BIOLOGICAL HEALTH RISKS
QUALITY OF LABORATORIES

COMMITEE OF EXPERTS

PROFICIENCY TEST
IN VETERINARY DIAGNOSIS

DEFINITIVE GLOBAL REPORT

VETERINARY MEDICINE

CHRONIC RESPIRATORY DISEASE – MYCOPLASMA GALLISEPTICUM (MYC)

PROFICIENCY TEST 2023-9

Sciensano/PT VET MYC/2023-9/E
Biological health risks
Quality of laboratories
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All the reports are available on our webpage:

- NL: https://www.sciensano.be/nl/kwaliteit-van-laboratoria
- FR: https://www.sciensano.be/fr/qualite-des-laboratoires
- EN: https://www.sciensano.be/en/quality-laboratories

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1 INTRODUCTION

Details relevant to the proficiency test are available in the procedure SOP 2.5/01 'Management of the proficiency tests organized by the scientific directorate infectious diseases in animals'. The PT was organized according to the ISO17043 'Conformity assessment - General requirements for proficiency testing' norm.

2 AIM

This proficiency test was dedicated to the detection of the causative agent of the Chronic Respiratory Disease (CRD), i.e. *Mycoplasma gallisepticum*, in swab by Real-time Polymerase Chain Reaction (RT-PCR).

3 MATERIALS AND METHODS

3.1 Bacteriology (swab)

3.1.1 THE PARTICIPANTS

Four laboratories participated in the proficiency test of Chronic respiratory disease - *Mycoplasma Gallisepticum* (MYC) bacteriology on swab samples. The names of the participating laboratories are:

- Sciensano, service of Veterinary Bacteriology
- Dierengezondheidszorg Vlaanderen (DGZ)
- IDVET
- Poulpharm

3.1.2 THE SAMPLES

The samples were prepared by the National Reference Laboratory (NRL) *Mycoplasma* spp., Veterinary Bacteriology Service, Infectious diseases in animals Directorate, Sciensano.

Three different samples were used: PT2023CRDBAC_PSW1, PT2023CRDBAC_PSW2 and PT2023CRDBAC_NSW1. The samples were prepared as follows: sterile swabs were spiked with poultry DNA and with either a pure culture of *M. gallisepticum* (PT2023CRDBAC_PSW1), or a 100-fold dilution of the pure culture of *M. gallisepticum* (PT2023CRDBAC_PSW2) or SP4-Z culture medium (PT2023CRDBAC_NSW1). The reference swab samples were lyophilized directly after their preparation.

3.1.3 HOMOGENEITY

The homogeneity of the samples was tested by the NRL on three aliquots (three swabs) of each sample using RT-PCR before and after the PT. The NRL obtained each time the same qualitative result. Therefore, the samples were considered as homogeneous.

3.1.4 TARGET VALUES

The target values were determined by the NRL based on the homogeneity tests. The panel consisted of three different samples. However, positive sample PSW2 and negative sample NSW1 were replicated two times. Therefore, the panel included five samples in total.

Sample content	Repetition	Expected result
PT2023CRDBAC_PSW1	1	POS (STRONG)
PT2023CRDBAC_PSW2	2	POS (WEAK)
PT2023CRDBAC_NSW1	2	NEG

(POS = positive; NEG = negative)

3.1.5 STABILITY

The stability was determined by comparison of the pre-proficiency test results with the results obtained by the NRL during and after the proficiency test. The samples were considered as stable.

3.1.6 RANDOMISATION AND PANEL COMPOSITION

Since a specific number has been assigned to each laboratory, the randomisation has been performed as follows:

Sample content	97505	97508	97522	97540
PT2023CRDBAC_PSW1	MYC23-2	MYC23-2	MYC23-1	MYC23-2
PT2023CRDBAC_PSW2 (1)	MYC23-3	MYC23-4	MYC23-5	MYC23-3
PT2023CRDBAC_PSW2 (2)	MYC23-4	MYC23-3	MYC23-2	MYC23-4
PT2023CRDBAC_NSW1 (1)	MYC23-1	MYC23-1	MYC23-3	MYC23-1
PT2023CRDBAC_NSW1 (2)	MYC23-5	MYC23-5	MYC23-4	MYC23-5

4 TIMELINE

Transfer of the samples from NRL to QL: 11/09/2023
Randomization of the samples by QL: 12/09/2023
Sending samples to participants: 18/09/2023

- Storage of the samples : at room temperature

Deadline for submitting the results: 06/10/2023
Individual report to the participants: 23/11/2023

5 RESULTS

5.1 Bacteriology (swab)

5.1.1 RESULTS PER SAMPLE

The panel consisted of three different samples. However, positive sample PSW2 and negative sample NSW1 were replicated two times. Therefore, the panel included five samples in total.

Sample content	Status	Number of repetitions (total results)	Observed result
PSW1	POS	1 (4)	4 POS
PSW2	POS	2 (8)	8 POS
NSW1	NEG	2 (8)	8 NEG

5.1.2 USED RT-PCR PROTOCOL/KIT

In the table below, the RT-PCR protocols/kits used are listed along with the number of laboratories that have used this protocol/kit with their achieved score.

Manufacturer RT-qPCR protocol / kit	Name RT-qPCR protocol / kit	N	NR	NCR	%
Thermo Fisher	VetMAX Avian Triplex Mycoplasmosis Kit	1	5	5	100
Thermo Fisher	VetMAX™ Avian M. gallisepticum & M. synoviae Kit	1	5	5	100
IDVET	ID Gene® MG/MS Triplex	1	5	5	100
Kylt	Kylt® MGS Triplex	1	5	5	100
TOTAL		**4**	**20**	**20**	**100**

(N= number of laboratories; NR = number of results; NCR = number of correct results).

5.1.3 USED EXTRACTION PROTOCOL/KIT

In the table below, the extraction protocols/kits used are listed along with the number of laboratories that have used this protocol/kit with their achieved score.

Manufacturer extraction protocol / kit	Name extraction protocol / kit	N	NR	NCR	%
Qiagen	QIAamp DNA Mini kit	1	5	5	100
Indical Bioscience	IndiMag Pathogen Kit	1	5	5	100
IDVET	ID Gene Mag Universal Extraction kit	1	5	5	100
Kylt	Kylt® DNA Extraction-Mix II	1	5	5	100
TOTAL		**4**	**20**	**20**	**100**

(N= number of laboratories; NR = number of results; NCR = number of correct results).

5.1.4 CONCLUSION

In 2023, four laboratories participated in the proficiency test “Chronic respiratory disease - *Mycoplasma Gallisepticum* (MYC) bacteriology (swab)” organized by Sciensano. According to the procedure currently in force, the performance of a participating laboratory is satisfactory if all the results (100%) provided by this laboratory are in agreement with the status of the reference samples assigned by the NRL of the Scientific Directorate Infectious Diseases in Animals of Sciensano. All laboratories succeeded in achieving the maximum score (100%) for this test.

6 ANNEXES (NOT UNDER ACCREDITATION)

This quantitative data is not under BELAC-accreditation and is solely for the information of the laboratories.

6.1 Annex 1: Quantitative results

Boxplots are generated exclusively for the positive samples that exhibited repetitions within the panel.

The boxplots, shown down below, were created by using the following software programme: shiny.chemgrid.org/boxplotr/

6.1.1 BACTERIOLOGY (SWAB)

PT2023CRDBACPSW2

Lab number	97504	97508	97522	97540
Method (RT-qPCR protocol/kit)	M_1	M_2	M_3	M_4
Ct (REP1)	26,02	24,85	22,90	26,94
Ct (REP2)	25,91	24,97	22,30	26,91
Mean	25,97	24,91	22,60	26,93
SD	0,078	0,085	0,42	0,021
CV (%)	0,30	0,34	1,88	0,079

Numbers were rounded to two significant decimal place. (Ct = crossing threshold; REP = repetition; SD = standard deviation; CV = coefficient of variation, M_1 = Thermo Fisher - VetMAX Avian Triplex Mycoplasmosis Kit, M_2 = Thermo Fisher - VetMAX™ Avian M. gallisepticum & M. synoviae Kit , M_3 = IDVET - ID Gene® MG/MS Triplex; M_4 = Kylt - Kylt® MGS Triplex).

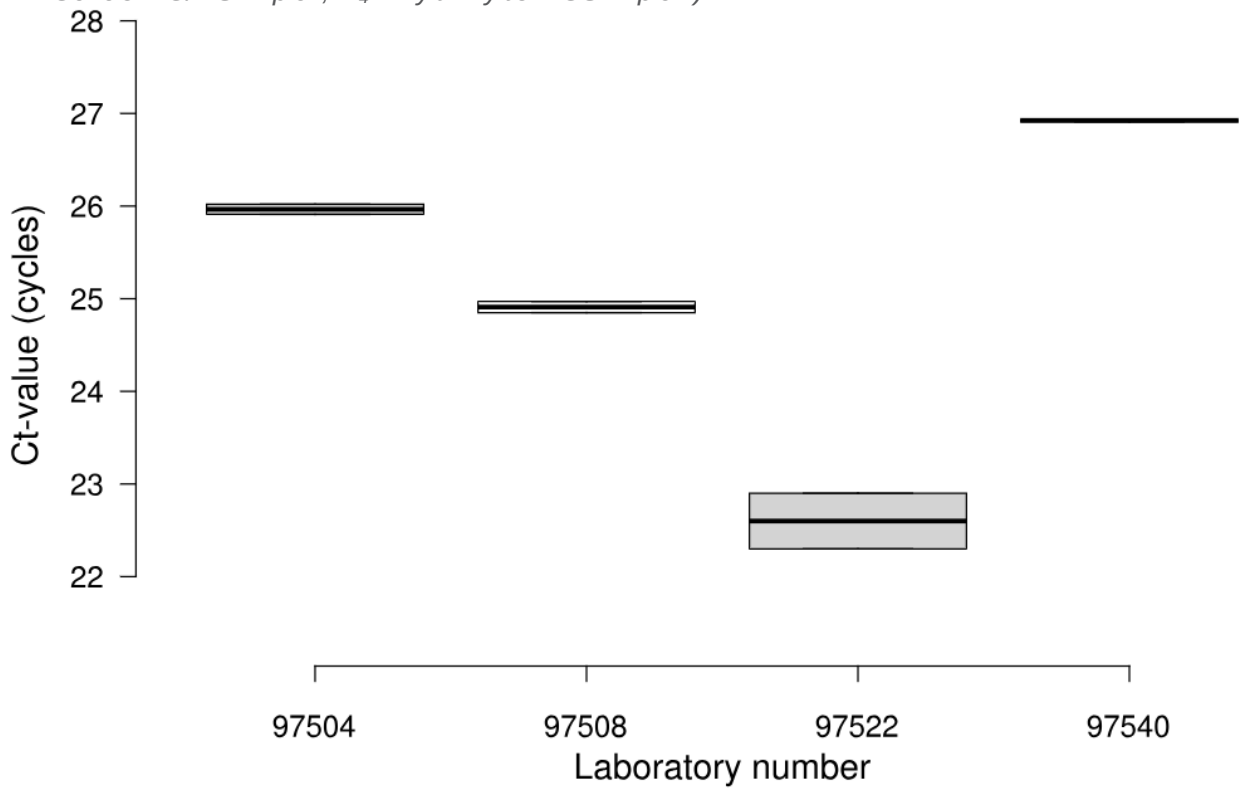


Figure 1. Distribution of the Ct-values (box-plots) per laboratory.

6.2 Annex 2: Additional information

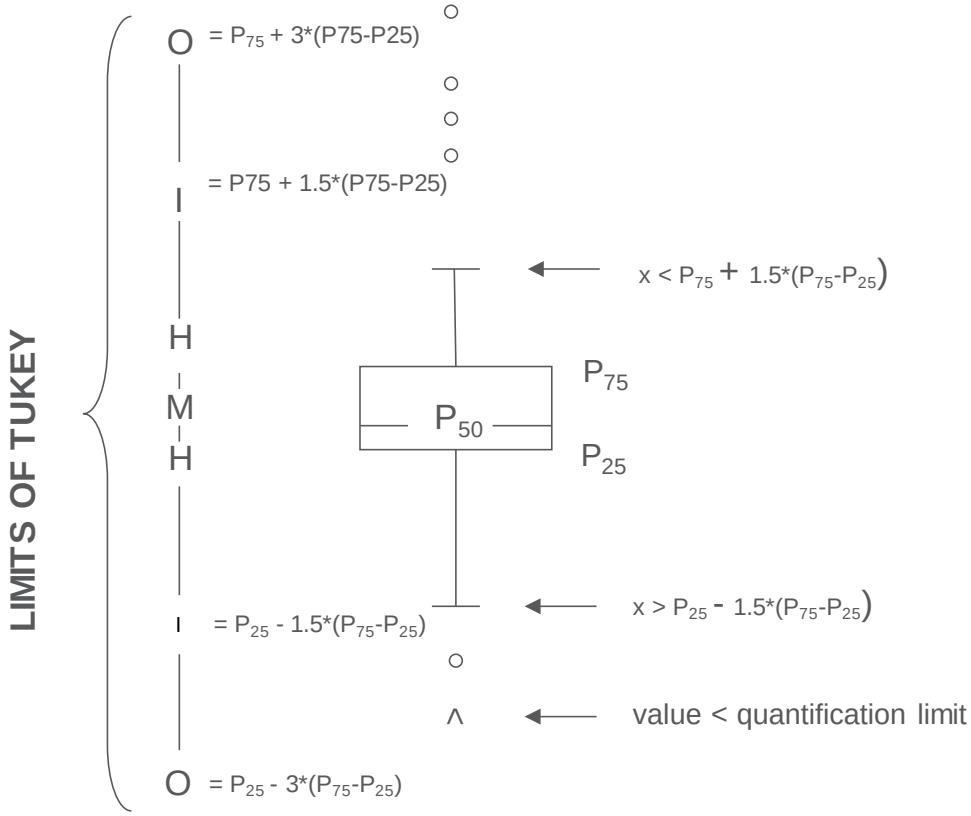
The **calendar** for Proficiency Testing in Veterinary diagnosis is available on our website:

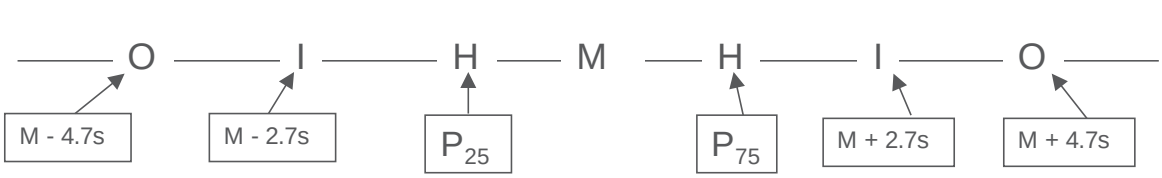
- NL: https://www.sciensano.be/fr/biblio/eke-kalender-2023
- FR: https://www.sciensano.be/en/biblio/calendrier-eeq-2023
- EN: https://www.sciensano.be/en/biblio/eqa-calendar-2023

Graphical representation

Besides the tables with the results a "Box and whisker" plot is added. It contains the following elements for the methods with at least 3 participants:

- a rectangle ranging from percentile 25 ($\mathbf{P_{25}}$) to percentile 75 ($\mathbf{P_{75}}$)
- a central line representing the median of the results ($\mathbf{P_{50}}$)
- a lower limit showing the smallest value $\mathbf{x > P_{25} - 1.5 * (P_{75} - P_{25})}$
- an upper limit representing the largest value $\mathbf{x < P_{75} + 1.5 * (P_{75} - P_{25})}$
- all points outside this interval are represented by a dot.





Corresponding limits in case of normal distribution

END