



CODA-CERVA

VETERINARY AND AGROCHEMICAL RESEARCH CENTRE

GROESELBERG 99 – B 1180 BRUSSELS (UKKEL)

TEL: +32 (0)2 379 04 11

FAX : + 32 (0)2 379 06 70

HTTP: // WWW.CODA-CERVA.BE



172-PT

PROFICIENCY TESTING 2017

MYCOPLASMA GALLISEPTICUM

***Detection of Mycoplasma gallisepticum in swab by
Real-time Polymerase Chain Reaction (RT-PCR)***

CODA-CERVA-UCCLE

DATE BEGIN PT: 04 DECEMBER 2017

DATE REPORT: 25 JANUARY 2018

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 *Mycoplasma gallisepticum* in swabs of poultry origin by real-time PCR (RT-PCR).

III. Materials and methods

III.1. Conduct of diagnostic tests

In the framework of this PT, predefined reference swab samples must be tested by means of a RT-PCR. The procedure for the RT-PCR must be fully described in the SOP of the participating laboratories.

III.2. Reference samples

Replicates of 3 reference swab samples, either free from *M. gallisepticum* (n=1 coded 'PT2017CRDBACNSW1') or containing detectable *M. gallisepticum* (n=2 coded 'PT2017CRDBACPSW1' and 'PT2017CRDBACPSW2'), were used. In total, 15 aliquots were distributed to 3 participating laboratories. All participants received 2 aliquots of the reference swab samples PT2017CRDBACNSW1 and PT2017CRDBACPSW1 and 1 aliquot of the reference swab sample PT2017CRDBACPSW2. The positions of the reference swab samples were randomized for each participant (Table 3).

The swab samples were prepared as follows: sterile swabs were spiked with poultry DNA and with either $5.7 \cdot 10^7$ CFU of *M. gallisepticum* (PT2017CRDBACPSW1), or $5.7 \cdot 10^5$ CFU of *M. gallisepticum* (PT2017CRDBACPSW2) or SP4-Z culture medium (PT2017CRDBACNSW1). The reference swab samples were lyophilized directly after their preparation.

The status and the homogeneity of the reference swab samples were checked before (1 aliquot of each sample) and after (5 aliquots of each sample) freeze-drying using the LSI VetMAX™ Triplex Avian Mycoplasmosis – *M. gallisepticum* & *M. synoviae* RT-PCR kit. The status and homogeneity controls gave results in agreement with the expectations: all aliquots of the samples PT2017CRDBACPSW1 and PT2017CRDBACPSW2 were positive for *M. gallisepticum* by RT-PCR and all aliquots of the sample PT2017CRDBACNSW1 gave negative results for *M. gallisepticum* and positive results for the Internal Positive Control by RT-PCR.

Consequently, all reference swab samples were considered as reliable samples in order to evaluate the ability of laboratories to correctly identify the absence or presence of *Mycoplasma gallisepticum* in poultry swab samples by RT-PCR. In addition, 3 aliquots of each reference swab sample were tested after the PT in order to confirm their stability and status (post-verification) using the LSI VetMAX™ Triplex Avian Mycoplasmosis – *M. gallisepticum* & *M. synoviae* RT-PCR kit.

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 a participating laboratory is expressed as the percentage *success* for the 5 aliquots of reference swab samples used in this PT.

III.3.3. Threshold for qualification

Following the procedure, a participating laboratory is only qualified if the level of agreement for the 5 aliquots of reference swab samples is 100%.

IV. Results

For confidentiality reasons, the participating laboratories are quoted anonymously and the concordance table is safely kept at the CODA-CERVA-Uccle.

IV.1. Transfer and start of the analyses of the reference samples

The 5 reference swab samples were sent to each of the 3 participating laboratories by national courier on 4th of December 2017. All laboratories acknowledged receipt of the samples on the same day. Analyses were performed between 4th and 14th of December 2017 (Table 1).

IV.2. Dates at which results were returned to the CODA-CERVA-Uccle

Results were submitted to the CODA-CERVA-Uccle on 5th, 14th and 15th of December 2017 (Table 1). All participants hereby respected the deadline of 15th of December 2017 for submission of the results.

Table 1. Overview of the dates on which (i) the reference samples were received and analyzed by the participating laboratories, and (ii) the obtained results were submitted to the CODA-CERVA-Uccle.

Laboratory	Reference samples received	Start of analysis	Submission of the results (Excel file)
LAB1	04/12/2017	04/12/2017 & 05/12/2017	05/12/2017
LAB2	04/12/2017	12/12/2017	14/12/2017
LAB3	04/12/2017	14/12/2017	15/12/2017

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

All participating laboratories provided qualitative results that were in full agreement with the assigned status of the reference swab samples and hence obtained 100% of agreement (Table 2).

Table 2. Agreement between results obtained by the participating laboratories (LABNR) and the status of the reference swab samples assigned by the *Mycoplasma gallisepticum* reference laboratory of CODA-CERVA-Uccle. All participating laboratories received 5 reference swab samples. Results are presented as absolute values and percentages (in parentheses).

	LABNR		
	1	2	3
failure	0 (0)	0 (0)	0 (0)
success	5 (100)	5 (100)	5 (100)

IV.4.2. Variability among participating laboratories

Since all participating laboratories reached 100% of agreement, no variability between qualitative laboratory results could be observed.

For each participating laboratory, the obtained results and the assigned statuses for the reference swab samples are shown in Table 3.

Table 3. The responses (RESULT) of the participating laboratories (LABNR) with the identification of the reference swab samples (SAMPLE), the external identification of the reference swab samples (LABPOSIT), and the status assigned by the *Mycoplasma gallisepticum* reference laboratory of CODA-CERVA-Uccle (STATUS).

	LABNR	LABPOSIT	SAMPLE	STATUS	RESULT	SUCCESS
1	1	1	PT2017CRDBACNSW1	NEG	NEG	1
2	1	2	PT2017CRDBACPSW1	POS	POS	1
3	1	3	PT2017CRDBACNSW1	NEG	NEG	1
4	1	4	PT2017CRDBACPSW2	POS	POS	1
5	1	5	PT2017CRDBACPSW1	POS	POS	1
6	2	1	PT2017CRDBACPSW1	POS	POS	1
7	2	2	PT2017CRDBACPSW2	POS	POS	1
8	2	3	PT2017CRDBACNSW1	NEG	NEG	1
9	2	4	PT2017CRDBACPSW1	POS	POS	1
10	2	5	PT2017CRDBACNSW1	NEG	NEG	1
11	3	1	PT2017CRDBACNSW1	NEG	NEG	1
12	3	2	PT2017CRDBACPSW1	POS	POS	1
13	3	3	PT2017CRDBACNSW1	NEG	NEG	1
14	3	4	PT2017CRDBACPSW2	POS	POS	1
15	3	5	PT2017CRDBACPSW1	POS	POS	1

V. Discussion

The purpose of this PT was to assess the performance of the participating laboratories when analyzing reference swab samples for the detection of *Mycoplasma gallisepticum* by RT-PCR.

All participating laboratories provided qualitative results that were in full agreement with the assigned status of the reference swab samples (100% of agreement).

VI. Conclusions

According to the procedure currently in force, the performance of a participating laboratory is satisfactory if 100% of the results provided by this laboratory is in agreement with the status of the reference swab samples assigned by the *Mycoplasma gallisepticum* reference laboratory of CODA-CERVA-Uccle (see III.3.3.). Consequently, all participants achieved a satisfactory performance for detection of *Mycoplasma gallisepticum* by RT-PCR in reference swab samples.

Coordinator proficiency tests

Katia Knapen



Appendix

Name 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)