT07-CRDNS3	NEG	NEG	1
72	3	12	ILT07-CRDPS3	POS	POS	1
73	3	13	ILT07-CRDPS3	POS	POS	1
74	3	14	ILT07-CRDPS1	POS	POS	1
75	3	15	ILT07-CRDNS1	NEG	NEG	1
76	3	16	ILT07-CRDNS2	NEG	NEG	1
77	3	17	ILT07-CRDPS1	POS	POS	1
78	3	18	ILT07-CRDNS3	NEG	NEG	1
79	3	19	ILT07-CRDPS2	POS	POS	1
80	3	20	ILT07-CRDNS1	NEG	NEG	1
81	3	21	ILT07-CRDPS3	POS	POS	1
82	3	22	ILT07-CRDPS3	POS	POS	1
83	3	23	ILT07-CRDNS1	NEG	NEG	1
84	3	24	ILT07-CRDPS1	POS	POS	1
85	3	25	ILT07-CRDNS2	NEG	NEG	1
86	3	26	ILT07-CRDNS3	NEG	NEG	1
87	3	27	ILT07-CRDPS2	POS	POS	1
88	3	28	ILT07-CRDNS1	NEG	NEG	1
89	3	29	ILT07-CRDPS1	POS	POS	1
90	3	30	ILT07-CRDPS2	POS	POS	1

(CONTINUED)

	LABNR	LABPOSIT	SAMPLE	STATUS	RESULT	SUCCESS
91	4	1	ILT07-CRDNS3	NEG	NEG	1
92	4	2	ILT07-CRDPS2	POS	POS	1
93	4	3	ILT07-CRDNS1	NEG	NEG	1
94	4	4	ILT07-CRDPS1	POS	POS	1
95	4	5	ILT07-CRDPS2	POS	POS	1
96	4	6	ILT07-CRDNS2	NEG	NEG	1
97	4	7	ILT07-CRDPS1	POS	POS	1
98	4	8	ILT07-CRDNS3	NEG	NEG	1
99	4	9	ILT07-CRDNS2	NEG	NEG	1
100	4	10	ILT07-CRDPS3	POS	POS	1
101	4	11	ILT07-CRDNS1	NEG	NEG	1
102	4	12	ILT07-CRDNS3	NEG	NEG	1
103	4	13	ILT07-CRDPS2	POS	POS	1
104	4	14	ILT07-CRDNS2	NEG	NEG	1
105	4	15	ILT07-CRDPS2	POS	POS	1
106	4	16	ILT07-CRDNS3	NEG	NEG	1
107	4	17	ILT07-CRDPS3	POS	POS	1
108	4	18	ILT07-CRDPS3	POS	POS	1
109	4	19	ILT07-CRDPS1	POS	POS	1
110	4	20	ILT07-CRDNS1	NEG	NEG	1
111	4	21	ILT07-CRDNS2	NEG	NEG	1
112	4	22	ILT07-CRDPS1	POS	POS	1
113	4	23	ILT07-CRDNS3	NEG	NEG	1
114	4	24	ILT07-CRDPS2	POS	POS	1
115	4	25	ILT07-CRDNS1	NEG	NEG	1
116	4	26	ILT07-CRDPS3	POS	POS	1
117	4	27	ILT07-CRDPS3	POS	POS	1
118	4	28	ILT07-CRDNS1	NEG	NEG	1
119	4	29	ILT07-CRDPS1	POS	POS	1
120	4	30	ILT07-CRDNS2	NEG	NEG	1

**Table 6.** The responses (RESULT), for the ELISA tests, of the participating laboratories (LABNR) with the identification (SAMPLE) of the reference serum samples, the position (LABPOSIT) of the reference serum samples in the block, and the results (STATUS) obtained by repeated screening. Reference samples include samples free from *Mycoplasma gallisepticum*-specific antibodies ('ILT07-CRDNS1', 'ILT07-CRDNS2', and 'ILT07-CRDNS3') or containing *Mycoplasma gallisepticum*-specific antibodies ('ILT07-CRDPS1', 'ILT07-CRDPS2', and 'ILT07-CRDPS3').

	LABNR	LABPOSIT	SAMPLE	STATUS	RESULT	SUCCESS
1	1	1	ILT07-CRDNS3	NEG	NEG	1
2	1	2	ILT07-CRDPS3	POS	POS	1
3	1	3	ILT07-CRDPS3	POS	POS	1
4	1	4	ILT07-CRDPS1	POS	POS	1
5	1	5	ILT07-CRDNS1	NEG	NEG	1
6	1	6	ILT07-CRDNS2	NEG	NEG	1
7	1	7	ILT07-CRDPS1	POS	POS	1
8	1	8	ILT07-CRDNS3	NEG	NEG	1
9	1	9	ILT07-CRDPS2	POS	POS	1
10	1	10	ILT07-CRDNS1	NEG	NEG	1
11	1	11	ILT07-CRDPS3	POS	POS	1
12	1	12	ILT07-CRDPS3	POS	POS	1
13	1	13	ILT07-CRDNS1	NEG	NEG	1
14	1	14	ILT07-CRDPS1	POS	POS	1
15	1	15	ILT07-CRDNS2	NEG	NEG	1
16	1	16	ILT07-CRDNS3	NEG	NEG	1
17	1	17	ILT07-CRDPS2	POS	POS	1
18	1	18	ILT07-CRDNS1	NEG	NEG	1
19	1	19	ILT07-CRDPS1	POS	POS	1
20	1	20	ILT07-CRDPS2	POS	POS	1
21	1	21	ILT07-CRDNS2	NEG	NEG	1
22	1	22	ILT07-CRDPS1	POS	POS	1
23	1	23	ILT07-CRDNS3	NEG	NEG	1
24	1	24	ILT07-CRDNS2	NEG	NEG	1
25	1	25	ILT07-CRDPS3	<b>POS</b>	<b>NEG</b>	<b>0</b>
26	1	26	ILT07-CRDNS1	NEG	NEG	1
27	1	27	ILT07-CRDNS3	NEG	NEG	1
28	1	28	ILT07-CRDPS2	POS	POS	1
29	1	29	ILT07-CRDNS2	NEG	NEG	1
30	1	30	ILT07-CRDPS2	POS	POS	1

(CONTINUED)

	LABNR	LABPOSIT	SAMPLE	STATUS	RESULT	SUCCESS
31	4	1	ILT07-CRDNS3	NEG	NEG	1
32	4	2	ILT07-CRDPS2	POS	POS	1
33	4	3	ILT07-CRDNS1	NEG	NEG	1
34	4	4	ILT07-CRDPS1	POS	POS	1
35	4	5	ILT07-CRDPS2	POS	POS	1
36	4	6	ILT07-CRDNS2	NEG	NEG	1
37	4	7	ILT07-CRDPS1	POS	POS	1
38	4	8	ILT07-CRDNS3	NEG	NEG	1
39	4	9	ILT07-CRDNS2	NEG	NEG	1
40	4	10	ILT07-CRDPS3	<b>POS</b>	<b>NI</b>	<b>0</b>
41	4	11	ILT07-CRDNS1	NEG	NEG	1
42	4	12	ILT07-CRDNS3	NEG	NEG	1
43	4	13	ILT07-CRDPS2	POS	POS	1
44	4	14	ILT07-CRDNS2	NEG	NEG	1
45	4	15	ILT07-CRDPS2	POS	POS	1
46	4	16	ILT07-CRDNS3	NEG	NEG	1
47	4	17	ILT07-CRDPS3	POS	POS	1
48	4	18	ILT07-CRDPS3	POS	POS	1
49	4	19	ILT07-CRDPS1	POS	POS	1
50	4	20	ILT07-CRDNS1	NEG	NEG	1
51	4	21	ILT07-CRDNS2	NEG	NEG	1
52	4	22	ILT07-CRDPS1	POS	POS	1
53	4	23	ILT07-CRDNS3	NEG	NEG	1
54	4	24	ILT07-CRDPS2	POS	POS	1
55	4	25	ILT07-CRDNS1	NEG	NEG	1
56	4	26	ILT07-CRDPS3	POS	POS	1
57	4	27	ILT07-CRDPS3	POS	POS	1
58	4	28	ILT07-CRDNS1	NEG	NEG	1
59	4	29	ILT07-CRDPS1	POS	POS	1
60	4	30	ILT07-CRDNS2	NEG	NEG	1

## V. Discussion

The purpose of this proficiency-test is to assess performances of participating laboratories when analyzing:

- (i) reference serums free from *Salmonella* Pullorum-specific antibodies,
- (ii) reference serums containing *Salmonella* Pullorum-specific antibodies.
- (iii) reference serums free from *Mycoplasma gallisepticum*-specific antibodies,
- (iv) reference serums containing *Mycoplasma gallisepticum*-specific antibodies.

For the agglutination test for *Salmonella* Pullorum, three participating laboratories provided responses that were in full agreement with the true status of the reference serums for *Salmonella* Pullorum. One participating laboratory (LAB3) reached 90% of agreement for the detection of *Salmonella* Pullorum-specific antibodies. No explanation could be found for this misclassification.

For the agglutination test for *Mycoplasma gallisepticum*, three participating laboratories provided responses that were in full agreement with the true status of the reference serums for *Mycoplasma gallisepticum*. One participating laboratory (LAB1) reached 96.7% of agreement for the detection of *Mycoplasma gallisepticum*-specific antibodies. This was due to the misclassification of 1 sample (=ILT07-CRDPS3).

For the ELISA test for *Mycoplasma gallisepticum*, the two participating laboratories (LAB1 and LAB4) reached 96.7% of agreement for the detection of *Mycoplasma gallisepticum*-specific antibodies. This was due to the misclassification of 1 sample (=ILT07-CRDPS3) with a % inhibition of 25.7 and 38.0 respectively. The range in % inhibition for 'ILT07-CRDPS3' during the 10 tests performed before the start of the proficiency-test (validation by the VAR) was 44% to 54% (weak positive sample).

## VI. Conclusions

According to the procedure currently in force, the performances of a participating laboratory are 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 four participating laboratories achieved a satisfactory performance.

The Committee

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