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172-PT

PROFICIENCY TESTING 2012

SALMONELLA PULLORUM (PUL) - MYCOPLASMA GALLISEPTICUM (CRD)

Detection of antibodies in serum by a rapid plate agglutination test

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

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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 either the absence or presence of *Salmonella enterica* subspecies *enterica* serotype Pullorum (*Salmonella* Pullorum) and *Mycoplasma gallisepticum* specific antibodies in serum of poultry origin by a rapid plate agglutination test.

III. Materials and methods

III.1. Conduct of diagnostic tests

In the framework of this PT, predefined reference serum samples must be tested by means of a rapid plate agglutination test. The procedure 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 samples

Replicates of 6 reference serum samples of poultry origin, either free from detectable PUL-specific antibodies (n=2; coded 'PT2012PULSERNS1' and 'PT2012PULSERNS2') or containing detectable PUL-specific antibodies (n=4; coded 'PT2012PULSERPS1', 'PT2012PULSERPS2', 'PT2012PULSERPS3' and 'PT2012PULSERPS4'), were used. In total, 60 aliquots were distributed to 3 participating laboratories. All participants received 20 aliquots: 3 aliquots of the reference serum samples PT2012PULSERNS1, PT2012PULSERNS2, PT2012PULSERPS3 and PT2012PULSERPS4, and 4 aliquots of the reference serum samples PT2012PULSERPS1 and PT2012PULSERPS2. The positions of the reference serum samples in the sent blocks were randomized for each participant (Table 4).

For each reference serum sample, a certificate containing the status of the sample (= 'golden standard') was made. The status of the reference serum samples was based on (i) the historical background of the animals and (ii) the results obtained by a rapid plate agglutination test using the PUL antigen from LDA[22] (pre-verification). The reference serum samples PT2012PULSERNS1 and PT2012PULSERNS2 were commercial chicken sera obtained from Life Technologies (Gibco) and Sigma-Aldrich, respectively. The reference serum samples PT2012PULSERPS1, PT2012PULSERPS2, PT2012PULSERPS3 and PT2012PULSERPS4 were obtained by diluting the first line PUL positive control serum made by the PUL reference laboratory of CODA-CERVA (batch: Sappos 05-7) 1/6, 1/8, 1/16 and 1/32, respectively, in the commercial chicken serum obtained from Life Technologies (Gibco). Each reference serum sample was tested 4 times before the start of the PT (pre-verification) by a rapid plate agglutination test, hereby obtaining 4 times the same qualitative result for each reference serum sample. Taken together, the reference serum samples PT2012PULSERNS1 and PT2012PULSERNS2 were considered as negative sera, and the reference serum samples PT2012PULSERPS1, PT2012PULSERPS2, PT2012PULSERPS3 and PT2012PULSERPS4 as positive sera in a rapid plate agglutination test using a PUL antigen.

After aliquoting the different reference serum samples, a homogeneity check was performed on 10 aliquots of each reference serum sample by a rapid plate agglutination test using the PUL antigen from LDA[22], hereby obtaining the same qualitative result for all 10 aliquots of the same reference serum sample. Consequently, all reference serum samples were considered as reliable samples in order to evaluate the ability of laboratories to correctly identify the absence or presence of PUL-specific antibodies in chicken serum. In addition, all reference serum samples were tested once after the PT in order to confirm their stability and status (post-verification) by a rapid plate agglutination test using the PUL antigen from LDA[22].

III.2.2. Mycoplasma gallisepticum reference samples

Replicates of 6 reference serum samples of poultry origin, either free from detectable CRD-specific antibodies (n=2; coded 'PT2012CRDSERNS1' and 'PT2012CRDSERNS2') or containing detectable CRD-specific antibodies (n=4; coded 'PT2012CRDSERPS1', 'PT2012CRDSERPS2', 'PT2012CRDSERPS3' and 'PT2012CRDSERPS4'), were used. In total, 60 aliquots were distributed to 3 participating laboratories. All participants received 20 aliquots: 3 aliquots of the reference serum samples PT2012CRDSERPS1, PT2012CRDSERPS2, PT2012CRDSERPS3 and PT2012CRDSERPS4, and 4 aliquots of the reference serum samples PT2012CRDSERNS1 and PT2012CRDSERNS2. The positions of the reference serum samples in the sent blocks were randomized for each participant (Table 5).

For each reference serum sample, a certificate containing the status of the sample (= 'golden standard') was made. The status of the reference serum samples was based on (i) the historical background of the animals and (ii) the results obtained by a rapid plate agglutination test using the CRD antigen from Soleil (pre-verification). The reference serum samples PT2012CRDSERNS1 (=PT2012PULSERNS1) and PT2012CRDSERNS2 (=PT2012PULSERNS2) were commercial chicken sera obtained from Life Technologies (Gibco) and Sigma-Aldrich, respectively. The reference serum samples PT2012CRDSERPS1, PT2012CRDSERPS2, PT2012CRDSERPS3 and PT2012CRDSERPS4 were obtained by diluting the first line CRD positive control serum made by the CRD reference laboratory of CODA-CERVA (batch: Mycpos 11-01) 1/2, 1/4, 1/6 and 1/8, respectively, in the commercial chicken serum obtained from Life Technologies (Gibco). Each reference serum sample was tested 4 times before the start of the PT (pre-verification) by a rapid plate agglutination test, hereby obtaining 4 times the same qualitative result for each reference serum sample. Taken together, the reference serum samples PT2012CRDSERNS1 and PT2012CRDSERNS2 were considered as negative sera, and the reference serum samples PT2012CRDSERPS1, PT2012CRDSERPS2, PT2012CRDSERPS3 and PT2012CRDSERPS4 as positive sera in a rapid plate agglutination test using a CRD antigen.

After aliquoting the different reference serum samples, a homogeneity check was performed on 10 aliquots of each reference serum sample by a rapid plate agglutination test using the CRD antigen from Soleil, hereby obtaining the same qualitative result for all 10 aliquots of the same reference serum sample. Consequently, all reference serum samples were considered as reliable samples in order to evaluate the ability of laboratories to correctly identify the absence or presence of CRD-specific antibodies in chicken serum. In addition, all reference serum samples were tested once after the PT in order to confirm their stability and status (post-verification) by a rapid plate agglutination test using the CRD antigen from Soleil.

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

III.3.2. Level of agreement

The level of agreement achieved by the participating laboratories is expressed as the percentage of *success* (i.e., the reported result matches with the assigned status) for the 20 aliquots of reference serum samples used for either the PUL or the CRD 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 reference serum samples used for either PT 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. Transfer and start of the analyses of the reference samples

The 40 aliquots of the reference serum samples (20 for the PUL PT and 20 for the CRD PT) were sent at 5±3°C to each of the 3 participating laboratories by national courier on 2nd of July 2012 (120 aliquots in total). All laboratories acknowledged receipt of the samples on the same day. All analyses were performed on 2nd of July 2012 (=date of sample receipt) (Table 1).

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

Results were submitted to the operational unit CVD-ERA between 2nd and 6th of July 2012 (Table 1). All participants hereby respected the deadline of 9th of July 2012 for submission of the results.

Table 1. Overview of the dates on which (i) the reference serum 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 PUL	Start of analysis CRD	Submission of the results (Excel file)
LAB1	02/07/2012	02/07/2012	02/07/2012	06/07/2012
LAB2	02/07/2012	02/07/2012	02/07/2012	04/07/2012
LAB3	02/07/2012	02/07/2012	02/07/2012	02/07/2012

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 all participating laboratories provided qualitative results that were in full agreement with the true status of the reference serum samples (100% of agreement) for both the PUL PT (Table 2) and the CRD PT (Table 3).

Table 2. Agreement between results generated by the participating laboratories (LABNR) and the status of the **PUL** reference serum samples assigned by the PUL reference laboratory of CODA-CERVA. All participating laboratories received 20 aliquots of reference serum samples. Results are presented as absolute values and percentages (in parentheses).

	LABNR		
	1	**2**	**3**
failure	0 (0.0)	0 (0.0)	0 (0.0)
success	20 (100.0)	20 (100.0)	20 (100.0)

Table 3. Agreement between results generated by the participating laboratories (LABNR) and the status of the **CRD** reference serum samples assigned by the CRD reference laboratory of CODA-CERVA. All participating laboratories received 20 aliquots of reference serum samples. Results are presented as absolute values and percentages (in parentheses).

	LABNR		
	1	**2**	**3**
failure	0 (0.0)	0 (0.0)	0 (0.0)
success	20 (100.0)	20 (100.0)	20 (100.0)

IV.4.2. Variability among participating laboratories

Since all participating laboratories reached 100% of agreement for the detection of PUL- and CRD-specific antibodies in reference serum samples by a rapid plate agglutination test, no variability between qualitative laboratory results could be observed.

For each participating laboratory, the obtained results and the assigned statuses for the reference serum samples are shown in Table 4 for the PUL PT and in Table 5 for the CRD PT.

Table 4. The responses (RESULT) of the participating laboratories (LABNR) with the identification of the **PUL** reference serum samples (SAMPLE), the positions of the PUL reference serum samples as placed in the block (LABPOSIT), and the status assigned by the PUL reference laboratory of CODA-CERVA (STATUS). NEG: negative; POS: positive

	LABNR	LABPOSIT	SAMPLE	STATUS	RESULT	SUCCESS
1	1	1	PT2012PULSERPS1	POS	POS	1
2	1	2	PT2012PULSERPS4	POS	POS	1
3	1	3	PT2012PULSERNS1	NEG	NEG	1
4	1	4	PT2012PULSERPS3	POS	POS	1
5	1	5	PT2012PULSERPS2	POS	POS	1
6	1	6	PT2012PULSERPS4	POS	POS	1
7	1	7	PT2012PULSERNS1	NEG	NEG	1
8	1	8	PT2012PULSERPS4	POS	POS	1
9	1	9	PT2012PULSERPS2	POS	POS	1
10	1	10	PT2012PULSERPS1	POS	POS	1
11	1	11	PT2012PULSERNS2	NEG	NEG	1
12	1	12	PT2012PULSERPS2	POS	POS	1
13	1	13	PT2012PULSERPS2	POS	POS	1
14	1	14	PT2012PULSERNS2	NEG	NEG	1
15	1	15	PT2012PULSERPS1	POS	POS	1
16	1	16	PT2012PULSERPS3	POS	POS	1
17	1	17	PT2012PULSERNS1	NEG	NEG	1
18	1	18	PT2012PULSERPS3	POS	POS	1
19	1	19	PT2012PULSERNS2	NEG	NEG	1
20	1	20	PT2012PULSERPS1	POS	POS	1
21	2	1	PT2012PULSERPS3	POS	POS	1
22	2	2	PT2012PULSERNS2	NEG	NEG	1
23	2	3	PT2012PULSERPS1	POS	POS	1
24	2	4	PT2012PULSERPS1	POS	POS	1
25	2	5	PT2012PULSERPS4	POS	POS	1
26	2	6	PT2012PULSERNS1	NEG	NEG	1
27	2	7	PT2012PULSERPS3	POS	POS	1
28	2	8	PT2012PULSERPS2	POS	POS	1
29	2	9	PT2012PULSERPS4	POS	POS	1
30	2	10	PT2012PULSERNS1	NEG	NEG	1
31	2	11	PT2012PULSERPS4	POS	POS	1
32	2	12	PT2012PULSERPS2	POS	POS	1
33	2	13	PT2012PULSERPS1	POS	POS	1
34	2	14	PT2012PULSERNS2	NEG	NEG	1
35	2	15	PT2012PULSERPS2	POS	POS	1
36	2	16	PT2012PULSERPS2	POS	POS	1
37	2	17	PT2012PULSERNS2	NEG	NEG	1
38	2	18	PT2012PULSERPS1	POS	POS	1
39	2	19	PT2012PULSERPS3	POS	POS	1
40	2	20	PT2012PULSERNS1	NEG	NEG	1

(Table 4 - CONTINUED)

	LABNR	LABPOSIT	SAMPLE	STATUS	RESULT	SUCCESS
41	3	1	PT2012PULSERPS1	POS	POS	1
42	3	2	PT2012PULSERPS3	POS	POS	1
43	3	3	PT2012PULSERNS1	NEG	NEG	1
44	3	4	PT2012PULSERPS3	POS	POS	1
45	3	5	PT2012PULSERNS2	NEG	NEG	1
46	3	6	PT2012PULSERPS1	POS	POS	1
47	3	7	PT2012PULSERPS1	POS	POS	1
48	3	8	PT2012PULSERPS4	POS	POS	1
49	3	9	PT2012PULSERNS1	NEG	NEG	1
50	3	10	PT2012PULSERPS3	POS	POS	1
51	3	11	PT2012PULSERPS2	POS	POS	1
52	3	12	PT2012PULSERPS4	POS	POS	1
53	3	13	PT2012PULSERNS1	NEG	NEG	1
54	3	14	PT2012PULSERPS4	POS	POS	1
55	3	15	PT2012PULSERPS2	POS	POS	1
56	3	16	PT2012PULSERPS1	POS	POS	1
57	3	17	PT2012PULSERNS2	NEG	NEG	1
58	3	18	PT2012PULSERPS2	POS	POS	1
59	3	19	PT2012PULSERPS2	POS	POS	1
60	3	20	PT2012PULSERNS2	NEG	NEG	1

Table 5. The responses (RESULT) of the participating laboratories (LABNR) with the identification of the **CRD** reference serum samples (SAMPLE), the positions of the CRD reference serum samples as placed in the block (LABPOSIT), and the status assigned by the CRD reference laboratory of CODA-CERVA (STATUS). NEG: negative; POS: positive

	LABNR	LABPOSIT	SAMPLE	STATUS	RESULT	SUCCESS
1	1	1	PT2012CRDSERPS1	POS	POS	1
2	1	2	PT2012CRDSERNS2	NEG	NEG	1
3	1	3	PT2012CRDSERPS3	POS	POS	1
4	1	4	PT2012CRDSERPS1	POS	POS	1
5	1	5	PT2012CRDSERNS1	NEG	NEG	1
6	1	6	PT2012CRDSERNS1	NEG	NEG	1
7	1	7	PT2012CRDSERNS2	NEG	NEG	1
8	1	8	PT2012CRDSERPS3	POS	POS	1
9	1	9	PT2012CRDSERPS2	POS	POS	1
10	1	10	PT2012CRDSERPS4	POS	POS	1
11	1	11	PT2012CRDSERNS1	NEG	NEG	1
12	1	12	PT2012CRDSERPS2	POS	POS	1
13	1	13	PT2012CRDSERNS2	NEG	NEG	1
14	1	14	PT2012CRDSERPS3	POS	POS	1
15	1	15	PT2012CRDSERNS2	NEG	NEG	1
16	1	16	PT2012CRDSERPS4	POS	POS	1
17	1	17	PT2012CRDSERPS1	POS	POS	1
18	1	18	PT2012CRDSERPS4	POS	POS	1
19	1	19	PT2012CRDSERNS1	NEG	NEG	1
20	1	20	PT2012CRDSERPS2	POS	POS	1
21	2	1	PT2012CRDSERPS4	POS	POS	1
22	2	2	PT2012CRDSERNS1	NEG	NEG	1
23	2	3	PT2012CRDSERPS2	POS	POS	1
24	2	4	PT2012CRDSERPS1	POS	POS	1
25	2	5	PT2012CRDSERNS2	NEG	NEG	1
26	2	6	PT2012CRDSERPS3	POS	POS	1
27	2	7	PT2012CRDSERPS1	POS	POS	1
28	2	8	PT2012CRDSERNS1	NEG	NEG	1
29	2	9	PT2012CRDSERNS1	NEG	NEG	1
30	2	10	PT2012CRDSERNS2	NEG	NEG	1
31	2	11	PT2012CRDSERPS3	POS	POS	1
32	2	12	PT2012CRDSERPS2	POS	POS	1
33	2	13	PT2012CRDSERPS4	POS	POS	1
34	2	14	PT2012CRDSERNS1	NEG	NEG	1
35	2	15	PT2012CRDSERPS2	POS	POS	1
36	2	16	PT2012CRDSERNS2	NEG	NEG	1
37	2	17	PT2012CRDSERPS3	POS	POS	1
38	2	18	PT2012CRDSERNS2	NEG	NEG	1
39	2	19	PT2012CRDSERPS4	POS	POS	1
40	2	20	PT2012CRDSERPS1	POS	POS	1

(Table 5 - CONTINUED)

	LABNR	LABPOSIT	SAMPLE	STATUS	RESULT	SUCCESS
41	3	1	PT2012CRDSERNS2	NEG	NEG	1
42	3	2	PT2012CRDSERPS4	POS	POS	1
43	3	3	PT2012CRDSERPS1	POS	POS	1
44	3	4	PT2012CRDSERPS4	POS	POS	1
45	3	5	PT2012CRDSERNS1	NEG	NEG	1
46	3	6	PT2012CRDSERPS2	POS	POS	1
47	3	7	PT2012CRDSERPS1	POS	POS	1
48	3	8	PT2012CRDSERNS2	NEG	NEG	1
49	3	9	PT2012CRDSERPS3	POS	POS	1
50	3	10	PT2012CRDSERPS1	POS	POS	1
51	3	11	PT2012CRDSERNS1	NEG	NEG	1
52	3	12	PT2012CRDSERNS1	NEG	NEG	1
53	3	13	PT2012CRDSERNS2	NEG	NEG	1
54	3	14	PT2012CRDSERPS3	POS	POS	1
55	3	15	PT2012CRDSERPS2	POS	POS	1
56	3	16	PT2012CRDSERPS4	POS	POS	1
57	3	17	PT2012CRDSERNS1	NEG	NEG	1
58	3	18	PT2012CRDSERPS2	POS	POS	1
59	3	19	PT2012CRDSERNS2	NEG	NEG	1
60	3	20	PT2012CRDSERPS3	POS	POS	1

V. Discussion

The purpose of this PT was to assess the performances of the participating laboratories when analyzing reference serum samples of poultry origin for the detection of PUL- and CRD-specific antibodies by a rapid plate agglutination test.

For the detection of PUL-specific antibodies in reference serum samples, all 3 participating laboratories obtained results that were in full agreement with the true status of the reference serum samples (Table 2 and Table 4). All participating laboratories used the PUL antigen from LDA[22], but 2 different batches were used: LAB1 and LAB3 used batch 05-2012, whereas LAB2 used batch 11-41.

For the detection of CRD-specific antibodies in reference serum samples, all 3 participating laboratories obtained results that were in full agreement with the true status of the reference serum samples (Table 3 and Table 5). All participating laboratories used batch 00713101 of the CRD antigen from Soleil.

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 reference serum samples assigned by the corresponding reference laboratory of CODA-CERVA (see III.3.3.). Consequently, all participants achieved a satisfactory performance for the detection of PUL- and CRD-specific antibodies in reference serum samples.

Head CVD-ERA
Yves Van der Stede

Appendix

Names of the participating laboratories

Association Régionale de Santé et d'Identification Animales (ARSIA) (Ciney, Belgium)

Dierengezondheidszorg Vlaanderen (DGZ) (Torhout, Belgium)

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