BIOLOGICAL HEALTH RISKS
QUALITY OF LABORATORIES

PROFICIENCY TEST IN VETERINARY DIAGNOSIS

DEFINITIVE GLOBAL REPORT

PT-PROGRAM 2024-5

ENZOOTIC BOVINE LEUKOSIS (EBL)

Corrected version

Sciensano/PT-program EBL/2024-5/E-cv

Biological health risks
Quality of laboratories
Rue Juliette Wytsmanstraat, 14
1050 Brussels | Belgium

www.sciensano.be

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NATIONAL REFERENCE LABORATORY

Sciensano					
Secretariat		Phone:	02/642.55.22	Fax:	02/642.56.45
		E-mail	ql_secretariat@sciensano.be		
Ynse Van de Maele	Coordinator	Phone:	02/642 55 24		
		E-mail:	Ynse.vandemaele@sciensano.be		
Bernard China	Alternate coordinator	Phone:	02/642 53 85		
		E-mail:	Bernard.china@sciensano.be		
Experts	**Institute**				
Gaëtan De Gryse	Sciensano – NRL Viral reemerging enzootic and bee diseases				
Marylène Tignon	Sciensano – NRL Viral reemerging enzootic and bee diseases				

A draft version of this report was submitted to the experts on 30/07/2024.

The experts were invited to send their comments via e-mail.

Responsibilities:
The National Reference Laboratory (NRL) of Sciensano was consulted for advice about the content of the global report, the interpretation of the results and the evaluation criteria. The responsibility for the choice of the samples used was carried out by the NRL.

A corrected version of the report had been published for the following raison: an incorrect laboratory number was mentioned during the randomisation (not LAB97510 but LAB97508). The following change is made in the report in colour (blue) on page five.

This report replaces the previous version of the report of 22/08/2024.

Authorization of the report: by Ynse Van de Maele, coordinator

Date of publication: 03/09/2024

All the global reports are also available on our webpage:

- NL: https://www.sciensano.be/nl/externe-kwaliteitsevaluatie/diergezondheid-pt-vet
- FR: https://www.sciensano.be/fr/evaluation-externe-de-la-qualite/sante-animale-pt-vet
- EN: https://www.sciensano.be/en/external-quality-assessment/animal-health-pt-vet

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1 INTRODUCTION

Details relevant to the proficiency test (PT) 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

The aim of this PT was to evaluate the ability of the participating laboratories to detect the absence or presence of enzootic bovine leukosis (EBL)-specific antibodies in serum of ruminants.

3 MATERIALS AND METHODS

3.1 Serology (serum)

3.1.1 THE PARTICIPANTS

Four laboratories participated in the proficiency test of enzootic bovine leukosis serology on serum samples. The laboratory numbers of the participating laboratories are:

- 97505
- 97507
- 97508
- 97516

3.1.2 THE SAMPLES

The National Reference Laboratory (NRL) of Sciensano, within the scientific service of 'Viral reemerging enzootic and bee diseases' in the department of 'Infectious diseases in animals Directorate', prepared the liquid sera gB samples. Participants were instructed to store the samples at 4°C until the analysis was carried out.

Information about the origin and preparation of the samples:

- Samples originate from historically infected animals from the field and from animals experimentally infected with BLV.

3.1.3 HOMOGENEITY

The homogeneity of the samples was tested by the NRL using three aliquots (250 µL each) of each sample, both before and after the PT, via ELISA. The NRL consistently obtained the same qualitative results, confirming the samples' homogeneity.

3.1.4 TARGET VALUES

The target values were determined by the NRL based on the homogeneity tests.

Sample content	Expected result
PT2024EBLSER_PS1	POS
PT2024EBLSER_PS2	POS
PT2024EBLSER_PS3	POS/NEG/NI*
PT2024EBLSER_NS1	POS
PT2024EBLSER_ NS2	POS

(POS = positive; NEG = negative; NI = not interpretable)

* = The positive sample PS3 represents a positive sample diluted to the limit of detection, implying that the result can be doubtful. Therefore, for this sample, POS. NEG or NI are accepted as correct results.

3.1.5 STABILITY

The stability of the samples was confirmed by comparing the pre-PT results with those obtained by the NRL during and after the PT. The samples were deemed 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: PT2024 EBLSER_	97505	97507	97508	97516
PS1 (1)	EBLSER24-4	EBLSER24-4	EBLSER24-3	EBLSER24-3
PS1 (2)	EBLSER24-5	EBLSER24-8	EBLSER24-5	EBLSER24-9
PS2 (1)	EBLSER24-2	EBLSER24-9	EBLSER24-6	EBLSER24-1
PS2 (2)	EBLSER24-8	EBLSER24-10	EBLSER24-9	EBLSER24-6
PS3 (1)	EBLSER24-7	EBLSER24-1	EBLSER24-1	EBLSER24-2
PS3 (2)	EBLSER24-9	EBLSER24-5	EBLSER24-7	EBLSER24-5
PS3 (3)	EBLSER24-10	EBLSER24-7	EBLSER24-8	EBLSER24-10
NS1 (1)	EBLSER24-3	EBLSER24-3	EBLSER24-2	EBLSER24-4
NS1 (2)	EBLSER24-6	EBLSER24-6	EBLSER24-4	EBLSER24-7
NS2	EBLSER24-1	EBLSER24-2	EBLSER24-10	EBLSER24-8

3.1.7 THRESHOLD FOR QUALIFICATION

Following the procedure, a participating laboratory is only qualified if the level of agreement for the ten reference samples is at least 90%.

4 TIMELINE

Transfer of the samples from NRL to QL: 17/06/2024
Randomisation of the samples by QL: 21/06/2024
Sending of samples to participants: 25/06/2024
Deadline for submitting the results: 19/07/2024
Individual report to the participants: 25/07/2024

5 RESULTS

5.1 Serology (serum)

5.1.1 RESULTS PER SAMPLE

The panel consisted of five different samples. However, samples PS1, PS2 and NS1 were replicated twice. In addition, sample PS3 was replicated three times. Therefore, the panel included ten samples in total.

Sample content	Expected results	Total results	Observed results
PS1	POS	8	8 POS
PS2	POS	8	8 POS
PS3	POS/NEG/NI	12	7 POS 5 NEG
NS1	NEG	8	8 NEG
NS2	NEG	4	4 NEG

(POS = positive; NEG = negative; NI = not interpretable)

5.1.2 RESULTS PER METHOD

Below, the table displays the results for each method.

Method	Name producer	Name kit	N	NR	NCR	%
ELISA Competition	IDEXX	Test IDEXX Leukosis Blocking Ab	**3**	**30**	**30**	**100**
ELISA Competition	ID.VET	ID Screen BLV Competition	**1**	**10**	**10**	**100**
TOTAL			**4**	**40**	**40**	**100**

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

5.1.3 CONCLUSION

In 2024, four laboratories participated in the proficiency test enzootic bovine leukosis serology (serum) organised by Sciensano. According to the procedure currently in force, the performance of a participating laboratory is satisfactory if at least 90% of the results provided by the 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 : 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 SEROLOGY (SERUM)

Sample PT2024EBLSER-PS1

Lab number	97505	97507	97508	97516
Method (ELISA protocol/kit)	M_1	M_1	M_2	M_1
Cut-off	40	40	50	40
	40	40	60	40
S/P % (1)	6.70	7.40	5.70	6.60
S/P % (2)	5.50	7.82	5.59	7.00
Mean	6.10	7.61	5.65	6.80
SD	0.85	0.30	0.07	0.28
CV (%)	13.91	3.90	1.32	4.16

S/P-values (%) were rounded to two significant decimal place. (S/P = Signal-to-Positive ratio; SD = standard deviation; CV = coefficient of variation; M_1 = IDEXX - Test IDEXX Leukosis Blocking Ab; M_2 = IDVet - ID Screen BLV Competition).

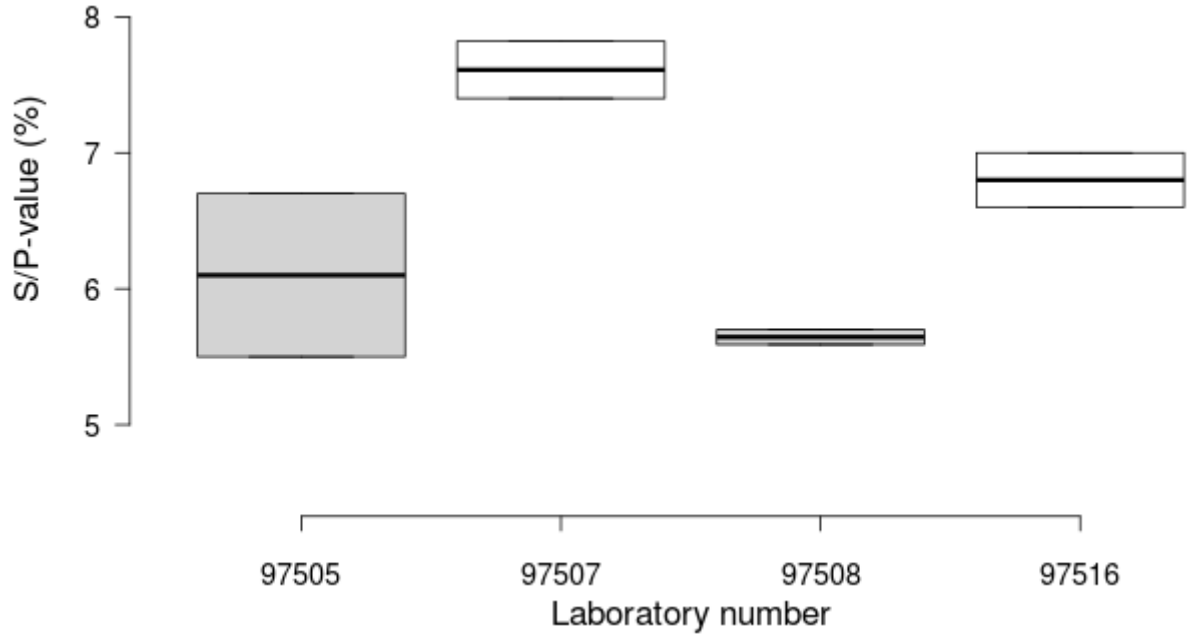


Figure 1. Distribution of the S/P-values (%) (box-plots) per laboratory.

Sample PT2024EBLSER-PS2

Lab number	97505	97507	97508	97516
Method (ELISA protocol/kit)	M_1	M_1	M_2	M_1
Cut-off	40	40	50	40
	40	40	60	40
S/P % (1)	5.73	10.20	6.44	8.10
S/P % (2)	5.24	10.63	12.67	7.40
Mean	5.49	10.42	9.55	7.75
SD	0.35	0.30	4.40	0.49
CV (%)	6.32	2.92	46.10	6.39

S/P-values (%) were rounded to two significant decimal place. (S/P = Signal-to-Positive ratio; SD = standard deviation; CV = coefficient of variation; M_1 = IDEXX - Test IDEXX Leukosis Blocking Ab; M_2 = IDVet - ID Screen BLV Competition).

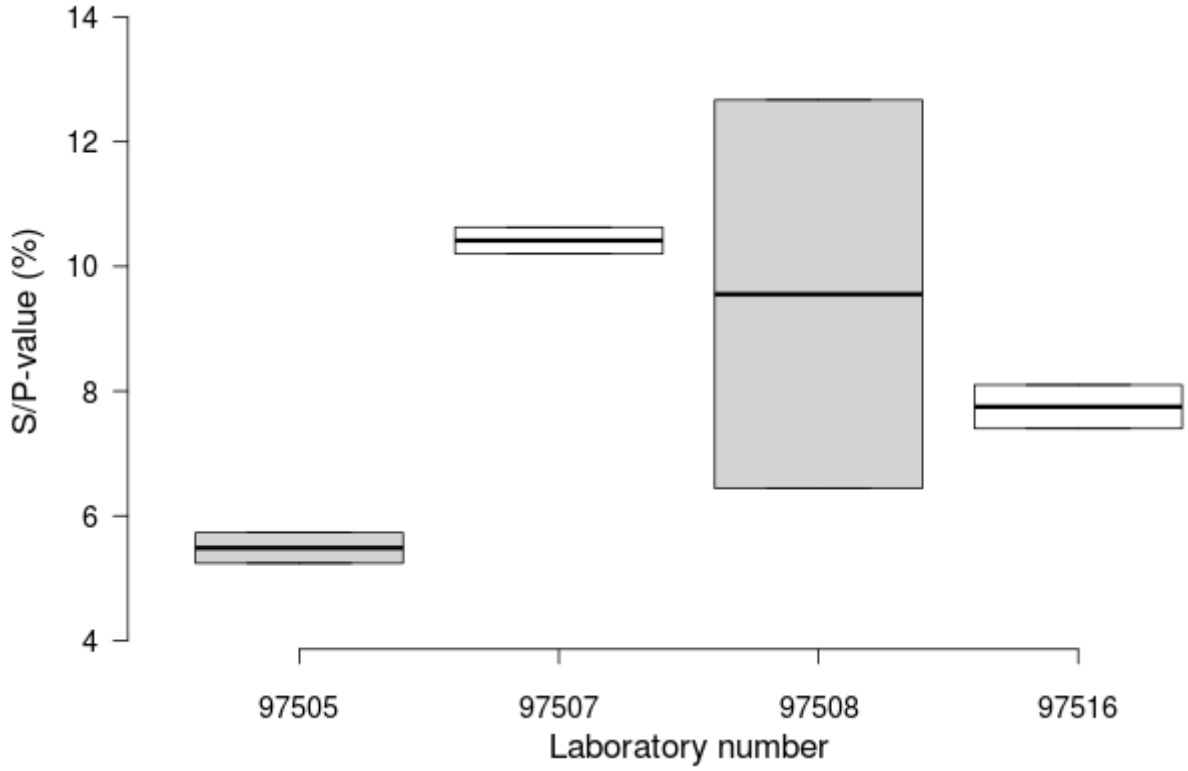


Figure 2. Distribution of the S/P-values (%) (box-plots) per laboratory.

Sample PT2024EBLSER-PS3

Lab number	97505	97507	97508	97516
Method (ELISA protocol/kit)	M_1	M_1	M_2	M_1
Cut-off	40	40	50	40
	40	40	60	40
S/P % (1)	37.77	42.26	31.14	44.20
S/P % (2)	37.00	43.88	28.92	38.10
S/P % (3)	36.77	40.90	29.87	40.20
Mean	37.18	42.35	29.97	40.83
SD	0.52	1.49	1.11	3.10
CV (%)	1.41	3.52	3.71	7.59

S/P-values (%) were rounded to two significant decimal place. (S/P = Signal-to-Positive ratio; SD = standard deviation; CV = coefficient of variation; M_1 = IDEXX - Test IDEXX Leukosis Blocking Ab; M_2 = IDVet - ID Screen BLV Competition).

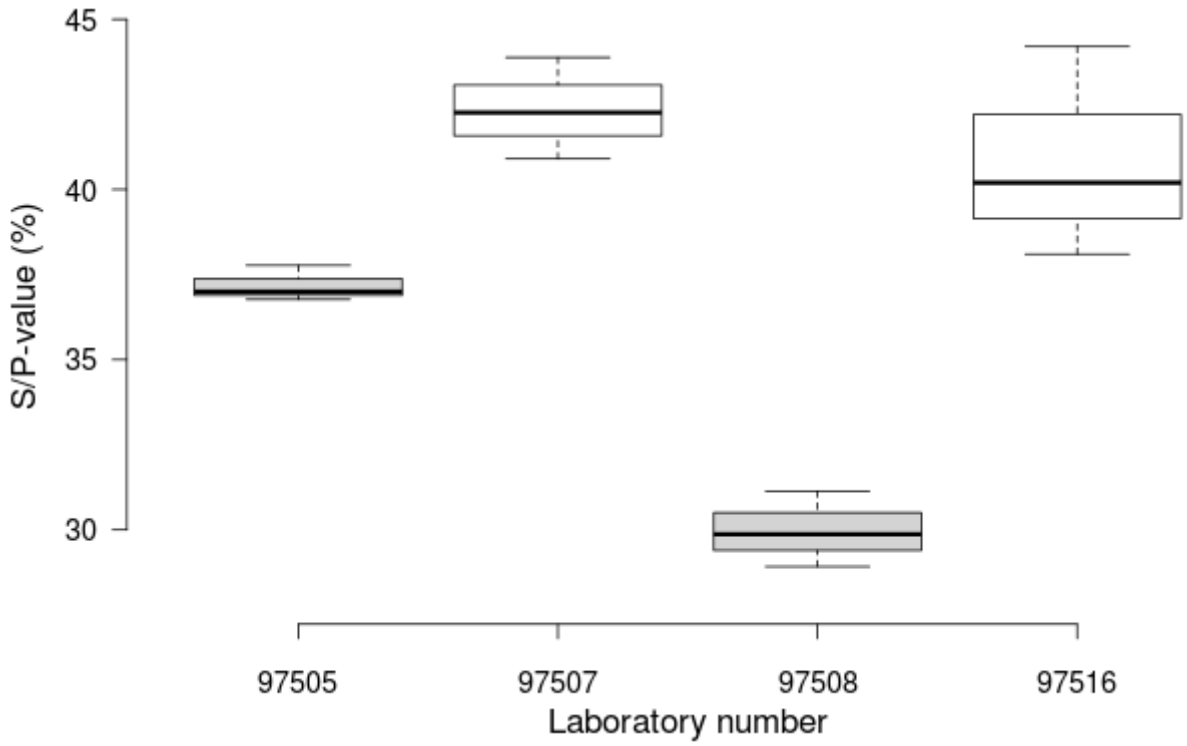


Figure 3. Distribution of the S/P-values (%) (box-plots) per laboratory.

6.2 Annex: Additional information

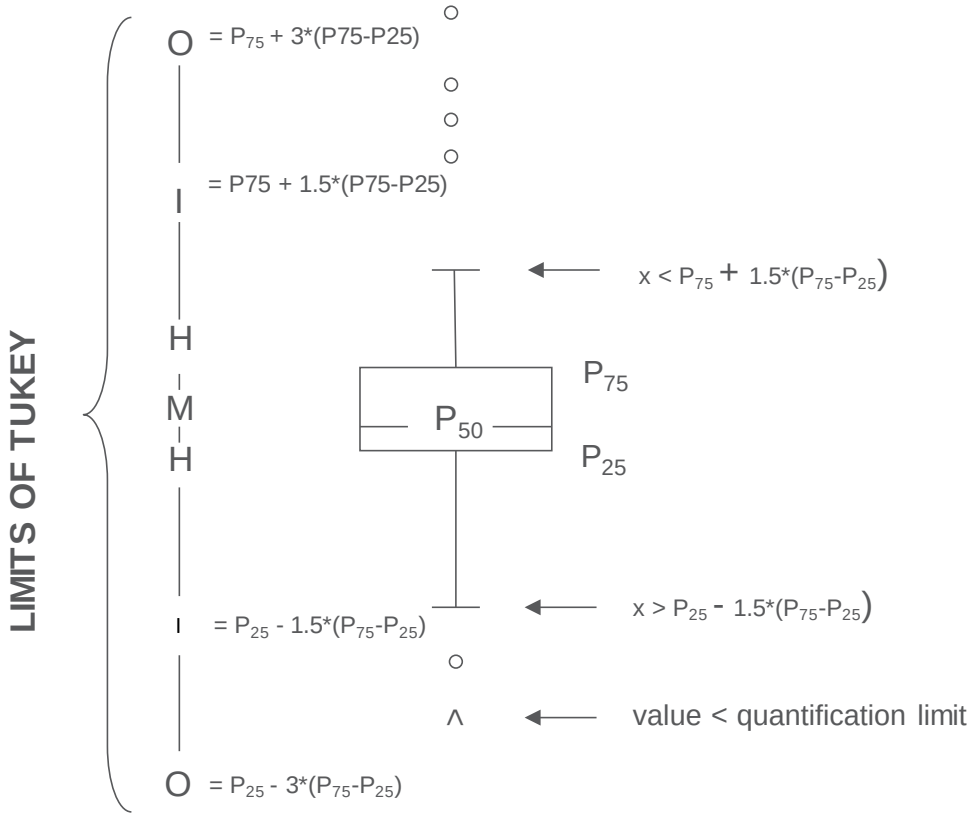
The **calendar** for Proficiency Testing in Veterinary diagnosis is available on our website:

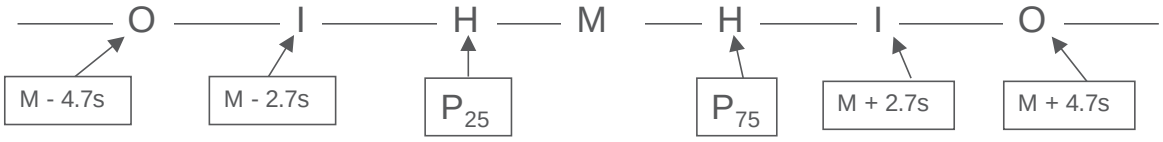
- NL: https://www.sciensano.be/nl/biblio/eke-kalender-2024
- FR: https://www.sciensano.be/fr/biblio/calendrier-eeq-2024
- EN: https://www.sciensano.be/nl/biblio/eqa-calendar-2024

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