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081-PT

# PROFICIENCY TESTING 2018

***VISNA MAEDI (MAE)***

***DETECTION OF SRLV-SPECIFIC ANTIBODIES IN OVINE AND CAPRINE SERA BY***

***ENZYME LINKED IMMUNOSORBENT ASSAY (ELISA)***

## SCIENTIFIC DIRECTORATE INFECTIOUS DISEASES IN ANIMALS
## SCIENSANO

**DATE BEGIN PT: 3 DECEMBER 2018**

**DATE REPORT: 6 FEBRUARY 2019**

# I. Introduction

Details relevant to the proficiency test (PT) are available in the procedure SOP 25/01 'Beheer van de proficiency testen georganiseerd door de Wetenschappelijke Directie Infectieziekten Dier/Gestion des essais d'aptitude organisés par la Direction Scientifique Maladies Infectieuses Animales', 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 the absence or presence of SRLV-specific antibodies in ovine and caprine sera by ELISA.

# III. Materials and methods

## *III.1. Conduct of diagnostic tests*

In the framework of this PT, predefined reference serum samples must be analyzed by means of an ELISA test. The procedures for the ELISA tests must be fully described in the SOPs of the participating laboratories.

## *III.2. Reference samples*

Replicates of 5 reference serum samples of ovine and caprine origin, either free from detectable SRLV-specific antibodies (n=2; coded 'PT2018MAEELINS1' and 'PT2018MAEELINS2') or containing detectable SRLV-specific antibodies (n=3; coded 'PT2018MAEELIPS1', 'PT2018MAEELIPS2', and 'PT2018MAEELIPS3'), were used. In total, 80 aliquots were distributed to 4 participating laboratories. All participants received 20 aliquots: 4 aliquots of each reference serum samples. The positions of the reference serum samples in the sent blocks were randomized for each participant (Table 3).

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 during pre-verification, hereby using immunodiffusion assays [Maeditect kit (Alph Scientific) and AGID CAEV P28 kit (Idexx)] and the ELISA kits from Hyphen BioMed (Elitest MVV/CAEV) and IDVet (ID Screen MVV/CAE).

The reference serum samples PT2018MAEELINS1 and PT2018MAEELINS2 were obtained from SRLV negative sheep from SRLV negative herds. The reference serum samples PT2018MAEELIPS1, PT2018MAEELIPS2, PT2018MAEELIPS3 were derived from 3 different animals. PT2018MAEELIPS1 was the serum from a sheep experimentally infected with a genotype A strain. PT2018MAEELIPS2 orginated from a naturally SRLV infected goat that was furthermore experimentally infected with a genotype B strain and PT2018MAEELIPS3 came from a SRLV negative goat that was experimentally infected with a genotype B strain.
Taken together, the reference serum samples PT2018MAEELINS1 and PT2018MAEELINS2 were considered as negative sera and the reference serum samples PT2018MAEELIPS1, PT2018MAEELIPS2 and PT2018MAEELIPS3 as positive sera in SRLV antibody ELISA.

After aliquoting the different reference serum samples, a homogeneity check was performed on 10 aliquots of each reference serum sample using the ELISA kits from Hyphen BioMed (Elitest MVV/CAEV) and IDVet (ID Screen MVV/CAE), 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 MAE-specific antibodies in bovine serum. In addition, 3 aliquots of each reference serum sample were tested after the PT in order to confirm their stability and status (post-verification) using the ELISA kits from Hyphen BioMed (Elitest MVV/CAEV) and IDVet (ID Screen MVV/CAE).

### *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 the participating laboratories is expressed as the percentage of *success* for the 20 aliquots of reference samples used for this 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 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 Scientific Directorate Infectious Diseases in Animals of Sciensano.

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

The 20 aliquots of reference serum samples were sent frozen (dry ice) to each of the 4 participating laboratories by national or international courier on 3th of December 2018 (80 aliquots in total). LAB2, LAB3 and LAB4 acknowledged receipt of the samples on the same day, whereas LAB1 acknowledged receipt of the samples on 6th of December 2018. Analyses were performed between 5th and 13th of December 2018 (Table 1).

### *IV.2. Dates at which results were returned to the Scientific Directorate Infectious Diseases in Animals of Sciensano*

Results were submitted to the Scientific Directorate Infectious Diseases in Animals of Sciensano between 11th and 19th of December 2018 (Table 1). All participants respected the deadline of 21st of December 2018 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 Scientific Directorate Infectious Diseases in Animals of Sciensano.

| Laboratory | Reference samples received | Start of analysis | Submission of the results (Excel file) |
|---|---|---|---|
| **LAB1** | 06/12/2018 | 11/12/2018 | 19/12/2018 |
| **LAB2** | 03/12/2018 | 13/12/2018 | 14/12/2018 |
| **LAB3** | 03/12/2018 | 05/12/2018 | 17/12/2018 |
| **LAB4** | 03/12/2018 | 07/12/2018 | 11/12/2018 |

### *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 LAB2, LAB3 and LAB4 provided qualitative results that were in full agreement with the true status of the reference serum samples (100% of agreement). In contrast, LAB1 misclassified 2 aliquots (90% of agreement) of reference serum samples. (Table 2).

A quantitative data analysis (box plots) is shown for educational purposes in Annex 1.

**Table 2.** Agreement between the results obtained by the participating laboratories (LABNR) and the status of the reference serum samples assigned by the MAE reference laboratory of the Scientific Directorate Infectious Diseases in Animals of Sciensano. All participating laboratories received 20 aliquots of reference serum samples. Results are presented as absolute values and percentages (in parentheses).

| | LABNR | | | |
|---|---|---|---|---|
| | 1 | 2 | 3 | 4 |
| failure | 2 (10) | 0 (0) | 0 (0) | 0 (0) |
| success | 18 (90) | 20 (100) | 20 (100) | 20 (100) |

### *IV.4.2. Variability among participating laboratories*

No variability between LAB2, LAB3 and LAB4 could be observed since these participants correctly identified all reference serum samples. In contrast LAB1 misclassified one aliquot of the reference serum samples PT2018MAEELINS1 and PT2018MAEELINS2 (positive instead of negative).

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

**Table 3.** The responses (RESULT) of the participating laboratories (LABNR) with the internal identification of the reference serum samples (SAMPLE), the external identification of the reference serum samples (LABPOSIT), and the status assigned by the MAE reference laboratory of the Scientific Directorate Infectious Diseases in Animals of Sciensano (STATUS). NEG: negative; POS: positive.

| | LABNR | LABPOSIT | SAMPLE | STATUS | RESULT | SUCCESS |
|---|---|---|---|---|---|---|
| 1 | 1 | 1 | PT2018MAEELINS2 | NEG | NEG | 1 |
| 2 | 1 | 2 | PT2018MAEELIPS1 | POS | POS | 1 |
| 3 | 1 | 3 | PT2018MAEELIPS3 | POS | POS | 1 |
| 4 | 1 | 4 | PT2018MAEELINS1 | NEG | NEG | 1 |
| 5 | 1 | 5 | PT2018MAEELIPS2 | POS | POS | 1 |
| 6 | 1 | 6 | PT2018MAEELINS2 | NEG | POS | 0 |
| 7 | 1 | 7 | PT2018MAEELIPS2 | POS | POS | 1 |
| 8 | 1 | 8 | PT2018MAEELINS1 | NEG | POS | 0 |
| 9 | 1 | 9 | PT2018MAEELIPS3 | POS | POS | 1 |
| 10 | 1 | 10 | PT2018MAEELINS1 | NEG | NEG | 1 |
| 11 | 1 | 11 | PT2018MAEELIPS1 | POS | POS | 1 |
| 12 | 1 | 12 | PT2018MAEELIPS2 | POS | POS | 1 |
| 13 | 1 | 13 | PT2018MAEELINS2 | NEG | NEG | 1 |
| 14 | 1 | 14 | PT2018MAEELIPS3 | POS | POS | 1 |
| 15 | 1 | 15 | PT2018MAEELIPS1 | POS | POS | 1 |
| 16 | 1 | 16 | PT2018MAEELINS1 | NEG | NEG | 1 |
| 17 | 1 | 17 | PT2018MAEELIPS2 | POS | POS | 1 |
| 18 | 1 | 18 | PT2018MAEELIPS3 | POS | POS | 1 |
| 19 | 1 | 19 | PT2018MAEELINS2 | NEG | NEG | 1 |
| 20 | 1 | 20 | PT2018MAEELIPS1 | POS | POS | 1 |

(Table 3 - CONTINUED)

| | LABNR | LABPOSIT | SAMPLE | STATUS | RESULT | SUCCESS |
|---|---|---|---|---|---|---|
| **21** | 2 | 1 | PT2018MAEELIPS1 | POS | POS | 1 |
| **22** | 2 | 2 | PT2018MAEELIPS2 | POS | POS | 1 |
| **23** | 2 | 3 | PT2018MAEELINS1 | NEG | NEG | 1 |
| **24** | 2 | 4 | PT2018MAEELIPS2 | POS | POS | 1 |
| **25** | 2 | 5 | PT2018MAEELIPS1 | POS | POS | 1 |
| **26** | 2 | 6 | PT2018MAEELINS2 | NEG | NEG | 1 |
| **27** | 2 | 7 | PT2018MAEELINS1 | NEG | NEG | 1 |
| **28** | 2 | 8 | PT2018MAEELIPS3 | POS | POS | 1 |
| **29** | 2 | 9 | PT2018MAEELIPS2 | POS | POS | 1 |
| **30** | 2 | 10 | PT2018MAEELINS2 | NEG | NEG | 1 |
| **31** | 2 | 11 | PT2018MAEELIPS1 | POS | POS | 1 |
| **32** | 2 | 12 | PT2018MAEELIPS3 | POS | POS | 1 |
| **33** | 2 | 13 | PT2018MAEELINS1 | NEG | NEG | 1 |
| **34** | 2 | 14 | PT2018MAEELINS1 | NEG | NEG | 1 |
| **35** | 2 | 15 | PT2018MAEELIPS2 | POS | POS | 1 |
| **36** | 2 | 16 | PT2018MAEELINS2 | NEG | NEG | 1 |
| **37** | 2 | 17 | PT2018MAEELIPS3 | POS | POS | 1 |
| **38** | 2 | 18 | PT2018MAEELINS2 | NEG | NEG | 1 |
| **39** | 2 | 19 | PT2018MAEELIPS1 | POS | POS | 1 |
| **40** | 2 | 20 | PT2018MAEELIPS3 | POS | POS | 1 |
| **41** | 3 | 1 | PT2018MAEELINS2 | NEG | NEG | 1 |
| **42** | 3 | 2 | PT2018MAEELIPS1 | POS | POS | 1 |
| **43** | 3 | 3 | PT2018MAEELIPS3 | POS | POS | 1 |
| **44** | 3 | 4 | PT2018MAEELINS1 | NEG | NEG | 1 |
| **45** | 3 | 5 | PT2018MAEELIPS2 | POS | POS | 1 |
| **46** | 3 | 6 | PT2018MAEELINS2 | NEG | NEG | 1 |
| **47** | 3 | 7 | PT2018MAEELIPS2 | POS | POS | 1 |
| **48** | 3 | 8 | PT2018MAEELINS1 | NEG | NEG | 1 |
| **49** | 3 | 9 | PT2018MAEELIPS3 | POS | POS | 1 |
| **50** | 3 | 10 | PT2018MAEELINS1 | NEG | NEG | 1 |
| **51** | 3 | 11 | PT2018MAEELIPS1 | POS | POS | 1 |
| **52** | 3 | 12 | PT2018MAEELIPS2 | POS | POS | 1 |
| **53** | 3 | 13 | PT2018MAEELINS2 | NEG | NEG | 1 |
| **54** | 3 | 14 | PT2018MAEELIPS3 | POS | POS | 1 |
| **55** | 3 | 15 | PT2018MAEELIPS1 | POS | POS | 1 |
| **56** | 3 | 16 | PT2018MAEELINS1 | NEG | NEG | 1 |
| **57** | 3 | 17 | PT2018MAEELIPS2 | POS | POS | 1 |
| **58** | 3 | 18 | PT2018MAEELIPS3 | POS | POS | 1 |
| **59** | 3 | 19 | PT2018MAEELINS2 | NEG | NEG | 1 |
| **60** | 3 | 20 | PT2018MAEELIPS1 | POS | POS | 1 |

(Table 3 - CONTINUED)

| | LABNR | LABPOSIT | SAMPLE | STATUS | RESULT | SUCCESS |
|---|---|---|---|---|---|---|
| **61** | 4 | 1 | PT2018MAEELIPS1 | POS | POS | 1 |
| **62** | 4 | 2 | PT2018MAEELIPS2 | POS | POS | 1 |
| **63** | 4 | 3 | PT2018MAEELINS1 | NEG | NEG | 1 |
| **64** | 4 | 4 | PT2018MAEELIPS2 | POS | POS | 1 |
| **65** | 4 | 5 | PT2018MAEELIPS1 | POS | POS | 1 |
| **66** | 4 | 6 | PT2018MAEELINS2 | NEG | NEG | 1 |
| **67** | 4 | 7 | PT2018MAEELINS1 | NEG | NEG | 1 |
| **68** | 4 | 8 | PT2018MAEELIPS3 | POS | POS | 1 |
| **69** | 4 | 9 | PT2018MAEELIPS2 | POS | POS | 1 |
| **70** | 4 | 10 | PT2018MAEELINS2 | NEG | NEG | 1 |
| **71** | 4 | 11 | PT2018MAEELIPS1 | POS | POS | 1 |
| **72** | 4 | 12 | PT2018MAEELIPS3 | POS | POS | 1 |
| **73** | 4 | 13 | PT2018MAEELINS1 | NEG | NEG | 1 |
| **74** | 4 | 14 | PT2018MAEELINS1 | NEG | NEG | 1 |
| **75** | 4 | 15 | PT2018MAEELIPS2 | POS | POS | 1 |
| **76** | 4 | 16 | PT2018MAEELINS2 | NEG | NEG | 1 |
| **77** | 4 | 17 | PT2018MAEELIPS3 | POS | POS | 1 |
| **78** | 4 | 18 | PT2018MAEELINS2 | NEG | NEG | 1 |
| **79** | 4 | 19 | PT2018MAEELIPS1 | POS | POS | 1 |
| **80** | 4 | 20 | PT2018MAEELIPS3 | POS | POS | 1 |
| **61** | 4 | 1 | PT2018MAEELIPS1 | POS | POS | 1 |

## V. Discussion

The purpose of this PT was to assess the performance of the participating laboratories when analyzing reference serum samples of ovine and caprine origin for the detection of SRLV-specific antibodies by ELISA.

For the detection of SRLV-specific antibodies in reference serum samples, LAB2, LAB3 aand LAB4 provided qualitative results that were in full agreement with the assigned status of the reference serum samples (100% of agreement). LAB1 misclassified 1 aliquot of the reference serum samples ‘PT2018MAEELINS1’ and ‘PT2018MAEELINS2’ (90% of agreement).

The participating laboratories used SRLV antibody ELISA kits from 3 different commercial producers : LAB1 Life Technologies (batch MC1711011), LAB2 IDVET (batch B98), LAB3 and LAB4 Hyphen Biomed (batch F1800245P1).

Although LAB1 obtained satisfactory results based on the criteria in force (90% of results in agreement with the reference status), it is suggested to evaluate whether the kit used is sufficiently specific.

## 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 MAE reference laboratory of the Scientific Directorate Infectious Diseases in Animals of Sciensano (see III.3.3.). Consequently, all participants achieved a satisfactory performance for the detection of SRLV-specific antibodies in reference serum samples by ELISA.

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)

Laboratoire National de Contrôle des Reproducteurs (LNCR / ACSEDIATE) (Maisons-Alfort, France)

Sciensano (Uccle, Belgium)

# Annex 1: Quantitative data analysis (Box plots)

Besides qualitative data analysis (positive or negative result), also quantitative data analysis was performed using the statistical software programs R (box plots).

Box plots represent the minimum and maximum value that are not considered as outliers, the 25th and 75th percentile (respectively P25 and P75), the median (P50), and possible outliers per sample and per laboratory. Values lower than (P25-1.5(P75-P25) and higher than (P75+1.5(P75-P25)) are considered as outliers. Note that due to the low number of data available, outliers cannot be detected when the number of data is smaller than 5 and P25=minimum and P75=maximum when the number data is 2.

The quantitative data analysis in this report was not used to evaluate the participants in this PT, but should only be considered as educational information for the participants in order to evaluate their performance and/or to standardize their different diagnostic tests.

The quantitative data analyse was performed on the normalized data according to the instructions of the PT provider per reference serum sample and per participating laboratory (Figure 1).

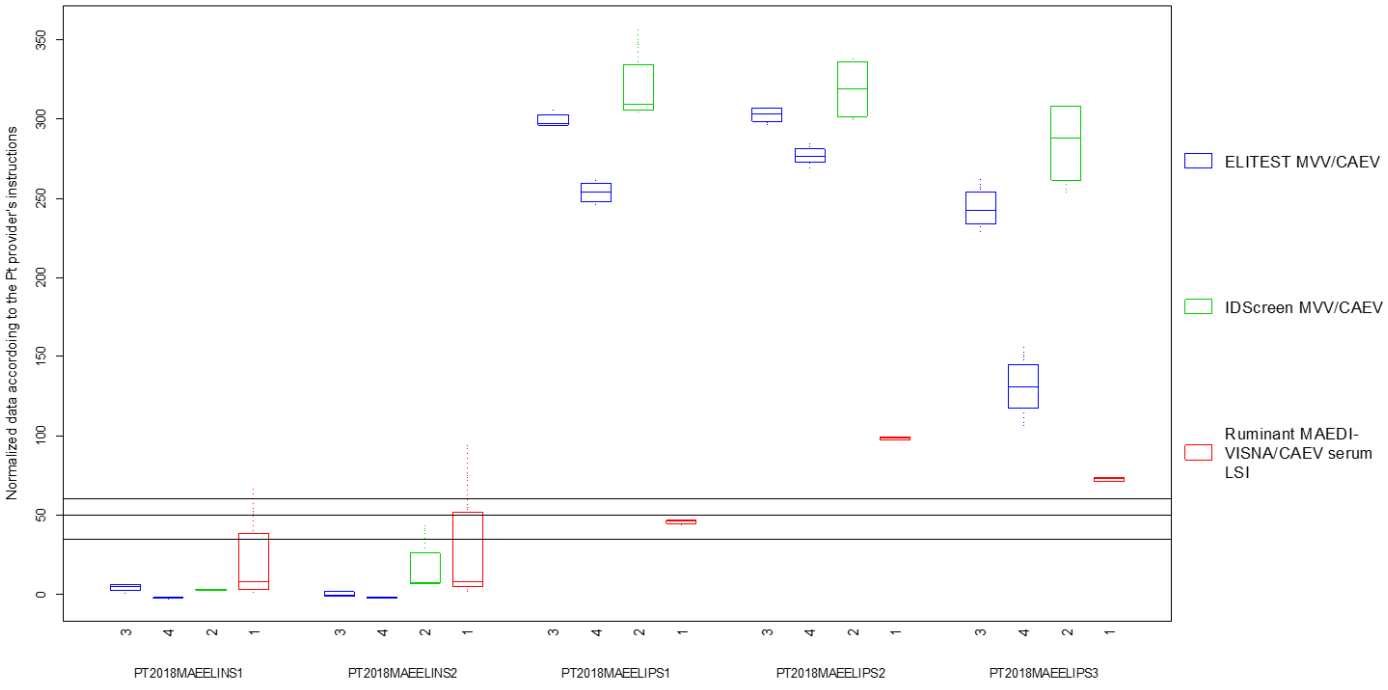


**Figure 1. Box plots showing the normalized OD values according the PT provider per reference serum and per participating laboratory.** The participating laboratories used SRLV antibody ELISA kits from 3 different commercial producers : Life Technologies IDVET and Hyphen Biomed. Cut-off values (Life Technologies 35 and IDVET 50-60) are shown by horizontal lines.