BIOLOGICAL HEALTH RISKS
QUALITY OF LABORATORIES

PROFICIENCY TEST IN VETERINARY DIAGNOSIS

DEFINITIVE GLOBAL REPORT

PT-PROGRAM 2025-3

CAPRIPOX (CAPX)

EURL

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Biological health risks
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Juliette Wytsmanstreet 14
1050 Brussels Belgium

www.sciensano.be

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EUROPEAN 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		
Expert	**Institute**				
Wannes Philips	Sciensano – EURL Capripox				

A draft version of this report was submitted to the expert on 22/08/2025.

Responsibilities:
The European Reference Laboratory (EURL) 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 EURL.

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

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- NL: https://www.sciensano.be/nl/externe-kwaliteitsevaluatie/diergezondheid-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 identify the absence or presence of antibodies to capripox (CAPX) viruses in serum of ruminants (serology component of the PT: PT2025CAPXSER) and/or to assess the ability of the participating laboratories to detect CAPX virus DNA in different matrices (virology component of the PT: PT2025CAPXVIR).

3 MATERIALS AND METHODS

3.1 Performance of diagnostic tests

Within the serology component of the PT, participants were asked to test predefined serum samples using their primary diagnostic assay(s) for serological diagnosis. Within the virology component of the PT, participants were asked to test predefined blood and tissue homogenate samples using their primary diagnostic assay(s) for molecular diagnosis of capripox virus infection. Furthermore, within this component, participants could submit additional results on capripox virus species differentiation and field or vaccine strain differentiation. The procedures for the assays must be fully described in the SOPs of the participating laboratories.

All tests in the serology part and all parts in the virology part are individually scored. These scores are determined based on the interpretation given by the laboratory.

All participants received a supporting document detailing the species and type of each collected sample.

3.2 Reference samples

Thirty-five laboratories were provided with the PT2025CAPXSER panel, each comprising ten serum aliquots. Also, thirty-five laboratories were supplied with the PT2025CAPXVIR panel, consisting of ten aliquots each of blood and tissue suspension.

The samples were prepared by the European Union Reference Laboratory (EURL) for diseases caused by capripox viruses, Scientific Directorate Infectious Diseases in Animals, Sciensano. Afterwards, the PT panels were prepared separately and within each panel samples were randomised from 1 to 10, by the Scientific Service Quality of Laboratories, Sciensano.

3.2.1 PT2025CAPXSER PANEL: REFERENCE SERUM SAMPLES

3.2.1.1 Origin of the samples

Replicates of eight reference serum samples, either free from detectable antibodies to capripox viruses (n=2; coded PT2025CAPXSER_N1 and PT2025CAPXSER_N2) or containing detectable antibodies to capripox viruses (n=6; coded PT2025CAPXSER_P1, PT2025CAPXSER_P2, PT2025CAPXSER_P3, PT2025CAPXSERP4, PT2025CAPXSER_P5, PT2025CAPXSER_P6) were used. Samples PT2025CAPXSER_P1 and PT2025CAPXSER_P6 were repeated twice in the panel. Therefore, the panel included ten samples in total.

In total, 350 aliquots were distributed to 35 participating laboratories. The positions of the reference samples were randomised for each participant.

For each serum sample, the status was determined based on the background of the animals from which the samples originated and the results obtained during pre-verification, hereby using the ELISA ID Screen Capripox Double Antigen Multi-species (ID.Vet), the virus neutralisation test (VNT) with the serum titrated against a constant titer of capripox virus as well as the immunoperoxidase monolayer assay (IPMA) (Haegeman *et al.* 2020).

Table 1: Origin of the individual serum samples in the PTCAPX2025 panel (LSDV = lumpy skin disease virus; SPPV = sheeppox virus; POS = positive; NEG = negative).

Sample ID	Origin	Background	Status
PT2025CAPXSER_P1 (repeated twice)	Bovine	LSDV infected (2.5)	POS
PT2025CAPXSER_P2	Ovine	SPPV infected	POS
PT2025CAPXSER_P3	Ovine	SPPV infected	POS
PT2025CAPXSER_P4	Bovine	LSDV vaccinated	POS
PT2025CAPXSER_P5	Bovine	LSDV infected (1.2)	POS
PT2025CAPXSER_P6 (repeated twice)	Bovine	LSDV vaccinated and infected (2.5)	POS
PT2025CAPXSER_N1	Ovine	Commercial serum	NEG
PT2025CAPXSER_N2	Bovine	Commercial serum	NEG

After aliquoting the different reference serum samples, a homogeneity check was performed on ten aliquots of each sample using the ELISA ID Screen Capripox Double Antigen Multi-species (ID.Vet), VNT and IPMA. For the ELISA, the same qualitative result was obtained for all ten aliquots of the same reference serum sample.

All serum samples were considered as reliable samples to evaluate the ability of laboratories to identify the absence or presence of antibodies to capripox viruses in serum. In addition, three more aliquots of each serum sample were tested after the PT in order to confirm their stability and status (post PT verification) using the ELISA ID Screen Capripox Double Antigen Multi-species (ID.Vet), VNT and IPMA.

The reference serum samples PT2025CAPXSER_N1 and PT2025CAPXSER_N2 were considered as negative samples in all tests. The reference serum samples, PT2025CAPXSER_P1, PT2025CAPXSER_P2, PT2025CAPXSER_P3, PT2025CAPXSER_P4, PT2025CAPXSER_P5 and PT2025CAPXSER_P6 as positive samples in all tests.

3.2.1.2 Final sample status

The final status of each sample was determined by the EURL for diseases caused by capripox viruses, based on the pre-PT verification.

Table 2: The final status of each sample in the PT2025CAPXSER panel (POS = positive; NEG = negative).

Sample ID	ELISA	IPMA	VNT
PT2025CAPXSER_P1 (repeated twice)	POS	POS	POS
PT2025CAPXSER_P2	POS	POS	POS
PT2025CAPXSER_P3	POS	POS	POS
PT2025CAPXSER_P4	POS	POS	POS
PT2025CAPXSER_P5	POS	POS	POS
PT2025CAPXSER_P6 (repeated twice)	POS	POS	POS
PT2025CAPXSER_N1	NEG	NEG	NEG
PT2025CAPXSER_N2	NEG	NEG	NEG

3.2.1.3 Randomisation and panel composition

The samples were randomised uniquely for each laboratory, and the PTCAPXSER2025 panel comprised ten samples, each containing 500 µL.

3.2.1.4 Stability

The stability was evaluated based on the comparison of the results obtained by the EURL before (homogeneity testing) and after (post PT verification) the proficiency test. The results of the post PT testing were comparable to the results of the homogeneity testing, indicating that the samples remained stable during the period of the PT.

3.2.2 PT2025CAPXVIR PANEL: REFERENCE BLOOD & TISSUE HOMOGENATE SAMPLES

3.2.2.1 Origin of the samples

Replicates of six reference samples were tissue homogenate samples. These included samples containing detectable capripox virus DNA (n=5; coded PT2025CAPX_TP1, PT2025CAPX_TP2, PT2025CAPX_TP3, PT2025CAPX_TP4, and PT2025CAPX_TP5) and one sample free from detectable capripox virus DNA (n=1; coded PT2025CAPX_TN1). However, sample PT2024CAPX_TP1 was repeated twice, resulting in a total of seven tissue samples.

The remaining three reference samples were blood samples, either containing detectable capripox virus DNA (n=2; coded PT2025CAPX_BP1 and PT2025CAPX_BP2) or free from detectable capripox virus DNA (n=1; coded PT2025CAPX_BN1).

In total, 350 aliquots were distributed to 35 participating laboratories. Each participant received ten aliquots.

For each sample, the status was determined based on the background of the sample and the results obtained during pre-verification, hereby using the real-time PCR for Capripox D5R (Haegeman *et al.* 2013) and DIVA tests (Agianniotaki *et al.* 2016; Haegeman *et al.* 2016; Chibbsa *et al.* 2018 and Haegeman *et al*. 2023).

Table 3: Origin of the samples in the PTCAPX2025 panel. (LSDV = lumpy skin disease virus; SPPV = sheeppox virus)

Sample ID	Origin	Background	Status
PT2025CAPX_TP1 (repeated twice)	Cattle tissue	LSDV recombinant spiked tissue	Capx positive LSDV recombinant (2.5) strain
PT2025CAPX_TP2	Sheep tissue	SPPV wild tissue	Capx positive SPPV wild strain
PT2025CAPX_TP3	Sheep tissue	SPPV Bakirkoy spiked tissue	Capx positive SPPV wild strain
PT2025CAPX_TP4	Cattle tissue	LSDV vaccine tissue	Capx positive LSDV vaccine (1.1) strain
PT2025CAPX_TP5	Cattle tissue	LSDV wild tissue	Capx positive LSDV wild (1.2) strain
PT2025CAPX_BP1	Cattle blood	LSDV recombinant spiked blood	Capx positive LSDV recombinant (2.5) strain
PT2025CAPX_BP2	Sheep blood	SPPV vaccine spiked blood	Capx positive SPPV vaccine strain
PT2025CAPX_TN1	Goat tissue	Negative tissue goat	Capx tissue negative
PT2025CAPX_BN1	Cattle blood	Negative blood cattle	Capx blood negative

After aliquoting the different samples, a homogeneity check was performed on ten aliquots of each sample. The homogeneity check was performed using the real-time PCR for capripox D5R (Haegeman *et al.* 2013) and DIVA tests (Agianniotaki *et al.* 2016; Haegeman *et al.* 2016; Chibssa *et al.* 2018 and Haegeman *et al.* 2023). For each sample, the same qualitative result was obtained for all ten aliquots. Consequently, all samples were considered as reliable samples in order to evaluate the ability of laboratories to identify the absence or presence of capripox virus DNA.

Moreover, three additional aliquots of each reference sample were tested once the PT deadline had passed using the real-time PCR for capripox D5R (Haegeman *et al.*, 2013) and DIVA tests (Agianniotaki *et al.* 2016; Haegeman *et al.* 2016; Chibssa et al. 2018 and Haegeman *et al.* 2023) in order to confirm the stability and status of the samples (post -PT verification). During stability testing, there were no differences detected for all the samples.

For the **detection of capripox virus DNA (primary PCR),** the samples PT2025CAPXVIR_TN1 and PT2025CAPXVIR_BN1 were considered as capripox negative samples. The samples PT2025CAPXVIR_TP1, PT2025CAPXVIR_TP2, PT2025CAPXVIR_TP3, PT2025CAPXVIR_TP4, PT2025CAPXVIR_TP5, PT2025CAPXVIR_BP1 and PT2025CAPXVIR_BP2 were identified as positive samples.

For the **capripox virus species differentiation**, the samples PT2025CAPXVIR_TN1 and PT2025CAPXVIR_BN1 were considered as capripox negative samples. The samples PT2025CAPXVIR_TP1, PT2025CAPXVIR_TP4, PT2025CAPXVIR_TP5 and PT2025CAPXVIR_BP1 were classified as LSDV positive samples (where LSDV was considered acceptable). The samples PT2025CAPXVIR_TP2, PT2025CAPXVIR_TP3 and PT2025CAPXVIR_BP2 were identified as SPPV positive sample.

Finally, for the **field or vaccine strain differentiation**, the samples PT2025CAPXVIR_TN1 and PT2025CAPXVIR_BN1 were considered as capripox negative samples. The sample PT2025CAPXVIR_TP5 was considered as LSDV wild (1.2) strain, while sample PT2025CAPX_TP1 and PT2025CAPX_BP1 was considered as LSDV recombinant (2.5) strain and PT2025CAPXVIR_TP4 was considered as LSDV vaccine (1.1) strain. Sample PT2025CAPXVIR_TP2 and PT2025CAPXVIR_TP3 were considered as SPPV wild strain. In addition, sample PT2025CAPXVIR_BP2 was classified as SPPV vaccine strain.

3.2.2.2 Final sample status

The final status of each sample was determined by the EURL for diseases caused by capripox viruses, based on the pre-PT verification.

Table 4: The final status of each sample in the PT2025CAPXVIR panel (LSDV = lumpy skin disease virus; SPPV = sheeppox virus; NEG = negative).

Sample ID	Primary PCR	Species differentiation	DIVA
PT2025CAPX_TP1 (repeated twice)	POS	LSDV	LSDV recombinant (2.5) strain
PT2025CAPX_TP2	POS	SPPV	SPPV wild strain
PT2025CAPX_TP3	POS	SPPV	SPPV wild strain
PT2025CAPX_TP4	POS	LSDV	LSDV vaccine (1.1) strain
PT2025CAPX_TP5	POS	LSDV	LSDV wild (1.2) strain
PT2025CAPX_BP1	POS	LSDV	LSDV recombinant (2.5) strain
PT2025CAPX_BP2	POS	SPPV	SPPV vaccine strain
PT2025CAPX_TN1	NEG	NEG	NEG
PT2025CAPX_BN1	NEG	NEG	NEG

3.2.2.3 Randomisation and panel composition

The samples were randomised uniquely for each laboratory, and the PTCAPXVIR2025 panel comprised ten samples, each containing 600 µL.

3.2.2.4 Stability

The stability was evaluated based on the comparison of the results of the EURL before (homogeneity testing) and after (post PT verification) the proficiency test. The results of the stability testing were comparable to the results of the homogeneity testing, indicating that the samples remained stable during the period of the PT.

3.3 Classification of results, level of agreement and threshold for qualification

3.3.1 CLASSIFICATION OF RESULTS

Results provided by the participating laboratories are categorised as correct when the reported result matches with the assigned status or failure when the reported result does not match with the assigned status.

3.3.2 LEVEL OF AGREEMENT

The level of agreement achieved by the participating laboratories is expressed as the percentage of correctness for each of the tested aliquots of reference samples used for this PT.

3.3.3 THRESHOLD FOR QUALIFICATION

Following the procedure, a participating laboratory is only qualified if the level of agreement for the tested aliquots of reference samples for each panel is at least 90% The threshold for qualification will be determined separately for each test. Therefore, the participants will achieve a satisfactory or unsatisfactory result for each test.

Please note that, for the DIVA part, if an LSDV recombinant field strain was present, it was expected to be correctly classified as LSDV rec. Any other answer was considered a failure.

4 THE PARTICIPANTS

Twenty-five National Reference Laboratories (NRLs) from European Union member states and eleven NRLs from non-EU member states took part in the capripox virus proficiency test.

Table 5: NRL's for Capripox virus from EU member states.

Country	Name of the laboratory	Participation PT serology	Participation PT virology
Austria	Austrian Agency for Health and Food Safety (NRL for CaPV)	1	1
Belgium	Sciensano; Scientific service 'Exotic viruses and particular diseases'	1	1
Bulgaria	National Diagnostic and Research Veterinary Medical Institute	1	1
Croatia	Croatian Veterinary Institute	1	1
Cyprus	Laboratory for animal health; virology section	0	1
Czech Republic	State Veterinary Institute Prague	1	1
Denmark	Statens Serum Institut; Department Veterinary Virology, VMS	1	1
Finland	Finnish Food Authority, Virology Unit	1	1
France	LNR poxviroses des ruminants, UMR Cirad-Inra ASTRE, "Anima, santé, Territoires, Risques et Ecosystèmes"	1	1
Germany	Friedrich-Loeffler-Institut	1	1
Greece	Dep. Mol. Diagnosis, F.M.D., Virol. Rik. & Exotic Diseases	1	1
Hungary	National Food Chain Safety; Department Veterinary Diagnostic Directorate, Laboratory for Molecular Biology	1	1
Ireland	Virology Division - CVR Laboratory; Department of Agriculture	1	1
Italy	Istituto Zooprofilattico Sperimentale dell'Abruzzo e del Molise -Centro di Referenza Nazionale per lo studio e l'accertamento delle malattie esotiche degli animali (CESME)	1	1
Latvia	Institute for Food Safety, Animal Health and Environment "BIOR", Animal Disease Diagnostic Laboratory	1	1

Lithuania	National Food and Veterinary Risk Assessment Institute (NFVRAI); Department molecular Biology and Genetically Modified organisms	1	1
Malta	Veterinary and Phytosanitary Regulation; Department National Veterinary Laboratory	1	0
Poland	National Veterinary Research Institute; Department of Virology	1	1
Portugal	Instituto Nacional de Investigaçao Agraria e Veterinaria (INIAV), Laboratório Nacional de Referência para a Saude animal	1	1
Romania	Institute for diagnosis and animal health	1	1
Slovakia	State veterinary and food institute, Veterinary institute in Zvolen	1	1
Slovenia	University of Ljubljana, Veterinary faculty/National Veterinary Institute, Institute of Microbiology and Parasitology, Department of Virology	1	1
Spain	Laboratorio Central De Veterinaria (LCV) (ALGETE) M.A.P.A.	1	1
Sweden	National Veterinary Institute (SVA)	1	1
The Netherlands	Wageningen Bioveterinary Research	1	1

Table 6: NRL's for Capripox virus from non-EU member states.

Country	Name of the laboratory	Participation PT serology	Participation PT virology
Albania	Food Safety and Veterinary Insitute; Department of Animal Health, Molecular Biology	1	1
Georgia	LEPL State Laboratory of agriculture (SLA) of Georgia	1	1
Kazakhstan	National Veterinary Reference Centre Astana	1	1
Kazakhstan	National Veterinary Reference Centre Almaty	1	1
Kosovo	Kosovo Food And Veterinary Laboratory, Kosovo Food And Veterinary Agency	1	1
Montenegro	Diagnostic Veterinary Laboratory	1	1

Republic of North Macedonia	Faculty of Veterinary Medicine Skopje, Laboratory for serology and molecular diagnostics	1	1
Republic of Moldova	Republican Veterinary Diagnostic Center	1	1
Serbia	Veterinary Specialized Institute Kraljevo	1	1
Turkey	Istanbul Pendik Veterinary Control Institute, Capripoxvirus National Laboratory	1	1
Ukraine	State Scientific Research Institute of Laboratory Diagnostics and Veterinary and Sanitary Expertise (SSRILDVSE)	1	1

5 PT TIMELINE

Transfer of the samples from EURL to QL: April 28, 2025
Randomization of the samples by QL: May 7, 2025 (serology) & May 9, 2025 (virology)
Sending samples (frozen at - 20 °C) to participants: May 12, 2025
Deadline for submitting results: June 27, 2025
Individual report: August 18, 2025

6 COMPLIANCE WITH THE PROCEDURE

All participating laboratories provided a duly dated copy of their results.

7 RESULTS – QUALITATIVE DATA ANALYSIS

7.1 Serology

The PT2025CAPXSER panel was composed of eight positive samples and two negative samples. The samples PT2025CAPSER_P1 and PT2025CAPSER_P6 were repeated twice in the panel.

7.1.1 ANTIBODY ELISA

7.1.1.1 Results per sample

In the table below, the results are presented per sample. A misinterpretation was reported only for sample P2, resulting in an overall correctness of 97% for that sample. All other samples were interpreted correctly. The raw data are provided in Annex 1.

Table 7: Results per sample (REP = repetition; POS = positive; NEG = negative; NI = non-interpretable; NA= not analysed).

Sample ID PT2025CAPXSER	REP	Expected results	# of POS	# of NEG	# of NI/NA	%
P1	Yes	POS	70	0	0	100
P2	No	POS	34	1	0	97
P3	No	POS	35	0	0	100
P4	No	POS	35	0	0	100
P5	No	POS	35	0	0	100
P6	Yes	POS	70	0	0	100
N1	No	NEG	0	35	0	100
N2	No	NEG	0	35	0	100

7.1.1.2 Results per method

In the table below, the results are presented per method. Among the participants, two different methods were applied. Only one false negative result was reported, while all other results were correctly interpreted. The raw data are provided in Annex 1.

Table 8: Results per method (N = number of laboratories; NR = number of results; NCR = number of correct results; FN = false negative; FP = false positive).

Kit or reference	N	NR	NCR	FN	FP
ID Screen Capripox Double Antigen Multi-species	33	330	329	1	0
BioStone Animal Health. AsurDx Capripox Antibody Test Kit Manual	2	20	20	0	0
TOTAL		**350**	**349**	**1**	**0**

7.1.1.3 Results per laboratory

In total, 35 laboratories participated and submitted their results. According to the procedure, a participating laboratory is qualified if the level of agreement is equal or more than 90%.

In total, 34 laboratories reached a 100% agreement, implying that the submitted qualitative results were in full agreement with the assigned status of the reference samples. One laboratory (97619) misinterpreted one sample resulting in a score of 90%. However this laboratory still met the minimum level of agreement and therefore achieved a satisfactory result. Raw data can be found in Annex 1.

Table 9: Results of the ELISA per participating laboratory. Correct answers are marked in green, while incorrect answers are indicated in red. In addition, non-interpretable answers are shown in orange.

Lab number	P1 (1)	P1 (2)	P2	P3	P4	P5	P6 (1)	P6 (2)	N1	N2	%
	POS								NEG		
97506	POS	POS	POS	POS	POS	POS	POS	POS	NEG	NEG	100
97600	POS	POS	POS	POS	POS	POS	POS	POS	NEG	NEG	100
97601	POS	POS	POS	POS	POS	POS	POS	POS	NEG	NEG	100
97602	POS	POS	POS	POS	POS	POS	POS	POS	NEG	NEG	100
97604	POS	POS	POS	POS	POS	POS	POS	POS	NEG	NEG	100
97605	POS	POS	POS	POS	POS	POS	POS	POS	NEG	NEG	100
97606	POS	POS	POS	POS	POS	POS	POS	POS	NEG	NEG	100
97607	POS	POS	POS	POS	POS	POS	POS	POS	NEG	NEG	100
97608	POS	POS	POS	POS	POS	POS	POS	POS	NEG	NEG	100
97609	POS	POS	POS	POS	POS	POS	POS	POS	NEG	NEG	100
97510	POS	POS	POS	POS	POS	POS	POS	POS	NEG	NEG	100
97611	POS	POS	POS	POS	POS	POS	POS	POS	NEG	NEG	100
97612	POS	POS	POS	POS	POS	POS	POS	POS	NEG	NEG	100
97613	POS	POS	POS	POS	POS	POS	POS	POS	NEG	NEG	100
97614	POS	POS	POS	POS	POS	POS	POS	POS	NEG	NEG	100
97615	POS	POS	POS	POS	POS	POS	POS	POS	NEG	NEG	100
97616	POS	POS	POS	POS	POS	POS	POS	POS	NEG	NEG	100
97617	POS	POS	POS	POS	POS	POS	POS	POS	NEG	NEG	100
97618	POS	POS	POS	POS	POS	POS	POS	POS	NEG	NEG	100
97619	POS	POS	NEG	POS	POS	POS	POS	POS	NEG	NEG	90
97620	POS	POS	POS	POS	POS	POS	POS	POS	NEG	NEG	100
97621	POS	POS	POS	POS	POS	POS	POS	POS	NEG	NEG	100
97622	POS	POS	POS	POS	POS	POS	POS	POS	NEG	NEG	100
97623	POS	POS	POS	POS	POS	POS	POS	POS	NEG	NEG	100
97624	POS	POS	POS	POS	POS	POS	POS	POS	NEG	NEG	100
97627	POS	POS	POS	POS	POS	POS	POS	POS	NEG	NEG	100

Lab number	P1 (1)	P1 (2)	P2	P3	P4	P5	P6 (1)	P6 (2)	N1	N2	%
	POS								NEG		
97628	POS	POS	POS	POS	POS	POS	POS	POS	NEG	NEG	**100**
97629	POS	POS	POS	POS	POS	POS	POS	POS	NEG	NEG	**100**
97630	POS	POS	POS	POS	POS	POS	POS	POS	NEG	NEG	**100**
97631	POS	POS	POS	POS	POS	POS	POS	POS	NEG	NEG	**100**
97632	POS	POS	POS	POS	POS	POS	POS	POS	NEG	NEG	**100**
97634	POS	POS	POS	POS	POS	POS	POS	POS	NEG	NEG	**100**
97636	POS	POS	POS	POS	POS	POS	POS	POS	NEG	NEG	**100**
97637	POS	POS	POS	POS	POS	POS	POS	POS	NEG	NEG	**100**
97638	POS	POS	POS	POS	POS	POS	POS	POS	NEG	NEG	**100**

7.1.2 VIRUS NEUTRALISATION (VN)

7.1.2.1 Results per sample

In the table below, the results are presented per sample. All samples were interpreted correctly. The raw data are provided in Annex 1.

Table 10: Results per sample (REP = repetition; POS = positive; NEG = negative; NI = non-interpretable; NA= not analysed).

Sample ID PT2025CAPXSER	REP	Expected results	# of POS	# of NEG	# of NI/NA	%
P1	Yes	POS	6	0	0	100
P2	No	POS	3	0	0	100
P3	No	POS	3	0	0	100
P4	No	POS	3	0	0	100
P5	No	POS	3	0	0	100
P6	Yes	POS	6	0	0	100
N1	No	NEG	0	3	0	100
N2	No	NEG	0	3	0	100

7.1.2.2 Results per method

In the table below, the results are presented per method. Among the participants, three different methods were applied. All samples were interpreted correctly. The raw data can be found in Annex 1.

Table 11: Results per method (N = number of laboratories; NR = number of results; NCR = number of correct results; FN = false negative; FP = false positive).

Protocol/SOP	N	NR	NCR	FN	FP
Homemade protocol (Sciensano)	1	10	10	0	0
WOAH terrestrial manual chapter 3.4.12 LSD (2021)	1	10	10	0	0
Homemade protocol (IZS)	1	10	10	0	0
TOTAL		**30**	**30**	**0**	**0**

7.1.2.3 Results per laboratory

Three laboratories submitted results for the VN (dilution antibody) tests. According to the procedure, a participating laboratory is qualified if the level of agreement is equal or more than 90%.

All laboratories provided qualitative results that were in full agreement with the assigned status of the reference serum samples and hence reached 100% agreement. Raw data can be found in Annex 1.

Table 12: Results of the VN-test per participating laboratory. Correct answers are marked in green, while incorrect answers are indicated in red. In addition, non-interpretable answers are shown in orange.

Lab number	P1 (1)	P1 (2)	P2	P3	P4	P5	P6 (1)	P6 (2)	N1	N2	%
	POS								NEG		
97506	POS	POS	POS	POS	POS	POS	POS	POS	NEG	NEG	**100**
97600	POS	POS	POS	POS	POS	POS	POS	POS	NEG	NEG	**100**
97612	POS	POS	POS	POS	POS	POS	POS	POS	NEG	NEG	**100**

7.1.3 IMMUNOPEROXYDASE MONOLAYER ASSAY (IPMA)

7.1.3.1 Results per sample

In the table below, the results are presented per sample. All samples were interpreted correctly. The raw data are provided in Annex 1.

Table 13: Results per sample (REP = repetition; POS = positive; NEG = negative; NI = non-interpretable; NA= not analysed).

Sample ID PT2025CAPXSER	REP	Expected results	# of POS	# of NEG	# of NI/NA	%
P1	Yes	POS	2	0	0	100
P2	No	POS	1	0	0	100
P3	No	POS	1	0	0	100
P4	No	POS	1	0	0	100
P5	No	POS	1	0	0	100
P6	Yes	POS	2	0	0	100
N1	No	NEG	0	1	0	100
N2	No	NEG	0	1	0	100

7.1.3.2 Results per method

In the table below, the results are presented per method. All samples were interpreted correctly. The raw data are provided in Annex 1.

Table 14: Results per method (N = number of laboratories; NR = number of results; NCR = number of correct results; FN = false negative; FP = false positive).

Kit or reference	N	NR	NCR	FN	FP
Haegeman *et al.* 2020	1	10	10	0	0
TOTAL		**10**	**10**	**0**	**0**

7.1.3.3 Results per laboratory

Only one laboratory submitted results for the immunoperoxydase monolayer assay (IPMA). According to the procedure, a participating laboratory is qualified if the level of agreement is equal or more than 90%.

This laboratory provided qualitative results that were in full agreement with the assigned status of the reference serum samples and hence reached 100% agreement. Raw data can be found in Annex 1.

Table 15: Results of the IPMA per participating laboratory. Correct answers are marked in green, while incorrect answers are indicated in red. In addition, non-interpretable answers are shown in orange.

Lab number	P1 (1)	P1 (2)	P2	P3	P4	P5	P6 (1)	P6 (2)	N1	N2	%
	POS								NEG		
97506	POS	POS	POS	POS	POS	POS	POS	POS	NEG	NEG	100

7.2 Virology

The PT2025CAPXVIR panel was composed of eight positive samples and two negative samples. The sample PT2025CAPVIR_TP1 was repeated twice in the panel.

7.2.1 PRIMARY PCR

7.2.1.1 Results per sample

In total, 35 laboratories participated in the primary PCR virology part of the proficiency test. As 31 laboratories submitted one dataset and four laboratories submitted two datasets, the total number of datasets registered is thus 39. Raw data can be found in Annex 1.

Table 16: Results per sample (REP = repetition; POS = positive; NEG = negative; NI = non-interpretable).

Sample ID PT2025CAPXVIR	REP	Expected results	# of POS	# of NEG	# of NI	Status*
TP1	Yes	POS	78	0	0	Frequently detected
TP2	No	POS	39	0	0	Frequently detected
TP3	No	POS	39	0	0	Frequently detected
TP4	No	POS	39	0	0	Frequently detected
TP5	No	POS	39	0	0	Frequently detected
BP1	No	POS	39	0	0	Frequently detected
BP2	No	POS	39	0	0	Frequently detected
TN1	No	NEG	0	39	0	NEG
BN1	No	NEG	0	39	0	NEG

**: for positive sample a frequently detected sample is detected by more than 95% of the participants, a detected sample is detected by more than 65% of the participants and a infrequently detected sample is detected by less than 65% of the participants (https://www.qcmd.org/).*

7.2.1.2 Results per method

In the table below, results are listed by PCR method. The target gene, if known, is also mentioned.

Table 17: Results per method (N = number of laboratories; NR = number of results; NCR = number of correct results; FN = false negative; FP = false positive).

Kit or reference	Target gene	N	NR	NCR	FN	FP
Bowden *et al*. (2008)	P32	20	200	200	0	0
Haegeman *et al*. (2013)	D5R/E3L	3	30	30	0	0
ID.VET - ID GENE® CAPRIPOX VIRUS TRIPLEX	/	7	70	70	0	0
Qiagen - MO-TO 45	P32	1	10	10	0	0
Qiagen - MO-TE 45	P32	1	10	10	0	0
Haegeman *et al*. (2013) & Bowden *et al*. (2008)	D5R/E3L/P32	1	10	10	0	0
Lamien *et al.* 2010	/	1	10	10	0	0
Bowden *et al.* (2008) & Babiuk *et al.* (2008)	P32	1	10	10	0	0
Capripox-POL	/	1	10	10	0	0
Stubbs *et al.* (2012)	/	1	10	10	0	0
In house	/	2	20	20	0	0
TOTAL		**39**	**390**	**390**	**0**	**0**

7.2.1.3 Results per laboratory

For the detection of capripox virus DNA in the PT panel: all laboratories provided results that were in full agreement with the assigned status of the ten reference samples (100% agreement). Four laboratories (LAB97602, LAB97605, LAB97608 and LAB97609) performed a secondary PCR and provided results that were in full agreement with the assigned status of the reference samples.

Table 18: Results per laboratory. Correct answers are marked in green, while incorrect answers are indicated in red. In addition, non-interpretable or not analysed answers are shown in orange. Orange answers will not be evaluated. (POS = positive; NEG = negative; NI = non-interpretable; NA = not analysed)

Lab number	TP1 (1)	TP1 (2)	TP2	TP3	TP4	TP5	BP1	BP2	TN1	BN1	%
	POS								NEG		
97506	POS	POS	POS	POS	POS	POS	POS	POS	NEG	NEG	**100**
97600	POS	POS	POS	POS	POS	POS	POS	POS	NEG	NEG	**100**
97601	POS	POS	POS	POS	POS	POS	POS	POS	NEG	NEG	**100**
97602 (1)	POS	POS	POS	POS	POS	POS	POS	POS	NEG	NEG	**100**
97602 (2)	POS	POS	POS	POS	POS	POS	POS	POS	NEG	NEG	**100**
97603	POS	POS	POS	POS	POS	POS	POS	POS	NEG	NEG	**100**

Lab number	TP1 (1)	TP1 (2)	TP2	TP3	TP4	TP5	BP1	BP2	TN1	BN1	%
	POS								NEG		
97604	POS	POS	POS	POS	POS	POS	POS	POS	POS	NEG	100
97605 (1)	POS	POS	POS	POS	POS	POS	POS	POS	POS	NEG	100
97605 (2)	POS	POS	POS	POS	POS	POS	POS	POS	POS	NEG	100
97606	POS	POS	POS	POS	POS	POS	POS	POS	NEG	NEG	100
97607	POS	POS	POS	POS	POS	POS	POS	POS	POS	NEG	100
97608 (1)	POS	POS	POS	POS	POS	POS	POS	POS	NEG	NEG	100
97608 (2)	POS	POS	POS	POS	POS	POS	POS	POS	NEG	NEG	100
97609 (1)	POS	POS	POS	POS	POS	POS	POS	POS	NEG	NEG	100
97609 (2)	POS	POS	POS	POS	POS	POS	POS	POS	NEG	NEG	100
97510	POS	POS	POS	POS	POS	POS	POS	POS	NEG	NEG	100
97611	POS	POS	POS	POS	POS	POS	POS	POS	POS	NEG	100
97612	POS	POS	POS	POS	POS	POS	POS	POS	NEG	NEG	100
97613	POS	POS	POS	POS	POS	POS	POS	POS	POS	NEG	100
97614	POS	POS	POS	POS	POS	POS	POS	POS	NEG	NEG	100
97616	POS	POS	POS	POS	POS	POS	POS	POS	NEG	NEG	100
97617	POS	POS	POS	POS	POS	POS	POS	POS	NEG	NEG	100
97618	POS	POS	POS	POS	POS	POS	POS	POS	NEG	NEG	100
97619	POS	POS	POS	POS	POS	POS	POS	POS	NEG	NEG	100
97620	POS	POS	POS	POS	POS	POS	POS	POS	POS	NEG	100
97621	POS	POS	POS	POS	POS	POS	POS	POS	POS	NEG	100
97622	POS	POS	POS	POS	POS	POS	POS	POS	NEG	NEG	100
97623	POS	POS	POS	POS	POS	POS	POS	POS	NEG	NEG	100
97624	POS	POS	POS	POS	POS	POS	POS	POS	NEG	NEG	100
97627	POS	POS	POS	POS	POS	POS	POS	POS	POS	NEG	100
97628	POS	POS	POS	POS	POS	POS	POS	POS	POS	NEG	100
97629	POS	POS	POS	POS	POS	POS	POS	POS	POS	NEG	100
97630	POS	POS	POS	POS	POS	POS	POS	POS	POS	NEG	100
97631	POS	POS	POS	POS	POS	POS	POS	POS	POS	NEG	100
97632	POS	POS	POS	POS	POS	POS	POS	POS	POS	NEG	100
97634	POS	POS	POS	POS	POS	POS	POS	POS	NEG	NEG	100

Lab number	TP1 (1)	TP1 (2)	TP2	TP3	TP4	TP5	BP1	BP2	TN1	BN1	%
	POS								NEG		
97636	POS	POS	POS	POS	POS	POS	POS	POS	NEG	NEG	**100**
97637	POS	POS	POS	POS	POS	POS	POS	POS	NEG	NEG	**100**
97638	POS	POS	POS	POS	POS	POS	POS	POS	NEG	NEG	**100**

7.2.2 SPECIES DIFFERENTIATION

7.2.2.1 Results per sample

A total of 25 laboratories participated in the Species Differentiation virology component of the proficiency test. Eighteen laboratories each submitted one dataset, five laboratories submitted two datasets, and two laboratories submitted three datasets. Although seven laboratories provided multiple datasets, only two or three distinct kit types were used: one for detecting LSDV samples and another for SPPV samples. As a result, the table below presents these combined results, maintaining the total number of datasets at 25. Raw data can be found in Annex 1.

Table 19: Results per sample (REP = repetition; LSDV = lumpy skin disease virus; SPPV = sheeppox virus; NEG = negative; NI = non-interpretable; NA= not analysed).

Sample ID PT2025CAPXVIR	REP	Expected results	# of LSDV	# of SPPV	# of SPPV/GTPV	# of NEG	# of NI/NA	%
TP1	Yes	LSDV	48	0	0	0	2	96
TP2	No	SPPV	0	22	2	0	1	88
TP3	No	SPPV	0	22	2	0	1	88
TP4	No	LSDV	25	0	0	0	0	100
TP5	No	LSDV	25	0	0	0	0	100
BP1	No	LSDV	24	0	0	0	1	96
BP2	No	SPPV	0	22	2	0	1	88
TN1	No	NEG	0	0	0	25	0	100
BN1	No	NEG	0	0	0	25	0	100

7.2.2.2 Results per method

In the table below, results are listed by method. The target gene, if known, is also mentioned.

Table 20: Results per method (N = number of laboratories; NR = number of results; NCR = number of correct results; F = false results; NI = non-interpretable; NA = not analysed).

Kit or reference	N	NR	NCR	F	NI/NA
Lamien *et al.* (2011)	9	90	90	0	0
Lamien *et al.* (2011) & Chibssa *et al.* (2018)	1	10	10	0	0
Agiannioaki *et al*. (2016) & Chibssa *et al.* (2018) & Wolff *et al*. (2021) & Haegeman *et al*. (2016)	1	10	10	0	0
Qiagen MO-TO 47	2	20	20	0	0
Wolff *et al*. (2021) (Both duplexes) & in house	1	10	10	0	0
Wolff *et al*. (2021) (Both duplexes)	3	30	27	0	3
Galaye *et al.* (2017) & Wolff *et al.* (2021)	1	10	10	0	0
Galaye *et al.* (2017) & Wolff *et al.* (2021) & Bio-T kit Lumpy Skin Disease- Biosellal	1	10	10	0	0
Wolff *et al.* (2021) & Sprygin *et al.* (2019)	1	10	10	0	0
Wolff *et al.* (2021) & Vidanovic *et al.* (2021)	1	10	10	0	0
Commercial kit	1	10	7	0	3

Kit or reference	N	NR	NCR	F	NI/NA
ID GENE LSD DIVA Triplex	2	20	14	6	0
In house	1	10	10	0	0
TOTAL	**25**	**250**	**238**	**6**	**6**

7.2.2.3 Results per laboratory

For the part Species Differentiation, 21 out of 25 participating laboratories provided qualitative results that were in full agreement with the assigned status of the ten reference samples (100% of agreement). Two laboratories used diagnostic kits that were unable to fully differentiate between the capripox virus species. These were laboratories 97630 and 97638. Laboratory 97630 failed to detect all LSDV Rec (clade 2) samples, while laboratory 97638 failed to detect all SPPV samples. Two laboratories (97628 and 97629) used a method that could only detect LSDV samples. Therefore, the SPPV strains could not be detected and these were misclassified as SPPV/GTPV.

Table 21: Results per laboratory. Correct answers are marked in green, while incorrect answers are indicated in red. In addition, non-interpretable or not analysed answers are shown in orange. Orange answers will not be evaluated. (REP = repetition; GTPV = goatpox virus; LSDV = lumpy skin disease virus; SPPV = sheeppox virus; NEG = negative; NI = non-interpretable; NA = not analysed).

Lab number	TP1 (1)	TP1 (2)	TP2	TP3	TP4	TP5	BP1	BP2	TN1	BN1	%
	LSDV	LSDV	SPPV	SPPV	LSDV	LSDV	LSDV	SPPV	NEG	NEG	
97506	LSDV	LSDV	SPPV	SPPV	LSDV	LSDV	LSDV	SPPV	NEG	NEG	100
97600	LSDV	LSDV	SPPV	SPPV	LSDV	LSDV	LSDV	SPPV	NEG	NEG	100
97602	LSDV	LSDV	SPPV	SPPV	LSDV	LSDV	LSDV	SPPV	NEG	NEG	100
97603	LSDV	LSDV	SPPV	SPPV	LSDV	LSDV	LSDV	SPPV	NEG	NEG	100
97604	LSDV	LSDV	SPPV	SPPV	LSDV	LSDV	LSDV	SPPV	NEG	NEG	100
97607	LSDV	LSDV	SPPV	SPPV	LSDV	LSDV	LSDV	SPPV	NEG	NEG	100
97608	LSDV	LSDV	SPPV	SPPV	LSDV	LSDV	LSDV	SPPV	NEG	NEG	100
97610	LSDV	LSDV	SPPV	SPPV	LSDV	LSDV	LSDV	SPPV	NEG	NEG	100
97611	LSDV	LSDV	SPPV	SPPV	LSDV	LSDV	LSDV	SPPV	NEG	NEG	100
97612	LSDV	LSDV	SPPV	SPPV	LSDV	LSDV	LSDV	SPPV	NEG	NEG	100
97613	LSDV	LSDV	SPPV	SPPV	LSDV	LSDV	LSDV	SPPV	NEG	NEG	100
97614	LSDV	LSDV	SPPV	SPPV	LSDV	LSDV	LSDV	SPPV	NEG	NEG	100
97617	LSDV	LSDV	SPPV	SPPV	LSDV	LSDV	LSDV	SPPV	NEG	NEG	100
97618	LSDV	LSDV	SPPV	SPPV	LSDV	LSDV	LSDV	SPPV	NEG	NEG	100
97619	LSDV	LSDV	SPPV	SPPV	LSDV	LSDV	LSDV	SPPV	NEG	NEG	100
97620	LSDV	LSDV	SPPV	SPPV	LSDV	LSDV	LSDV	SPPV	NEG	NEG	100
97621	LSDV	LSDV	SPPV	SPPV	LSDV	LSDV	LSDV	SPPV	NEG	NEG	100
97628	LSDV	LSDV	SPPV/ GTPV	SPPV/ GTPV	LSDV	LSDV	LSDV	SPPV/ GTPV	NEG	NEG	70

Lab number	TP1 (1)	TP1 (2)	TP2	TP3	TP4	TP5	BP1	BP2	TN1	BN1	%
	LSDV	LSDV	SPPV	SPPV	LSDV	LSDV	LSDV	SPPV	NEG	NEG	
97629	LSDV	LSDV	SPPV/ GTPV	SPPV/ GTPV	LSDV	LSDV	LSDV	SPPV/ GTPV	NEG	NEG	**70**
97630	NI	NI	SPPV	SPPV	LSDV	LSDV	NI	SPPV	NEG	NEG	**70**
97631	LSDV	LSDV	SPPV	SPPV	LSDV	LSDV	LSDV	SPPV	NEG	NEG	**100**
97632	LSDV	LSDV	SPPV	SPPV	LSDV	LSDV	LSDV	SPPV	NEG	NEG	**100**
97634	LSDV	LSDV	SPPV	SPPV	LSDV	LSDV	LSDV	SPPV	NEG	NEG	**100**
97637	LSDV	LSDV	SPPV	SPPV	LSDV	LSDV	LSDV	SPPV	NEG	NEG	**100**
97638	LSDV	LSDV	NA	NA	LSDV	LSDV	LSDV	NA	NEG	NEG	**70**

7.2.3 DIVA PCR

7.2.3.1 Results per sample

In total, nineteen laboratories participated in the DIVA PCR virology part of the proficiency test. The datasets of the laboratories submitting multiple datasets were taken together to get a final result for this part. Raw data can be found in Annex 1.

Table 22: Results per sample (REP = repetition; LSDV = lumpy skin disease virus; SPPV = sheeppox virus; NEG = negative; NI = non-interpretable; NA = not analysed).

Sample ID	REP	Expected results	# of LSDV			# of SPPV		# of NEG	# of NI/NA
			Vac (clade 1.1)	Wild (clade 1.2)	Rec (clade 2)	Wild	Vac		
TP1	Yes	LSDV Rec (clade 2)	4	2	30	0	0	0	2
TP2	No	SPPV Wild	0	0	0	14	0	0	5
TP3	No	SPPV Wild	0	0	0	14	0	0	5
TP4	No	LSDV Vac (clade 1.1)	18	0	1	0	0	0	0
TP5	No	LSDV Wild (clade 1.2)	0	18	0	0	0	0	1
BP1	No	LSDV Rec (clade 2)	3	1	14	0	0	0	1
BP2	No	SPPV Vac (RM65/Romania)	0	0	0	0	14	0	5
TN1	No	NEG	0	0	0	0	0	19	0
BN1	No	NEG	0	0	0	0	0	19	0

7.2.3.2 Results per method

In the table below, results are listed by method.

Table 23: Results per method (N = number of laboratories; NR = number of results; NCR = number of correct results; F = false result; NI = non-interpretable; NA = not analysed).

Kit or reference	N	NR	NCR	F	NI/NA
Agiannioaki *et al.* 2016 & Chibssa *et al.* 2018 & Wolff *et al.* 2021 & Haegeman *et al.* 2023	1	10	10	0	0
Haegeman *et al.* 2023 & Chibssa *et al.* 2018	3	30	27	3	0
Haegeman *et al.* 2023 & Chibssa *et al.* 2018 & sequencing	1	10	10	0	0
Agianniotaki *et al.* 2017 & Gelaye *et al.* 2015	1	10	10	0	0
Agianniotaki *et al.* 2017 & Chibssa et al. 2018	1	10	8	2	0
Vidanovic *et al.* 2021	1	10	4	3	3
Agianniotaki *et al.* 2017	1	10	7	0	3
Chibssa *et al.* 2018 & Krotova *et al.* 2023	1	10	10	0	0
Agiannioaki *et al.* 2016 & Chibssa *et al.* 2018 & Gelaye *et al.* 2015 & Haegeman *et al.* 2023	1	10	10	0	0

Kit or reference	N	NR	NCR	F	NI/NA
Menasherow *et al.* 2014 & Haegeman *et al.* 2023	1	10	10	0	0
Haegeman *et al.* 2023 & Vidanovic *et al.* 2021	1	10	10	0	0
Haegeman *et al.* 2015 & Haegeman *et al.* 2023	2	20	20	0	0
Wolff *et al.* 2021	1	10	4	0	6
Haegeman *et al.* 2015 & Haegeman *et al.* 2023 & Gelaye *et al.* 2015	1	10	10	0	0
Haegeman *et al.* 2023	1	10	6	0	4
Bio-T Kit Lumpy Skin Disease - DIVA Biosellal	1	10	4	3	3
TOTAL	**19**	**190**	**160**	**11**	**19**

7.2.3.3 Results per laboratory

Twelve out of nineteen laboratories provided results that were in full agreement with the assigned status of the reference samples (100% agreement). One laboratory misclassified aliquots PTCAPXVIR2025_TP4 and PTCAPXVIR2025_BP1 as respectively LSDV Rec (Clade 2) and LSDV Vac (Clade 1.1) instead of LSDV Vac (Clade 1.1) and LSDV Rec (Clade 2). Two laboratories misclassified three aliquots. One laboratory misclassified the three LSDV Rec (clade 2) samples as LSDV Wild (clade 1.2). The other laboratory did not classify the SPPV samples and these were thus misclassified as NA. One laboratory misclassified 4 aliquots. These were the three SPPV samples as NA and sample PTCAPXVIR2025_TP5 as NA instead of LSDV Wild (Clade 1.2).The remaining three labs misclassified six samples. In all cases, these were the three SPPV and the three LSDV Rec (Clade 1.2) samples. All three misclassified the SPPV samples as NA. Two laboratories misclassified the LSDV Rec (Clade 2) samples as LSDV Vac (Clade 1.1) and one laboratory misclassified the same samples as NA instead of LSDV Rec (clade 2).

Datasets of the laboratories submitting multiple datasets were taken together to get a final result for this part. Raw data can be found in Annex 1.

Table 24: Results per laboratory. Correct answers are marked in green, while incorrect answers are indicated in red. In addition, non-interpretable or not analysed answers are shown in orange. Orange answers are not evaluated. (REP = repetition; LSDV = lumpy skin disease virus; SPPV = sheeppox virus; NEG = negative; NI = non-interpretable; NA = not analysed; vac = vaccine, rec = recombinant).

Lab number	TP1 (1)	TP1 (2)	TP2	TP3	TP4	TP5	BP1	BP2	TN1	BN1	%
	LSDV Rec (clade 2)		SPPV Wild		LSDV Vaccine (clade 1.1)	LSDV Wild (clade 1.2)	LSDV Rec (clade 2)	SPPV Vaccine (RM65/Romania)	NEG		
97506	LSDV rec	LSDV rec	SPPV wild	SPPV wild	LSDV vac	LSDV wild	LSDV rec	SPPV vac	NEG	NEG	**100**
97600	LSDV rec	LSDV rec	SPPV wild	SPPV wild	LSDV vac	LSDV wild	LSDV rec	SPPV vac	NEG	NEG	**100**
97602	LSDV rec	LSDV rec	SPPV wild	SPPV wild	LSDV vac	LSDV wild	LSDV rec	SPPV vac	NEG	NEG	**100**
97608	LSDV rec	LSDV rec	SPPV wild	SPPV wild	LSDV vac	LSDV wild	LSDV rec	SPPV vac	NEG	NEG	**100**
97609	LSDV rec	LSDV rec	SPPV wild	SPPV wild	LSDV vac	LSDV wild	LSDV rec	SPPV vac	NEG	NEG	**100**
97610	LSDV vac	LSDV vac	NA	NA	LSDV vac	LSDV wild	LSDV vac	NA	NEG	NEG	**40**

Lab number	TP1 (1)	TP1 (2)	TP2	TP3	TP4	TP5	BP1	BP2	TN1	BN1	%
	LSDV Rec (clade 2)		SPPV Wild		LSDV Vaccine (clade 1.1)	LSDV Wild (clade 1.2)	LSDV Rec (clade 2)	SPPV Vaccine (RM65/Romania)	NEG		
97611	LSDV rec	LSDV rec	NA	NA	LSDV vac	LSDV wild	LSDV rec	NA	NA	NEG	70
97612	LSDV rec	LSDV rec	SPPV wild	SPPV wild	LSDV vac	LSDV wild	LSDV rec	SPPV vac	NEG	NEG	100
97613	LSDV rec	LSDV rec	SPPV wild	SPPV wild	LSDV vac	LSDV wild	LSDV rec	SPPV vac	NEG	NEG	100
97617	LSDV rec	LSDV rec	SPPV wild	SPPV wild	LSDV vac	LSDV wild	LSDV rec	SPPV vac	NEG	NEG	100
97618	LSDV rec	LSDV rec	SPPV wild	SPPV wild	LSDV rec	LSDV wild	LSDV vac	SPPV vac	NEG	NEG	80
97619	LSDV rec	LSDV rec	SPPV wild	SPPV wild	LSDV vac	LSDV wild	LSDV rec	SPPV vac	NEG	NEG	100
97620	LSDV wild	LSDV wild	SPPV wild	SPPV wild	LSDV vac	LSDV wild	LSDV wild	SPPV vac	NEG	NEG	70
97621	LSDV rec	LSDV rec	SPPV wild	SPPV wild	LSDV vac	LSDV wild	LSDV rec	SPPV vac	NEG	NEG	100
97630	NA	NA	NA	NA	LSDV vac	LSDV wild	NA	NA	NEG	NEG	40
97632	LSDV rec	LSDV rec	SPPV wild	SPPV wild	LSDV vac	LSDV wild	LSDV rec	SPPV vac	NEG	NEG	100
97634	LSDV rec	LSDV rec	SPPV wild	SPPV wild	LSDV vac	LSDV wild	LSDV rec	SPPV vac	NEG	NEG	100
97637	LSDV rec	LSDV rec	NA	NA	LSDV vac	NA	LSDV rec	NA	NEG	NEG	60
97638	LSDV vac	LSDV vac	NA	NA	LSDV vac	LSDV wild	LSDV vac	NA	NEG	NEG	40

8 DISCUSSION

The purpose of this PT was to assess the performances of the participating laboratories when analysing reference serum samples of ruminant origin for the detection of antibodies to capripox viruses and/or analysing reference blood and tissue homogenate samples for the detection of capripox virus DNA.

8.1 Serology component of the PT

For the detection of **specific antibodies** to capripox virus in reference serum samples, using ELISA, and in some cases, virus neutralisation test (VNT) or an immunoperoxidase monolayer assay (IPMA) as well, the overall performance of participating laboratories was highly satisfactory.

In the ELISA test, which was performed by the majority of the participating laboratories (n=35), the level of concordance with the assigned reference status was excellent. Thirty-four laboratories obtained 100% agreement, indicating a robust reproducibility of the method across laboratories. Only one laboratory (97619) misclassified a single sample, which lowered its score to 90%. Nevertheless, this still met the acceptance criteria, confirming that ELISA is a reliable and broadly applicable method for antibody detection in this context.

The virus neutralisation test (VNT), submitted by three laboratories, also demonstrated full concordance with the expected results, with all laboratories reaching 100% agreement. This outcome confirms the strong specificity and accuracy of the method.

Finally, the immunoperoxidase monolayer assay (IPMA) was applied by only one laboratory, which achieved 100% agreement. While this confirms the validity of the method under the given conditions, the lack of inter-laboratory comparison means that reproducibility could not be evaluated.

Overall, the results underline the robustness of ELISA as the most widely used method. The IPMA and VNT are less used, but were still highly accurate.

8.2 Virology component of the PT

For the detection of capripox virus **DNA by real-time PCR (primary PCR)** in the PT panel: 35 out of 35 participating laboratories provided qualitative results that were in full agreement with the assigned status of the ten reference samples (100% of agreement). Four laboratories added a secondary PCR and they both provided qualitative results that were in full agreement with the assigned status of the ten reference samples (100% of agreement).

For the **differentiation of capripox virus species**, 21 out of 25 participating laboratories produced qualitative results that matched the assigned status of the ten reference samples, achieving a 100% agreement rate. When evaluating species differentiation, the majority of laboratories (21 out of 25) achieved full agreement with the assigned status of the reference samples, confirming the overall robustness of the methods applied. Nevertheless, two laboratories (97630 and 97638) encountered limitations linked to the diagnostic kits they employed. The kit used by laboratory 97630 was unable to reliably detect LSDV recombinant (clade 2) samples, while the kit applied by laboratory 97638 failed to identify SPPV samples, reaching a level of agreement of 70%. The remaining two labs misclassified the SPPV samples as SPPV/GTPV. Since there are methods available to discriminate these strains from each other SPPV/GTPV cannot be considered as a correct answer. Therefore, a level of agreement of 70% was obtained.

For the **DIVA PCR**, there are methods available for the discrimination of LSDV recombinant field strains from other LSDV strains, therefore only LSDV rec (Clade 2) was considered as correct for PTCAPXVIR2025_BP1 and PTCAPXVIR_TP1. NA or NI was only accepted if a laboratory has no method in place to discriminate an LSDV rec strain. In this case, this sample was not taken into account. If a wrong answer was given, this sample was still taken into account. The same holds true for the SPPV samples (PTCAPXVIR2025_BP2, PTCAPXVIR2025_TP2 and PTCAPXVIR_TP3). Twelve out of nineteen laboratories provided results that were in full agreement with the assigned status of the reference samples (100% agreement). One laboratory (97618) misclassified sample PTCAPXVIR2025_BP1 and PTCAPXVIR2025_TP4 as respectively LSDV vac (Clade 1.1) and LSDV Rec (Clade 2) instead of LSDV Rec (Clade 2) and LSDV Vac (Clade 1.1) reaching a level of agreement of 80%. Surprisingly, the other LSDV Rec strains were correctly classified. It might be possible that the results of these samples were switched. One laboratory (97620) misclassified all LSDV Rec (Clade 2) samples (PTCAPXVIR2025_BP1 and PTCAPXVIR_TP1 (2x)) as LSDV Wild (Clade 1.2) reaching a level of agreement of 70%. The method used cannot discriminate Clade 1.2 strains from Clade 2 strains. One other laboratory (97611) misclassified three aliquots. Here all SPPV samples were misclassified. They classified PTCAPXVIR2025_BP2, PTCAPXVIR2025_TP2 and PTCAPXVIR_TP3 NA instead of respectively SPPV Vac, SPPV Wild and SPPV Wild, reaching a level of agreement of 70%. The method used was however not able to discriminate SPPV strains. One laboratory (97637) misclassified four samples. Samples PTCAPXVIR2025_BP2, PTCAPXVIR2025_TP2 and PTCAPXVIR2025_TP3 were misclassified as NA instead of SPPV Vac, SPPV Wild, and SPPV Wild. PTCAPXVIR2025_TP5 was misclassified as NI instead of LSDV Wild (Clade 1.2), reaching a level of agreement of 60% The method used cannot discriminate SPPV strains. The method used for can discriminate clade 1.1 LSDV strains from Clade 1.2 and Clade 2 strains. Surprisingly, the samples containing the Clade 2 strain were correctly classified, but the LSDV Wild (clade 1.2) strain was not classified. The remaining laboratories all used methods that cannot classify the SPPV strains and can also not correctly classify the Clade 2 LSDV strains. These three laboratories all misclassified the SPPV samples as NA. The LSDV Rec (Clade 2 strains) were misclassified as LSDV Vaccine by two laboratories (97610 and 97638). The remaining laboratory (97620) misclassified these samples as NA. These three labs reached thus a level of agreement of 40%.

9 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 samples assigned by the European Union Reference Laboratory (EURL) for disease caused by capripox viruses of the Scientific Directorate Infectious Diseases in Animals of Sciensano. Each part of the PT is separately evaluated.

Only laboratories that provided a complete dataset are rated and will be able to get a satisfactory performance. The remaining laboratories will be rated on the samples that could be analysed with the used assay. However, a satisfactory performance cannot be awarded, since not all aliquots were analysed for a specific part.

The **serology part** of the PT Capripox can be delineated into three distinct sections, namely ELISA, VNT, and IPMA. For the ELISA test, 35 laboratories submitted results, of which 34 achieved 100% agreement and one laboratory reached 90%, meeting the required qualification criteria. For the virus neutralisation test (VNT), three laboratories participated and all obtained 100% agreement. For the immunoperoxidase monolayer assay (IPMA), one laboratory submitted results and also achieved 100% agreement. These results demonstrate that all participating laboratories met the minimum performance criteria across the different serological methods applied.

The **virology part** of the PT Capripox can also be divided into three parts, namely Primary PCR, Species Differentiation and DIVA PCR. For the Primary PCR part, 35 out of 35 laboratories delivered results that entirely matched the expected status of the ten reference samples, achieving a 100% agreement rate and thus a satisfactory result. Furthermore, four laboratories conducted a secondary PCR analysis. All yielded results that perfectly aligned with the reference sample status.

For the species differentiation part, 21 out of 25 participating laboratories delivered qualitative outcomes that were in full agreement with the expected status of the ten reference samples, achieving a 100% agreement rate. All of these laboratories achieved a satisfactory result. The observed discrepancies in two laboratories were linked to diagnostic kits with limited discriminatory capacity, particularly regarding LSDV recombinant and SPPV samples, resulting each in a level of agreement of 70%. Because of the limitations of their kits, a satisfactory result cannot be rewarded. However, all samples that could be classified with the respective methods, were correctly classified.

For the DIVA PCR part, twelve out of nineteen laboratories achieved a perfect agreement with the expected status of the reference samples, resulting in a 100% agreement rate and a satisfactory result. Two labs (97611 and 97637) did not submit complete datasets and could therefore not reach a satisfactory result. However, the classified samples were correctly classified. The remaining labs misclassified at least two or more samples and therefore reached an unsatisfactory result.

10 ANNEXES (NOT UNDER ACCREDITATION)

10.1 Annex 1: Boxplots

Besides qualitative data analysis (positive, negative or doubtful result), also quantitative data analysis was performed. 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 standardise their different diagnostic tests. The boxplots, shown down below, were created by using the following software programme: shiny.chemgrid.org/boxplotr/.

10.1.1 SERO: ELISA

For the serology ELISA part, box plots of the optical density (OD) per reference sample and per participating laboratory. Boxplots are generated exclusively for the positive samples that exhibited repetitions within the panel.

Quantitative results for two replicate samples: PT2025CAPXSER-P1

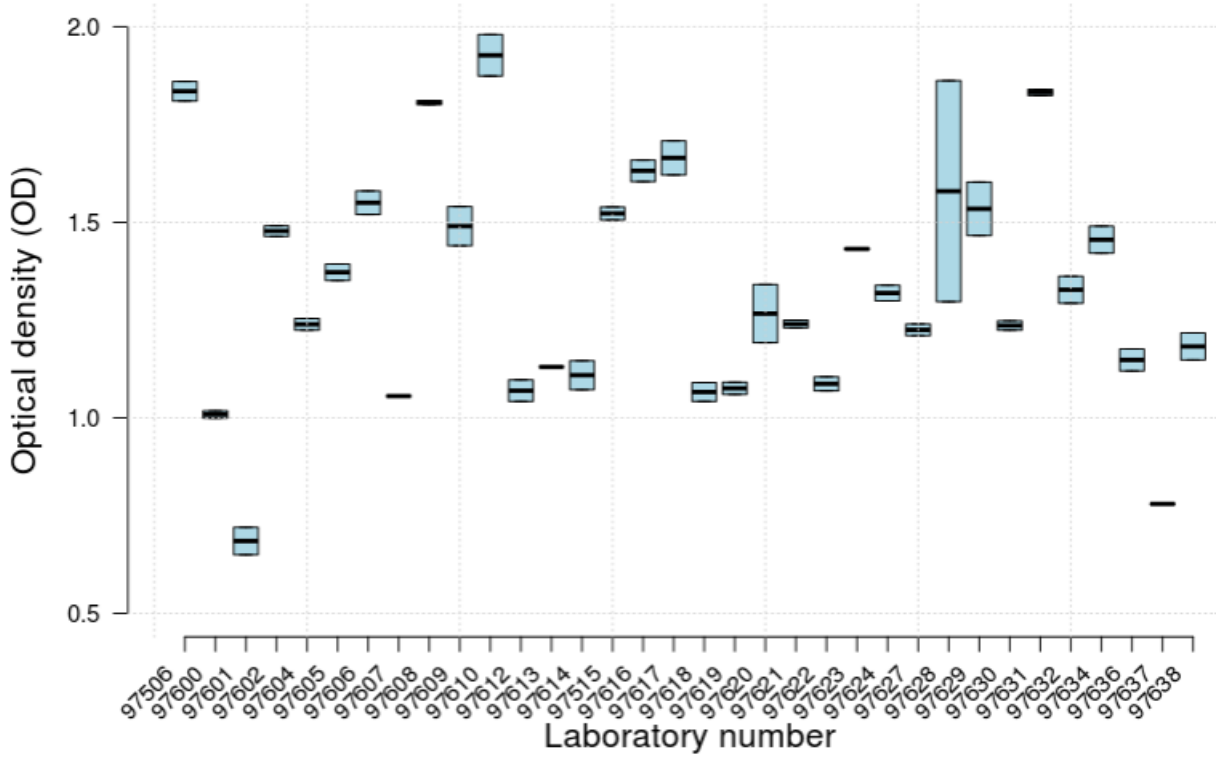


Fig I: Distribution of the optical density (OD) for the PT2025CAPXVIR_P1 samples (LAB97506-97638).

Quantitative results for two replicate samples: PT2025CAPXSER-P6

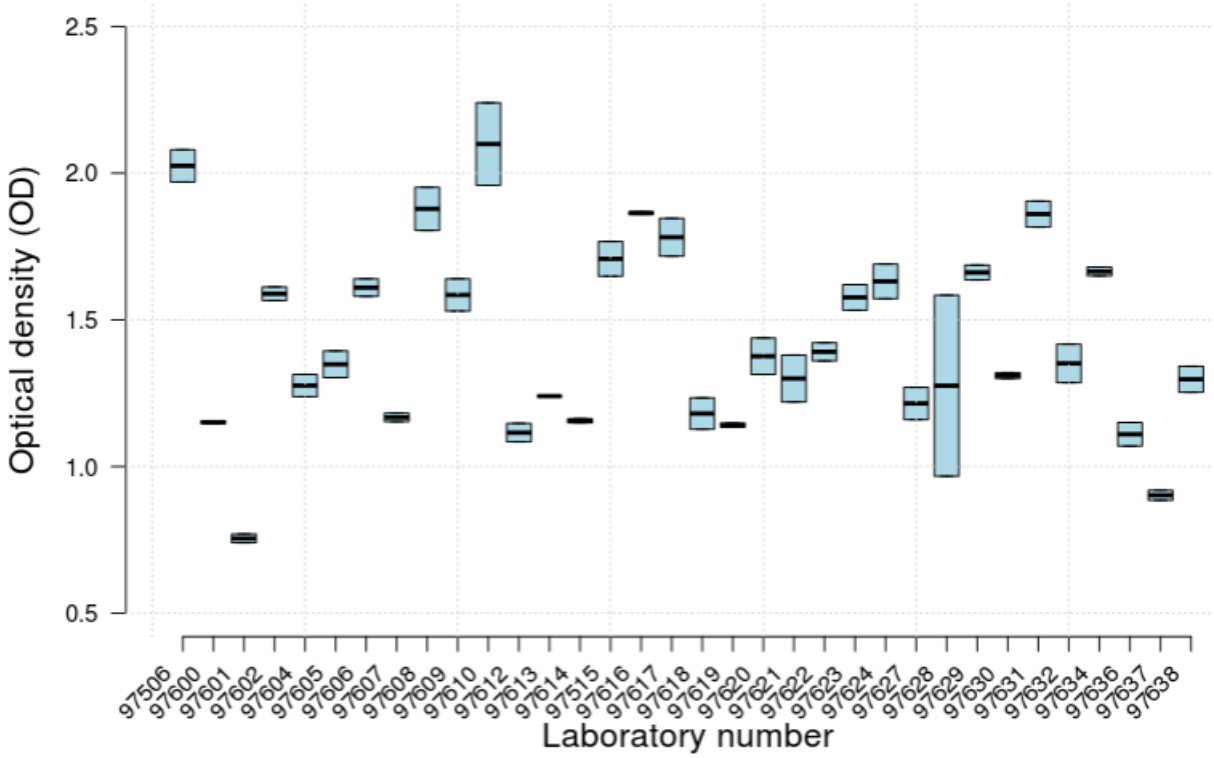


Fig II: Distribution of the optical density (OD) for the PT2025CAPXVIR_P6 samples (LAB97506-97638).

10.1.2 VIRO: PRIMARY PCR

For the virology Primary PCR part, box plots of the Ct-values per reference sample and per participating laboratory. Boxplots are generated exclusively for the positive samples that exhibited repetitions within the panel.

Laboratories that used two different methods are represented twice in the boxplot.

Quantitative results for two replicate samples: PT2025CAPXVIR-TP1

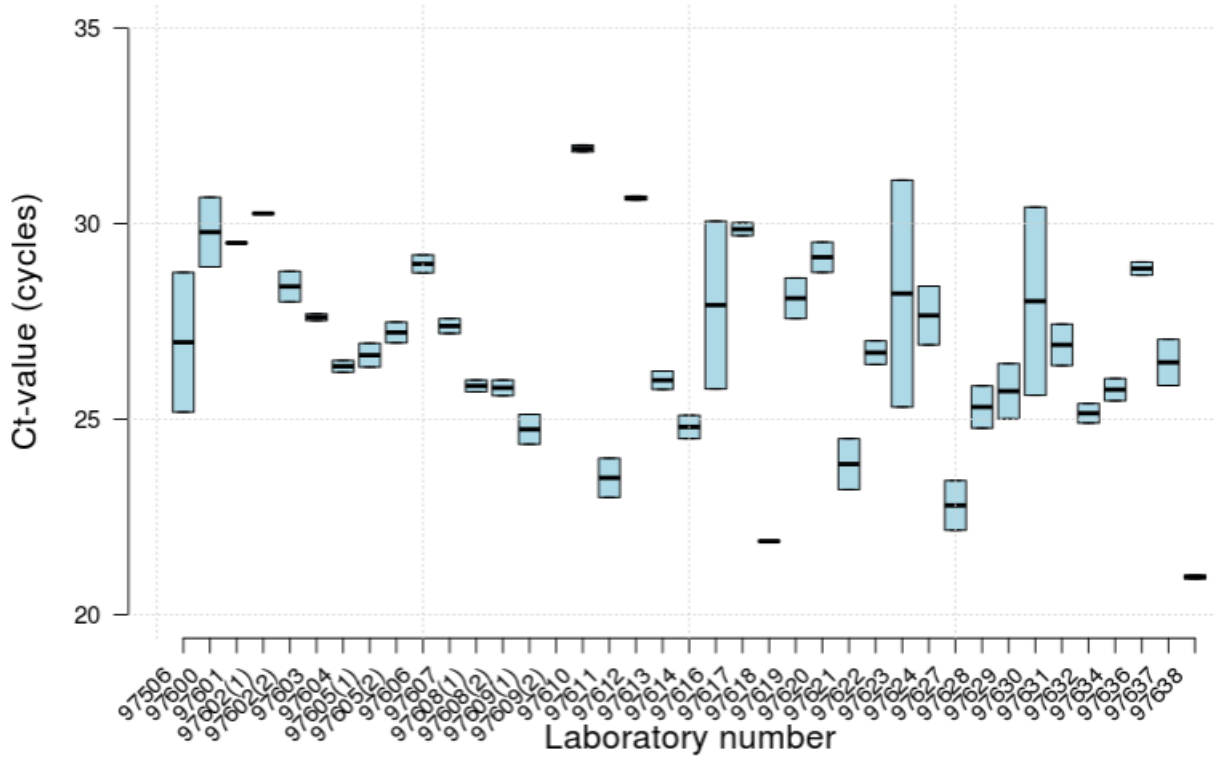


Fig III: Distribution of the Ct-values data for the PT2025CAPXVIR_TP1 samples (LAB97506-97638).

10.2 Annex 2: Raw data

10.2.1 SERO: ANTIBODY ELISA

lab ID	Sample ID	Reagens	Cut-off	Pos control	Neg control	OD	Interpretation	Comment
97506	N1	IDVet - ID SCREEN® CAPRIPOX DOUBLE ANTIGEN MULTI-SPECIES	30%	0,043	1,075	0,046	NEG	S/P%= (OD(sample)-OD(NC))/(OD(PC)-OD(NC))*100
	N2					0,0472	NEG	
	P1					1,8142	POS	
	P1					1,8627	POS	
	P2					1,1492	POS	
	P3					2,088	POS	
	P4					1,9603	POS	
	P5					2,7971	POS	
	P6					1,9785	POS	
	P6					2,0841	POS	
97600	N1	IDVet - ID SCREEN® CAPRIPOX DOUBLE ANTIGEN MULTI-SPECIES	30%	0,887	0,044	0,047	NEG	
	N2					0,046	NEG	
	P1					0,999	POS	
	P1					1,018	POS	
	P2					0,477	POS	
	P3					1,224	POS	
	P4					1,048	POS	
	P5					2,01	POS	
	P6					1,155	POS	
	P6					1,147	POS	
97601	N1	IDVet - ID SCREEN® CAPRIPOX DOUBLE ANTIGEN MULTI-SPECIES	30%	0,375	0,05	0,06	NEG	
	N2					0,063	NEG	
	P1					0,65	POS	
	P1					0,72	POS	
	P2					0,38	POS	
	P3					1,26	POS	
	P4					0,88	POS	
	P5					1,17	POS	
	P6					0,77	POS	
	P6					0,74	POS	
97602	N1	IDVet - ID SCREEN® CAPRIPOX DOUBLE ANTIGEN MULTI-SPECIES	30%	1,2245	0,072	0,062	NEG	
	N2					0,069	NEG	
	P1					1,464	POS	
	P1					1,491	POS	
	P2					0,731	POS	

lab ID	Sample ID	Reagens	Cut-off	Pos control	Neg control	OD	Interpretation	Comment
	P3					1,465	POS	
	P4					1,298	POS	
	P5					2,641	POS	
	P6					1,566	POS	
	P6					1,612	POS	
	N1					0,067	NEG	
	N2					0,07	NEG	
	P1					1,254	POS	
	P1					1,224	POS	
97604	P2	IDVet - ID SCREEN® CAPRIPOX DOUBLE ANTIGEN MULTI-SPECIES	30%	1,009	0,067	0,591	POS	((Sample - NC)/(PC - NC))*100
	P3					1,492	POS	
	P4					1,339	POS	
	P5					2,201	POS	
	P6					1,238	POS	
	P6					1,314	POS	
	N1					0,057	NEG	
	N2					0,058	NEG	
	P1					1,351	POS	
	P1					1,393	POS	
97605	P2	IDVet - ID SCREEN® CAPRIPOX DOUBLE ANTIGEN MULTI-SPECIES	30%	0,996	0,051	0,529	POS	S/P%=((ODsample-ODnc)/(ODpc-ODnc))*100
	P3					1,666	POS	
	P4					1,321	POS	
	P5					2,18	POS	
	P6					1,303	POS	
	P6					1,394	POS	
	N1					0,0575	NEG	
	N2					0,0593	NEG	
	P1					1,5805	POS	
	P1					1,5271	POS	
97606	P2	IDVet - ID SCREEN® CAPRIPOX DOUBLE ANTIGEN MULTI-SPECIES	30%	1,071	0,0529	0,6159	POS	
	P3					1,7947	POS	
	P4					1,4428	POS	
	P5					2,5248	POS	
	P6					1,5898	POS	
	P6					1,6428	POS	
	N1					0,0575	NEG	
97607	N2	IDVet - ID SCREEN® CAPRIPOX DOUBLE ANTIGEN MULTI-SPECIES	30%	0,8435	0,045	0,051	NEG	=100*(DOSample-DO NegativeControl)/(DOPositiveControl-DO NegativeControl
	P1					1,055	POS	
	P1					1,056	POS	

lab ID	Sample ID	Reagens	Cut-off	Pos control	Neg control	OD	Interpretation	Comment
	P2					0,475	POS	Given that samples were tested in duplicate data shown (DO value) for each sample correspond to a mean DO
	P3					1,236	POS	
	P4					0,977	POS	
	P5					1,935	POS	
	P6					1,153	POS	
	P6					1,182	POS	
97608	N1	IDVet - ID SCREEN® CAPRIPOX DOUBLE ANTIGEN MULTI-SPECIES	30%	1,28	0,054	0,059	NEG	
	N2					0,063	NEG	
	P1					1,801	POS	
	P1					1,811	POS	
	P2					0,841	POS	
	P3					1,847	POS	
	P4					1,619	POS	
	P5					2,931	POS	
	P6					1,952	POS	
	P6					1,805	POS	
97609	N1	IDVet - ID SCREEN® CAPRIPOX DOUBLE ANTIGEN MULTI-SPECIES	30%	1,028	0,083	0,0729	NEG	100x [(ODsample - ODNc)/(ODPc - ODNc)])
	N2					0,0715	NEG	
	P1					1,4495	POS	
	P1					1,5462	POS	
	P2					0,6552	POS	
	P3					1,8289	POS	
	P4					1,5863	POS	
	P5					2,3699	POS	
	P6					1,5321	POS	
	P6					1,6458	POS	
97610	N1	IDVet - ID SCREEN® CAPRIPOX DOUBLE ANTIGEN MULTI-SPECIES	30%	1,947	0,055	0,06	NEG	s/p%=(ODsample-ODNC/ODPC-ODNC)*100
	N2					0,065	NEG	
	P1					1,98	POS	
	P1					1,874	POS	
	P2					1,199	POS	
	P3					1,83	POS	
	P4					1,91	POS	
	P5					3,183	POS	
	P6					2,24	POS	
	P6					1,959	POS	
97611	N1	IDVet - ID SCREEN® CAPRIPOX DOUBLE ANTIGEN MULTI-SPECIES	30%	0,808	0,049		NEG	S/P%=((ODSample - OD Neg control)/(OD Positive Control- OD Negative Control)) *100
	N2						NEG	
	P1						POS	

lab ID	Sample ID	Reagens	Cut-off	Pos control	Neg control	OD	Interpretation	Comment
	P1						POS	
	P2						POS	
	P3						POS	
	P4						POS	
	P5						POS	
	P6						POS	
	P6						POS	
	N1					0,134	NEG	
	N2					0,086	NEG	
	P1					1,097	POS	
	P1					1,042	POS	
97612	P2	IDVet - ID SCREEN® CAPRIPOX DOUBLE ANTIGEN MULTI-SPECIES	30%	0,07	0,954	0,528	POS	S/P%=(ODsample-ODneg/ODpos-ODneg)*100
	P3					1,459	POS	
	P4					1,242	POS	
	P5					1,829	POS	
	P6					1,147	POS	
	P6					1,084	POS	
	N1					0,0538	NEG	
	N2					0,0756	NEG	
	P1					1,1318	POS	
	P1					1,1351	POS	
97613	P2	IDVet - ID SCREEN® CAPRIPOX DOUBLE ANTIGEN MULTI-SPECIES	30%	1,0638	0,0587	0,4558	POS	S/P%=((sample OD-NC OD)/(PC OD-NC OD))*100
	P3					1,31	POS	
	P4					1,1289	POS	
	P5					2,0764	POS	
	P6					1,2433	POS	
	P6					1,2472	POS	
	N1					0,051	NEG	
	N2					0,054	NEG	
	P1					1,072	POS	
	P1					1,146	POS	
97614	P2	IDVet - ID SCREEN® CAPRIPOX DOUBLE ANTIGEN MULTI-SPECIES	30%	0,9845	0,047	0,567	POS	S/P% = (OD sample - OD NC) / (OD PC - OD NC) x 100
	P3					1,354	POS	
	P4					0,974	POS	
	P5					2,05	POS	
	P6					1,163	POS	
	P6					1,149	POS	
97615	N1	IDVet - ID SCREEN® CAPRIPOX DOUBLE ANTIGEN MULTI-SPECIES	30%	1,205	0,046	0,049	NEG	((ODsample-Mean OD Neg Ctrl)/(Mean OD Pos ctrl-mean OD Neg Ctrl)*100)
	N2					0,049	NEG	

lab ID	Sample ID	Reagens	Cut-off	Pos control	Neg control	OD	Interpretation	Comment
	P1					1,506	POS	
	P1					1,539	POS	
	P2					0,728	POS	
	P3					1,899	POS	
	P4					1,391	POS	
	P5					2,533	POS	
	P6					1,767	POS	
	P6					1,649	POS	
	N1					0,052	NEG	
	N2					0,054	NEG	
	P1					1,604	POS	
	P1					1,659	POS	
97616	P2	IDVet - ID SCREEN® CAPRIPOX DOUBLE ANTIGEN MULTI-SPECIES	30%	1,35	0,049	0,798	POS	S/P=(Sample Mean OD - Negative Control Mean OD) / (Positive Control Mean OD - Negative Control Mean OD)
	P3					1,628	POS	
	P4					1,692	POS	
	P5					2,765	POS	
	P6					1,86	POS	
	P6					1,868	POS	
	N1					0,052	NEG	
	N2					0,056	NEG	
	P1					1,621	POS	
	P1					1,708	POS	
97617	P2	IDVet - ID SCREEN® CAPRIPOX DOUBLE ANTIGEN MULTI-SPECIES	30%	1,385	0,048	0,784	POS	S/P % = (ODsample-ODnegative)/(ODpositive-ODnegative)x100
	P3					1,863	POS	
	P4					1,514	POS	
	P5					2,73	POS	
	P6					1,846	POS	
	P6					1,717	POS	
	N1					0,055	NEG	
	N2					0,051	NEG	
	P1					1,09	POS	
	P1					1,042	POS	
97618	P2	IDVet - ID SCREEN® CAPRIPOX DOUBLE ANTIGEN MULTI-SPECIES	30%	0,896	0,048	0,518	POS	Indirect ELISA (S/P% = DOsample-DOnegative control / DOpositive control - DOnegative control)x 100)
	P3					1,365	POS	
	P4					1,086	POS	
	P5					2	POS	
	P6					1,234	POS	
	P6					1,127	POS	
97619	N1		30%	0,987	0,055	0,048	NEG	

lab ID	Sample ID	Reagens	Cut-off	Pos control	Neg control	OD	Interpretation	Comment
	N2	IDVet - ID SCREEN® CAPRIPOX DOUBLE ANTIGEN MULTI-SPECIES				0,044	NEG	
	P1					1,06	POS	
	P1					1,091	POS	
	P2					0,345	NEG	
	P3					1,113	POS	
	P4					0,859	POS	
	P5					1,968	POS	
	P6					1,134	POS	
	P6					1,148	POS	
97620	N1	IDVet - ID SCREEN® CAPRIPOX DOUBLE ANTIGEN MULTI-SPECIES	30%	1,006	0,041	0,047	NEG	S/P%=(ODsample-ODnc)/(ODpc-ODnc)*100
	N2					0,047	NEG	
	P1					1,192	POS	
	P1					1,341	POS	
	P2					0,547	POS	
	P3					1,57	POS	
	P4					1,349	POS	
	P5					2,173	POS	
	P6					1,438	POS	
	P6					1,314	POS	
97621	N1	IDVet - ID SCREEN® CAPRIPOX DOUBLE ANTIGEN MULTI-SPECIES	30%	1,04	0,05	0,05	NEG	S/P%= ((OD sample- OD NC)/(OD PC- OD NC))*100
	N2					0,05	NEG	
	P1					1,25	POS	
	P1					1,23	POS	
	P2					0,51	POS	
	P3					1,44	POS	
	P4					1,29	POS	
	P5					2,14	POS	
	P6					1,38	POS	
	P6					1,22	POS	
97622	N1	IDVet - ID SCREEN® CAPRIPOX DOUBLE ANTIGEN MULTI-SPECIES	30%	1,135	0,055	0,057	NEG	S/P% ; ((ODsample - OD neg control) / (OD pos control - ODneg control))*100
	N2					0,059	NEG	
	P1					1,105	POS	
	P1					1,069	POS	
	P2					0,494	POS	
	P3					1,683	POS	
	P4					1,249	POS	
	P5					2,301	POS	
	P6					1,422	POS	
	P6					1,36	POS	

lab ID	Sample ID	Reagens	Cut-off	Pos control	Neg control	OD	Interpretation	Comment
97623	N1	IDVet - ID SCREEN® CAPRIPOX DOUBLE ANTIGEN MULTI-SPECIES	30%	0,965	0,046	0,053	NEG	According to manual Samples were analysed in duplicates. Results given are the average. VNT nor IPMA have been performed.
	N2					0,053	NEG	
	P1					1,434	POS	
	P1					1,43	POS	
	P2					0,575	POS	
	P3					1,768	POS	
	P4					1,619	POS	
	P5					2,315	POS	
	P6					1,62	POS	
	P6					1,533	POS	
97624	N1	IDVet - ID SCREEN® CAPRIPOX DOUBLE ANTIGEN MULTI-SPECIES	450	1,257	0,0445	0,051	NEG	S/P% = ((sample OD - NCm.OD) / (PCm.OD - NCm.OD))*100
	N2					0,049	NEG	
	P1					1,299	POS	
	P1					1,339	POS	
	P2					0,574	POS	
	P3					1,664	POS	
	P4					1,377	POS	
	P5					2,451	POS	
	P6					1,69	POS	
	P6					1,572	POS	
97627	N1	IDVet - ID SCREEN® CAPRIPOX DOUBLE ANTIGEN MULTI-SPECIES	30%	1,12	0,04	0,054	NEG	S/P=(OD sample - ODNC)/(ODPC-ODNC)*100
	N2					0,05	NEG	
	P1					1,21	POS	
	P1					1,24	POS	
	P2					0,5	POS	
	P3					1,48	POS	
	P4					1,06	POS	
	P5					2,12	POS	
	P6					1,27	POS	
	P6					1,16	POS	
97628	N1	BioStone Animal Health. AsurDx Capripox Antibody Test Kit Manual.	PP>40 (pos)	1,5665	0,0495	0,155	NEG	PP=(OD450testsample- NC)/(PC-NC)*100%
	N2					0,244	NEG	
	P1					1,297	POS	
	P1					1,862	POS	
	P2					1,081	POS	
	P3					0,848	POS	
	P4					0,941	POS	
	P5					1,399	POS	
	P6					0,967	POS	

lab ID	Sample ID	Reagens	Cut-off	Pos control	Neg control	OD	Interpretation	Comment
	P6					1,584	POS	
97629	N1	BioStone Animal Health. AsurDx Capripox Antibody Test Kit Manual.	PP>40 (pos)	1,5665	0,0495	0,122	NEG	PP=(OD450testsample- NC)/(PC-NC)*100%
	N2					0,165	NEG	
	P1					1,603	POS	
	P1					1,466	POS	
	P2					1,029	POS	
	P3					1,175	POS	
	P4					1,206	POS	
	P5					2,088	POS	
	P6					1,687	POS	
	P6					1,636	POS	
97630	N1	IDVet - ID SCREEN® CAPRIPOX DOUBLE ANTIGEN MULTI-SPECIES	30%	0,9181	0,0437	0,04535	NEG	S/P%=100*((OD sample-ODnc)/(OD pc-ODnc))
	N2					0,0476	NEG	
	P1					1,22415	POS	
	P1					1,24835	POS	
	P2					0,49035	POS	
	P3					1,30855	POS	
	P4					1,0455	POS	
	P5					2,2022	POS	
	P6					1,3	POS	
	P6					1,3234	POS	
97631	N1	IDVet - ID SCREEN® CAPRIPOX DOUBLE ANTIGEN MULTI-SPECIES	30%	1,0665	0,0535	0,0465	NEG	S/P%=(OD sample -OD neg) /(OD pc- OD nc) x100
	N2					0,049	NEG	
	P1					1,824	POS	
	P1					1,8425	POS	
	P2					0,986	POS	
	P3					2,055	POS	
	P4					1,924	POS	
	P5					2,8165	POS	
	P6					1,816	POS	
	P6					1,905	POS	
97632	N1	IDVet - ID SCREEN® CAPRIPOX DOUBLE ANTIGEN MULTI-SPECIES	30%	0,997	0,042	0,047	NEG	S/P=(ODsample-ODnegctrl)/(ODposctrl-ODnegctrl)
	N2					0,046	NEG	
	P1					1,362	POS	
	P1					1,293	POS	
	P2					0,607	POS	
	P3					1,631	POS	
	P4					1,438	POS	
	P5					2,292	POS	

lab ID	Sample ID	Reagens	Cut-off	Pos control	Neg control	OD	Interpretation	Comment
	P6					1,417	POS	
	P6					1,286	POS	
97634	N1	IDVet - ID SCREEN® CAPRIPOX DOUBLE ANTIGEN MULTI-SPECIES	40%	1,1	0,05	0,1	NEG	
	N2					0,0535	NEG	
	P1					1,421	POS	
	P1					1,4975	POS	
	P2					0,668	POS	
	P3					0,696	POS	
	P4					0,926	POS	
	P5					2,535	POS	
	P6					1,6505	POS	
	P6					1,6895	POS	
97636	N1	IDVet - ID SCREEN® CAPRIPOX DOUBLE ANTIGEN MULTI-SPECIES	30%	0,5766	0,0087	0,0086	NEG	
	N2					0,0098	NEG	
	P1					1,1272	POS	
	P1					1,176	POS	
	P2					0,7587	POS	
	P3					0,3888	POS	
	P4					0,756	POS	
	P5					2,074	POS	
	P6					1,0759	POS	
	P6					1,1549	POS	
97637	N1	IDVet - ID SCREEN® CAPRIPOX DOUBLE ANTIGEN MULTI-SPECIES	30%	0,496	0,071	0,081	NEG	S/P%= [(OD sample-ODneg)/(ODpc-ODnc)]*100
	N2					0,062	NEG	
	P1					0,781	POS	
	P1					0,779	POS	
	P2					0,356	POS	
	P3					0,376	POS	
	P4					0,361	POS	
	P5					1,34	POS	
	P6					0,885	POS	
	P6					0,919	POS	
97638	N1	IDVet - ID SCREEN® CAPRIPOX DOUBLE ANTIGEN MULTI-SPECIES	30%	0,906	0,051	0,051	NEG	
	N2					0,06	NEG	
	P1					1,217	POS	
	P1					1,148	POS	
	P2					0,483	POS	
	P3					1,346	POS	
	P4					1,093	POS	

lab ID	Sample ID	Reagens	Cut-off	Pos control	Neg control	OD	Interpretation	Comment
	P5					2,114	POS	
	P6					1,342	POS	
	P6					1,253	POS	

10.2.2 SERO: VIRUS NEUTRALISATION

Lab ID	Sample ID	Protocol / SOP	Name (+ reference) cell type	Name (+ reference) virus strain	Starting dilution of PT serum samples tested	Dilution of PT serum samples tested	Virusdosis in test (TCID50)	Positive control serum	Expected antibody titer in positive control serum	Cut-off	Pos control	Neg control	Operator	Raw data (Ct cp value)	Interpretation	Comment
97506	N1	In House	OA3.Ts	LSDV Neethling	1/2	1/50	100	R6F 45dpi	1/400	1/50	400	2	=	2	NEG	
	N2												=	2	NEG	
	P1												=	6250	POS	
	P1												=	1250	POS	
	P2												=	150	POS	
	P3												=	250	POS	
	P4												=	250	POS	
	P5												=	1250	POS	
	P6												=	750	POS	
	P6												=	1250	POS	
97600	N1	After WAOH terrestrial chapter 3.4.12 LSD 2021	Other	LSDV Neethling	1/10	2	153	IAH Pirbright VN84	480	10	640	10	<	10	NEG	Celltype: SFT-R (CCLV-RIE 43)
	N2												<	10	NEG	
	P1												=	960	POS	
	P1												=	960	POS	
	P2												=	640	POS	
	P3												=	240	POS	
	P4												=	40	POS	
	P5												=	480	POS	
	P6												=	240	POS	
	P6												=	240	POS	
97612	N1	Home made protocol	MDBK	LSDV Neethling	1/5	From 1:5 to 1:640	100	laboratory reference material	1:80	≥1:10	80	10	<	10	NEG	
	N2												<	10	NEG	
	P1												=	320	POS	
	P1												=	320	POS	
	P2												=	160	POS	
	P3												=	160	POS	
	P4												=	20	POS	
	P5												=	320	POS	
	P6												=	160	POS	
	P6												=	160	POS	

10.2.3 SERO: IPMA

lab ID	Sample ID	Protocol / SOP	Name (+ ref) cell type	Name (+ ref) virus strain	Starting dilution of PT serum samples tested	Dilution of PT serum samples tested	Virusdosis in test (TCID50)	Pos control serum	Expected antibody titer in positive control serum	Secondary antibody (+ reference)	Dilution of secondary antibody	Cut-off	Pos control	Neg control	Operator	Raw data (Ct cp value)	Interpretation
97506	N1	Haegeman et al. 2020	OA3.Ts	LSDV Neethling	0,02	0,02	100	R6F 45dpi	0,02	Anti-Bovine Ig G Peroxidase antibody produced in rabbit A 5295 SIGMA	0,0001	0,02	50	1	=	1	NEG
	N2														=	1	NEG
	P1														=	300	POS
	P1														=	300	POS
	P2														=	300	POS
	P3														=	300	POS
	P4														=	300	POS
	P5														=	300	POS
	P6														=	300	POS
	P6														=	300	POS

10.2.4 VIRO: PRIMARY PCR

Lab ID	Sample ID	Protocol/ SOP	Producer Extraction protocol / kit	Extraction protocol / kit	RT-PCR protocol / kit	Target of RT primer	Cut-off	Pos control	Neg control	Operator	Raw data (Ct cp value)	Interpretation	General comments
97506	BN1	Haegeman et al. 2013	Other		Other - Haegeman et al. 2013	D5R	37	28,92	50	=	50	NEG	Extraction kit: Macherey-Nagel Nucleospin Blood kit
	BP1									=	28,04	POS	
	BP2									=	30	POS	
	TN1									=	50	NEG	
	TP1									=	28,75	POS	
	TP1									=	25,18	POS	
	TP2									=	25,15	POS	
	TP3									=	25,64	POS	
	TP4									=	24,79	POS	
	TP5									=	29,1	POS	
97600	BN1	Bowden et al. 2008 modified by FLI	Biosellal	"Biosellal Superball				Bowden et al. 2008 modified by FLI	Biosellal	>	45	NEG	qPCR-Kit: Qiagen QuantiTect Multiplex PCR Kit NoRox
	BP1									=	28,55	POS	
	BP2									=	30,94	POS	
	TN1									>	45	NEG	
	TP1									=	30,67	POS	
	TP1									=	28,89	POS	
	TP2									=	23,19	POS	
	TP3									=	26,99	POS	
	TP4									=	24,71	POS	
	TP5									=	28,12	POS	
97601	BN1	ID Gene	Indical Bioscience	Indispin pathogen kit	IDVet - ID GENE® CAPRIPOX VIRUS TRIPLEX	D5R	40	31,91	0	=	0	NEG	
	BP1									=	27,46	POS	
	BP2									=	27,42	POS	
	TN1									=	0	NEG	
	TP1									=	29,5	POS	
	TP1									=	29,51	POS	
	TP2									=	24,13	POS	
	TP3									=	24,82	POS	
	TP4									=	22,3	POS	
	TP5									=	26,99	POS	
97602 (1)	BN1	Bowden et al.	Indical Bioscience		Other - Bowden et al. 2008	ORF074 (P32)		23,74	45	>	45	NEG	
	BP1									=	31,67	POS	
	BP2									=	35,01	POS	
	TN1									>	45	NEG	
	TP1									=	30,24	POS	
	TP1									=	30,27	POS	
	TP2									=	24,39	POS	
	TP3									=	27,46	POS	
	TP4									=	25,29	POS	
	TP5									=	27,88	POS	
97602 (2)	BN1	ID Gene	Indical Bioscience		IDVet - ID GENE® CAPRIPOX VIRUS TRIPLEX	ORF074 (P32)		22,36	45	>	45	NEG	
	BP1									=	29,7	POS	
	BP2									=	33,08	POS	
	TN1									>	45	NEG	
	TP1									=	28	POS	
	TP1									=	28,78	POS	
	TP2									=	22,98	POS	
	TP3									=	26,3	POS	
	TP4									=	23,3	POS	
	TP5									=	26,42	POS	

Lab ID	Sample ID	Protocol/ SOP	Producer Extraction protocol / kit	Extraction protocol / kit	RT-PCR protocol / kit	Target of RT primer	Cut-off	Pos control	Neg control	Operator	Raw data (Ct cp value)	Interpretation	General comments
97603	BN1	Bowden et al. 2008; Babiuk et al. 2008; SOP from Pirbright	Roche	"Roche-MP 96/ Viral NA SV				Bowden et al. 2008; Babiuk et al. 2008; SOP from Pirbright	Roche	=	0	NEG	
	BP1									=	29,58	POS	
	BP2									=	30,8	POS	
	TN1									=	0	NEG	
	TP1									=	27,69	POS	
	TP1									=	27,52	POS	
	TP2									=	26,55	POS	
	TP3									=	24,62	POS	
	TP4									=	26,54	POS	
	TP5									=	29,92	POS	
97604	BN1	6.3.51	Roche	"Roche-MP 96/ Viral NA SV				6.3.51	Roche	=	0	NEG	
	BP1									=	32,2	POS	
	BP2									=	29,1	POS	
	TN1									=	0	NEG	
	TP1									=	26,2	POS	
	TP1									=	26,5	POS	
	TP2									=	22,8	POS	
	TP3									=	26,4	POS	
	TP4									=	22,4	POS	
	TP5									=	29,5	POS	
97605 (1)	BN1	Bowden et al. 2008	Roche	"Roche-MP 96/ Viral NA SV				Bowden et al. 2008	Roche	>	45	NEG	
	BP1									=	27,14	POS	
	BP2									=	29,13	POS	
	TN1									>	45	NEG	
	TP1									=	26,94	POS	
	TP1									=	26,33	POS	
	TP2									=	22,64	POS	
	TP3									=	24,15	POS	
	TP4									=	23,94	POS	
	TP5									=	28,23	POS	
97605 (2)	BN1	Haegeman et al. 2013	Roche	"Roche-MP 96/ Viral NA SV				Haegeman et al. 2013	Roche	>	45	NEG	
	BP1									=	27,53	POS	
	BP2									=	29,96	POS	
	TN1									>	45	NEG	
	TP1									=	27,48	POS	
	TP1									=	26,95	POS	
	TP2									=	23,27	POS	
	TP3									=	24,65	POS	
	TP4									=	24,22	POS	
	TP5									=	28,71	POS	
97606	BN1	DNA extraction and real time PCR (Bowden et al. 2008)	Qiagen	QIAamp 96 Virus QIAcube HT Kit	Other - Bowden et al. 2008	ORF074 (P32)		37,04	45	=	0	NEG	
	BP1									=	29,8	POS	
	BP2									=	31,9	POS	
	TN1									=	0	NEG	
	TP1									=	29,2	POS	
	TP1									=	28,74	POS	
	TP2									=	24,86	POS	
	TP3									=	25,33	POS	
	TP4									=	24,72	POS	
	TP5									=	27,24	POS	
97607	BN1		Qiagen						Qiagen	>	45	NEG	

Lab ID	Sample ID	Protocol/ SOP	Producer Extraction protocol / kit	Extraction protocol / kit	RT-PCR protocol / kit	Target of RT primer	Cut-off	Pos control	Neg control	Operator	Raw data (Ct cp value)	Interpretation	General comments
	BP1	MO-TE-45 Détection des Capripoxvirus par PCR en temps réel P32		"Qiagen DNAesay Blood and tissue				MO-TE-45 Détection des Capripoxvirus par PCR en temps réel P32		=	29,03	POS	Kit BIORAD SSoAdvanced Universal Probes Supermix (and not as mentionned above SsoFast EvaGreen Supermix 2X)
	BP2									=	31,09	POS	
	TN1									>	45	NEG	
	TP1									=	27,57	POS	
	TP1									=	27,19	POS	
	TP2									=	24,2	POS	
	TP3									=	24,99	POS	
	TP4									=	26,36	POS	
	TP5									=	29,09	POS	
97608 (1)	BN1	Capripox-P32-Bowden	Other		Other - Bowden et al. 2008	ORF074 (P32)	40	28,4	40	>	40	NEG	Extraction kit: NucleoMagVet (Macheray-Nagel) qPCR kit: Pefecta (Quanta Biosciences)
	BP1									=	26,7	POS	
	BP2									=	28,8	POS	
	TN1									>	40	NEG	
	TP1									=	26	POS	
	TP1									=	25,7	POS	
	TP2									=	25,1	POS	
	TP3									=	23,1	POS	
	TP4									=	22,6	POS	
	TP5									=	27,3	POS	
97608 (2)	BN1	Capripox-Pol	Other		Other - Other		40	28,1	40	>	40	NEG	Extraction kit: NucleoMagVet (Macheray-Nagel) qPCR kit: Pefecta (Quanta Biosciences) Target: poly (A) polymerase small subunit region (LSDV068 gene)
	BP1									=	26,7	POS	
	BP2									=	28,9	POS	
	TN1									>	40	NEG	
	TP1									=	26	POS	
	TP1									=	25,6	POS	
	TP2									=	24,9	POS	
	TP3									=	23	POS	
	TP4									=	22,7	POS	
	TP5									=	27,2	POS	
97609 (1)	BN1	Bowden et al Generic Capripoxvirus detection Real Time PCR	Indical Bioscience	Indispin pathogen kit	Other - Bowden et al. 2008	ORF074 (P32)	37	32,18	45	=	45	NEG	RT-PCR kit used:QuantiFast Pathogen+ IC PCR Kit
	BP1									=	27,18	POS	
	BP2									=	29,29	POS	
	TN1									=	45	NEG	
	TP1									=	24,36	POS	
	TP1									=	25,12	POS	
	TP2									=	21,11	POS	
	TP3									=	23,75	POS	
	TP4									=	21,92	POS	
	TP5									=	26,26	POS	
97609 (2)	BN1	Lamien et al 2011 conventional PCR partial GPCR	Indical Bioscience	Indispin pathogen kit	Other - Lamien et al. 2010				0	=	0	NEG	PCR kit used: HotStart Taq Polymerase Qiagen. Pos control GTPV: LSDV/GTPV band (172 bp) Pos control SPPV: SPPV band (151 bp).
	BP1									=	172	POS	
	BP2									=	151	POS	
	TN1									=	0	NEG	
	TP1									=	172	POS	
	TP1									=	172	POS	
	TP2									=	151	POS	
	TP3									=	151	POS	
	TP4									=	172	POS	
	TP5									=	172	POS	

Lab ID	Sample ID	Protocol/ SOP	Producer Extraction protocol / kit	Extraction protocol / kit	RT-PCR protocol / kit	Target of RT primer	Cut-off	Pos control	Neg control	Operator	Raw data (Ct cp value)	Interpretation	General comments
97610	BN1	Bowden et al	Other		Other - Bowden et al. 2008		>40	31,13	40	>	40	NEG	Extraction was made with Kingfisher Flex/Magnifiq Pathogen kit
	BP1									=	34,22	POS	
	BP2									=	36,07	POS	
	TN1									>	40	NEG	
	TP1									=	32,01	POS	
	TP1									=	31,83	POS	
	TP2									=	26,3	POS	
	TP3									=	30,86	POS	
	TP4									=	26,67	POS	
	TP5									=	31,29	POS	
97611	BN1	Hageman et al 2013	Qiagen	" QIAamp Viral RNA Mini Kit				Hageman et al 2013	Qiagen	>	38	NEG	
	BP1									=	24	POS	
	BP2									=	26	POS	
	TN1									>	38	NEG	
	TP1									=	24	POS	
	TP1									=	23	POS	
	TP2									=	18	POS	
	TP3									=	23	POS	
	TP4									=	20	POS	
	TP5									=	24	POS	
97612	BN1	Idvet	Invitrogen/Thermo Fisher Scientific	"MagMAX Core Nucleic Acid Purification Kit				Idvet	Invitrogen/Thermo Fisher Scientific	>	38	NEG	
	BP1									=	29,4	POS	
	BP2									=	31	POS	
	TN1									>	38	NEG	
	TP1									=	30,7	POS	
	TP1									=	30,6	POS	
	TP2									=	26,4	POS	
	TP3									=	25,5	POS	
	TP4									=	23,5	POS	
	TP5									=	27,3	POS	
97613	BN1	Bowden et al. 2008	Indical Bioscience	Indispin pathogen kit	Other - Bowden et al. 2008	ORF074 (P32)	40	25,22	40	>	40	NEG	
	BP1									=	27,07	POS	
	BP2									=	29,28	POS	
	TN1									>	40	NEG	
	TP1									=	26,23	POS	
	TP1									=	25,76	POS	
	TP2									=	21,91	POS	
	TP3									=	24,13	POS	
	TP4									=	20,22	POS	
	TP5									=	29,95	POS	
97614	BN1	SOP G.72	Qiagen	QIAamp Viral RNA Mini Kit				SOP G.72	Qiagen	=	40	NEG	
	BP1									=	25,5	POS	
	BP2									=	29,9	POS	
	TN1									=	40	NEG	
	TP1									=	24,5	POS	
	TP1									=	25,1	POS	
	TP2									=	20,9	POS	
	TP3									=	22,1	POS	
	TP4									=	20,7	POS	
	TP5									=	24,7	POS	
97616	BN1		Qiagen	"QIAmp DNA mini kit					Qiagen	=	0	NEG	

Lab ID	Sample ID	Protocol/ SOP	Producer Extraction protocol / kit	Extraction protocol / kit	RT-PCR protocol / kit	Target of RT primer	Cut-off	Pos control	Neg control	Operator	Raw data (Ct cp value)	Interpretation	General comments
	BP1									=	24,92	POS	
	BP2									=	27,14	POS	
	TN1									=	0	NEG	
	TP1	Path-ID qPCR						Path-ID qPCR		=	25,77	POS	
	TP1									=	30,06	POS	
	TP2									=	21,33	POS	
	TP3									=	24,6	POS	
	TP4									=	22,19	POS	
	TP5									=	27,15	POS	
	BN1									>	40	NEG	
	BP1									=	26,61	POS	
	BP2									=	30,49	POS	
	TN1									>	40	NEG	
97617	TP1	Bowden et al. 2008	Indical Bioscience		Other - Bowden et al. 2008	ORF07 4 (P32)	38	30,05	40	=	30,02	POS	
	TP1									=	29,69	POS	
	TP2									=	24,2	POS	
	TP3									=	23,74	POS	
	TP4									=	23,49	POS	
	TP5									=	26,73	POS	
	BN1									=	0	NEG	
	BP1									=	23,57	POS	
	BP2									=	26,43	POS	
	TN1									=	0	NEG	
97618	TP1	Bowden et al. 2008	Invitrogen/Thermo Fisher Scientific	"Pure Link Genomic DNA Mini Kit (Invitrogen)				Bowden et al. 2008	Invitrogen/Thermo Fisher Scientific	=	21,87	POS	
	TP1									=	21,89	POS	
	TP2									=	20,41	POS	
	TP3									=	19,44	POS	
	TP4									=	19,6	POS	
	TP5									=	25,44	POS	
	BN1									=	0	NEG	
	BP1									=	32,33	POS	
	BP2									=	32,08	POS	
	TN1									=	0	NEG	
97619	TP1	Stubbs et al. 2012			Invitrogen/Thermo Fisher Scientific - Invitrogen/Thermo Fisher Scientific kit	ORF07 4 (P32)	36	33	0	=	27,57	POS	
	TP1									=	28,61	POS	
	TP2									=	24,45	POS	
	TP3									=	26,6	POS	
	TP4									=	24,2	POS	
	TP5									=	28,35	POS	
	BN1									=	-	NEG	
	BP1									=	28,615	POS	
	BP2									=	31,684	POS	
	TN1									=	-	NEG	
97620	TP1	SOP487v2	Qiagen	" QIAamp Viral RNA Mini Kit				SOP487v2	Qiagen	=	29,524	POS	
	TP1									=	28,752	POS	
	TP2									=	23,624	POS	
	TP3									=	30,578	POS	
	TP4									=	24,436	POS	
	TP5									=	28,029	POS	
97621	BN1		Qiagen	MagAttract 96 cador Pathogen Kit		ORF07 4 (P32)	35	35	40	>	40	NEG	
	BP1									=	23,5	POS	

Lab ID	Sample ID	Protocol/ SOP	Producer Extraction protocol / kit	Extraction protocol / kit	RT-PCR protocol / kit	Target of RT primer	Cut-off	Pos control	Neg control	Operator	Raw data (Ct cp value)	Interpretation	General comments
	BP2	Bowden et al. 2008			Other - Bowden et al. 2008					=	24,4	POS	
	TN1									>	40	NEG	
	TP1									=	24,5	POS	
	TP1									=	23,2	POS	
	TP2									=	19,5	POS	
	TP3									=	19,5	POS	
	TP4									=	20,8	POS	
	TP5									=	25,2	POS	
97622	BN1	In-house AVS-0959	Roche	"Roche-MP 96/ Viral NA SV				In-house AVS-0959	Roche	=	45	NEG	Enzyme used: Luna DNA from NEB
	BP1									=	26,2	POS	
	BP2									=	26,5	POS	
	TN1									=	45	NEG	
	TP1									=	26,4	POS	
	TP1									=	27	POS	
	TP2									=	23,7	POS	
	TP3									=	23,8	POS	
	TP4									=	26,9	POS	
	TP5									=	28,6	POS	
97623	BN1	Bowden et al. 2008 and Haegeman et al. 2013	Indical Bioscience	Indispin pathogen kit	Other - Path_ID qPCR	E3L	45	32,41	45	>	45	NEG	We used AgPath-ID PCR kit (13 microlitre mastermix + 2 microlitre of nucleic acid of sample). E3L J6R D5R and GAPDH according to Haegeman et al. 2013 and O74 according to Bowden et al. 2008. Positive control: J6R: 31.66; O74: 32.28; D5R: 32.4; GAPDH: 28.33. Negative control: negative for all CPX genes; GAPDH: 22.56.
	BP1									=	26,2	POS	
	BP2									=	29,43	POS	
	TN1									>	45	NEG	
	TP1									=	31,11	POS	
	TP1									=	25,31	POS	
	TP2									=	22,06	POS	
	TP3									=	23,02	POS	
	TP4									=	25,09	POS	
	TP5									=	27,22	POS	
97624	BN1	SOP 10-BM-001/ Bowden et.al	Other		Invitrogen/Thermo Fisher Scientific - Invitrogen/Thermo Fisher Scientific kit	ORF074 (P32)	38	29	0	=	0	NEG	
	BP1									=	28,9	POS	
	BP2									=	31,3	POS	
	TN1									=	0	NEG	
	TP1									=	26,9	POS	
	TP1									=	28,4	POS	
	TP2									=	25,5	POS	
	TP3									=	27,4	POS	
	TP4									=	25,5	POS	
	TP5									=	29,6	POS	
97627	BN1	IDCPV-100	Qiagen	"Qiagen DNAesay Blood and tissue				IDCPV-100	Qiagen	=		NEG	
	BP1									=	23,35	POS	
	BP2									=	23,7	POS	
	TN1									=		NEG	
	TP1									=	22,16	POS	
	TP1									=	23,43	POS	
	TP2									=	19,56	POS	
	TP3									=	19,69	POS	
	TP4									=	20,1	POS	
	TP5									=	23,63	POS	
97628	BN1	V.N. Shurin.	ID VET	ID Gene Spin Universal Extraction Kit	IDVet - ID GENE® CAPRIPOX			29,63		=		NEG	
	BP1									=	26,77	POS	
	BP2									=	28,93	POS	

Lab ID	Sample ID	Protocol/ SOP	Producer Extraction protocol / kit	Extraction protocol / kit	RT-PCR protocol / kit	Target of RT primer	Cut-off	Pos control	Neg control	Operator	Raw data (Ct cp value)	Interpretation	General comments
	TN1				VIRUS TRIPLEX					=		NEG	
	TP1									=	24,77	POS	
	TP1									=	25,85	POS	
	TP2									=	22,41	POS	
	TP3									=	22,8	POS	
	TP4									=	21,14	POS	
	TP5									=	26,15	POS	
97629	BN1	V.N. Shurin. "Diagnostics of viral diseases of animals". Guide. 1991	ID VET	ID Gene Spin Universal Extraction Kit	IDVet - ID GENE® CAPRIPOX VIRUS TRIPLEX			29,3		=		NEG	
	BP1									=	27,98	POS	
	BP2									=	28,16	POS	
	TN1									=		NEG	
	TP1									=	25,01	POS	
	TP1									=	26,42	POS	
	TP2									=	20,03	POS	
	TP3									=	24,36	POS	
	TP4									=	21,64	POS	
	TP5									=	25,76	POS	
97630	BN1	Wolf et al. 2021	Indical Bioscience	Indispin pathogen kit	Other - Bowden et al. 2008	ORF074 (P32)	N/A	27,92	45	>	45	NEG	Protocol used: Wolf et al. 2021. Probe-Based Real-Time qPCR Assays for a Reliable Differentiation of Capripox Virus Species. Microorganisms. 2021 Apr 6;9(4):765 Master mix PerfeCTa®qPR ToughMix Low rox (Quantabio) Lot 66259208
	BP1									=	27,96	POS	
	BP2									=	28,09	POS	
	TN1									>	45	NEG	
	TP1									=	25,61	POS	
	TP1									=	30,42	POS	
	TP2									=	27,92	POS	
	TP3									=	27,42	POS	
	TP4									=	23,52	POS	
	TP5									=	27,92	POS	
97631	BN1	Bowden et al 2008	Roche	High Pure Viral Nucleic Acid Kit	Qiagen - MO-TO 46		38	28,2		=	-	NEG	
	BP1									=	25,34	POS	
	BP2									=	25,35	POS	
	TN1									=	-	NEG	
	TP1									=	26,37	POS	
	TP1									=	27,43	POS	
	TP2									=	22,46	POS	
	TP3									=	22,57	POS	
	TP4									=	24,07	POS	
	TP5									=	26	POS	
97632	BN1	SOP-685	Sacace Biotechnologies	"Sacace-viral Nucleic acid Extraction				SOP-685	Sacace Biotechnologies	=	0	NEG	
	BP1									=	29,3	POS	
	BP2									=	32	POS	
	TN1									=	0	NEG	
	TP1									=	24,9	POS	
	TP1									=	25,4	POS	
	TP2									=	21,3	POS	
	TP3									=	21,6	POS	
	TP4									=	22	POS	
	TP5									=	25,2	POS	
97634	BN1	Bowden	Biosellal	"Biosellal Superball				Bowden	Biosellal	>	45	NEG	
	BP1									=	28,69	POS	
	BP2									=	29,53	POS	
	TN1									>	45	NEG	

Lab ID	Sample ID	Protocol/ SOP	Producer Extraction protocol / kit	Extraction protocol / kit	RT-PCR protocol / kit	Target of RT primer	Cut-off	Pos control	Neg control	Operator	Raw data (Ct cp value)	Interpretation	General comments
	TP1									=	25,47	POS	
	TP1									=	26,04	POS	
	TP2									=	22,56	POS	
	TP3									=	22,91	POS	
	TP4									=	22,69	POS	
	TP5									=	29,88	POS	
97636	BN1	in-house	Indical Bioscience	Indispin pathogen kit	Invitrogen/Thermo Fisher Scientific - Invitrogen/Thermo Fisher Scientific kit			28,58	0	=	0	NEG	
	BP1									=	28,51	POS	
	BP2									=	29,22	POS	
	TN1									=	0	NEG	
	TP1									=	28,68	POS	
	TP1									=	29,02	POS	
	TP2									=	25,47	POS	
	TP3									=	26,83	POS	
	TP4									=	26,42	POS	
	TP5									=	30,49	POS	
97637	BN1	Bowden et al. 2008	Roche	High Pure Viral Nucleic Acid Kit	Roche - Roche kit			25		=	-	NEG	
	BP1									=	27,62	POS	
	BP2									=	31,89	POS	
	TN1									=	-	NEG	
	TP1									=	27,04	POS	
	TP1									=	25,86	POS	
	TP2									=	20,64	POS	
	TP3									=	21,42	POS	
	TP4									=	21,96	POS	
	TP5									=	26,7	POS	
97638	BN1	Commercial kit	Indical Bioscience	Indispin pathogen kit	IDVet - ID GENE® CAPRIPOX VIRUS TRIPLEX			26,49	0	=	0	NEG	
	BP1									=	21,03	POS	
	BP2									=	21,98	POS	
	TN1									=	0	NEG	
	TP1									=	20,92	POS	
	TP1									=	21,01	POS	
	TP2									=	16,75	POS	
	TP3									=	19,27	POS	
	TP4									=	17,64	POS	
	TP5									=	21,81	POS	

10.2.5 VIRO: SPECIES DIFFERENTIATION

Lab ID	Sample ID	Protocol/ SOP	Producer Extraction protocol / kit	RT-PCR protocol / kit	Methodology	Cut-off	Pos control	Neg control	Operator	Raw data (Ct cp value)	Interpretation	General comments
97506	BN1	Agianniotaki et al. (2016) Chibssa et al. (2018) Wolff et al. (2021) and Haegeman et al. (2016)	Other	Other - Other	Combination of different protocols	45	26,2	50	=	50	NEG	Extraction kit: Macherey-Nagel Nucleospin Blood kit
	BP1								=	29,19	LSDV	
	BP2								=	30,55	SPPV	
	TN1								=	50	NEG	
	TP1								=	29,72	LSDV	
	TP1								=	28,21	LSDV	
	TP2								=	25,53	SPPV	
	TP3								=	26,34	SPPV	
	TP4								=	23,18	LSDV	
	TP5								=	28,46	LSDV	
97600	BN1	Lamien et al. 2011	Biosellal	Other - Lamien et al. 2011	Melting-curve	not applicable			=		NA	Roche LC480 Mix Melt-Curve Analysis
	BP1								=		LSDV	
	BP2								=		SPPV	
	TN1								=		NA	
	TP1								=		LSDV	
	TP1								=		LSDV	
	TP2								=		SPPV	
	TP3								=		SPPV	
	TP4								=		LSDV	
	TP5								=		LSDV	
97602	BN1	Lamien et al. FRET	Indical Bioscience	Other - Lamien et al. 2011			26,38	45	>	45	NEG	
	BP1								=	31,03	LSDV	
	BP2								=	36,29	SPPV	
	TN1								>	45	NEG	
	TP1								=	29,84	LSDV	
	TP1								=	30,02	LSDV	
	TP2								=	27,16	SPPV	
	TP3								=	28,96	SPPV	
	TP4								=	25,84	LSDV	
	TP5								=	28,63	LSDV	
97603	BN1	Lamien et al. 2011	Roche	Other - Lamien et al. 2011	Classical PCR amplification of a portion of the RP030 gene: conventional	151 bp product for SPPV 172 bp for GTPV and LSDV	172	0	=	0	NEG	
	BP1								=	172	LSDV	
	BP2								=	151	SPPV	
	TN1								=	0	NEG	
	TP1								=	172	LSDV	
	TP1								=	172	LSDV	
	TP2								=	151	SPPV	
	TP3								=	151	SPPV	
	TP4								=	172	LSDV	
	TP5								=	172	LSDV	
97604	BN1	6.3.51	Roche	Qiagen - MO-TO 47					=		NEG	
	BP1								=		LSDV	
	BP2								=		SPPV	
	TN1								=		NEG	
	TP1								=		LSDV	
	TP1								=		LSDV	
	TP2								=		SPPV	
	TP3								=		SPPV	
	TP4								=		LSDV	
	TP5								=		LSDV	

Lab ID	Sample ID	Protocol/ SOP	Producer Extraction protocol / kit	RT-PCR protocol / kit	Methodology	Cut-off	Pos control	Neg control	Operator	Raw data (Ct cp value)	Interpretation	General comments
97607 (1)	BN1	MO-TE-47 Détection des DNCV par PCR en temps réel GPCR	QIAGEN	Bio-Rad - SsoFast™ EvaGreen® Supermix 2X	Taqman real-time PCR (home made)	45 cycles	32,73	45	>	45	NEG	Kit BIORAD SSoAdvanced Universal Probes Supermix (and not as mentionned above SsoFast EvaGreen Supermix 2X)
	BP1								=	30,98	LSDV	
	BP2								>	45	NEG	
	TN1								>	45	NEG	
	TP1								=	28,29	LSDV	
	TP1								=	28,29	LSDV	
	TP2								>	45	NEG	
	TP3								>	45	NEG	
	TP4								=	28,28	LSDV	
	TP5								=	30,64	LSDV	
97607 (2)	BN1	MO-TE-57 : Détection des SPPV et GTPV par PCR en temps réel	QIAGEN	Bio-Rad - SsoFast™ EvaGreen® Supermix 2X	Taqman RT-PCR (Wolff J. Microorganism 2021 9 765)	45 cycles	33,47	45	>	45	NEG	
	BP1								>	45	NEG	
	BP2								=	32,71	SPPV	
	TN1								>	45	NEG	
	TP1								>	45	NEG	
	TP1								>	45	NEG	
	TP2								=	24,16	SPPV	
	TP3								=	26,03	SPPV	
	TP4								>	45	NEG	
	TP5								>	45	NEG	
97608 (1)	BN1	LSDfield-ORF126-Mix11-Taq-FAM /LSDvac-Mix5-Taq-HEX und SPPV-ORF041-Mix1-MGB-FAM/GTPV-ORF095-Mix1-MGB-HEX	Other	Other - Wolff et al. 2021 (Both duplexes)	LSDfield-ORF126-Mix11-Taq-FAM /LSDvac-Mix5-Taq-HEX und SPPV-ORF041-Mix1-MGB-FAM/GTPV-ORF095-Mix1-MGB-HEX	40	24	40	>	40	NEG	Extraction kit: NucleoMagVet (Macheray-Nagel) qPCR kit: Pefecta (Quanta Biosciences)
	BP1								>	40	LSDV	
	BP2								=	31,2	SPPV	
	TN1								>	40	NEG	
	TP1								=	40	LSDV	
	TP1								>	40	LSDV	
	TP2								=	26,1	SPPV	
	TP3								=	24,6	SPPV	
	TP4								=	23,8	LSDV	
	TP5								=	28,4	LSDV	
97608 (2)	BN1	LSD-WTR-Mix-FAM/LSD-VR-Mix-HEX	Other	Other - Other		40	26,3	40	>	40	NEG	Extraction kit: NucleoMagVet (Macheray-Nagel) qPCR kit: Pefecta (Quanta Biosciences) Targe: Haegeman et al 2023
	BP1								=	29,1	LSDV	
	BP2								>	40	NI	
	TN1								>	40	NEG	
	TP1								=	28,1	LSDV	
	TP1								=	28	LSDV	
	TP2								>	40	NI	
	TP3								>	40	NI	
	TP4								=	23,6	LSDV	
	TP5								=	29,4	LSDV	
97610	BN1	Lamien et al 2011	Other	Other - Lamien et al. 2011	real-time PCR+melting	>40	29,2	40	>	40	NEG	Extraction was made with Kingfisher Flex/Magnifiq Pathogen kit
	BP1								=	36,8	LSDV	
	BP2								=	36,8	SPPV	
	TN1								>	40	NEG	
	TP1								=	33,8	LSDV	
	TP1								=	33,9	LSDV	
	TP2								=	29,8	SPPV	
	TP3								=	32,1	SPPV	

Lab ID	Sample ID	Protocol/ SOP	Producer Extraction protocol / kit	RT-PCR protocol / kit	Methodology	Cut-off	Pos control	Neg control	Operator	Raw data (Ct cp value)	Interpretation	General comments
	TP4								=	28,9	LSDV	
	TP5								=	33,4	LSDV	
97611 (1)	BN1 BP1 BP2 TN1 TP1 TP1 TP2 TP3 TP4 TP5	Multiplex real time Wolff et al 2021 and Galaye et al 2017	QIAGEN	Other - Galaye et al. 2017	In-house Melt curve PCR test using Qiagen Quantitect SYBR Green chemistry	HRM:Sheep: Tm 75.00Goat: Tm 74.40LSD= Tm76 Neg: Tm 70 or No Cq or > 37	75	70	= = = = = = = = = =	70 75,8 74,8 70 75,8 75,8 74,8 74,8 75,8 75,8	NEG LSDV SPPV NEG LSDV LSDV SPPV SPPV LSDV LSDV	SPPV: 74.5-75 LSD: 75.2-76
97611 (2)	BN1 BP1 BP2 TN1 TP1 TP1 TP2 TP3 TP4 TP5	wolff et al2021	QIAGEN	Other - Wolff et al. 2021 (SPPV/GTPV)	In-House Multiplex PCR	38	20	38	> > = > > > = = > >	38 38 27 38 38 38 19 24 38 38	NEG NEG SPPV NEG NEG NEG SPPV SPPV NEG NEG	SPPV=20
97612 (1)	BN1 BP1 BP2 TN1 TP1 TP1 TP2 TP3 TP4 TP5	IZS TE B456.1 SOP021	Thermo Fisher Scientific	Other - Other	Duplex PCR real time	>38	25,3	38	> = > > = = > > = =	38 29,5 38 38 28,3 31,4 38 38 24,6 26,4	NEG LSDV NEG NEG LSDV LSDV NEG NEG LSDV LSDV	The IZS TE B456.1 SOP021 uses the commercial kit Bio-T kit Lumpy Skin Disease- Biosellal. This assay detected only LSDV.
97612 (2)	BN1 BP1 BP2 TN1 TP1 TP1 TP2 TP3 TP4 TP5	E. Gelaye et al. 2017	Thermo Fisher Scientific	Other - Galaye et al. 2017	PCR HRM		30	32	= = = = = = = = = =	33,9 25,7 29,2 32,1 23,3 27,4 22,9 23,6 20,9 22,5	NEG LSDV SPPV NEG LSDV LSDV SPPV SPPV LSDV LSDV	
97612 (3)	BN1 BP1 BP2 TN1 TP1	J. Wolff et al. 2021	Thermo Fisher Scientific	Other - Other	PCR Real Time	>38			> > > > >	38 38 38 38 38	NEG NEG NEG NEG NEG	This assay (J. Wolff et al. 2021) is used in singleplex to detect only GTPV.

Lab ID	Sample ID	Protocol/ SOP	Producer Extraction protocol / kit	RT-PCR protocol / kit	Methodology	Cut-off	Pos control	Neg control	Operator	Raw data (Ct cp value)	Interpretation	General comments
	TP1								>	38	NEG	
	TP2								>	38	NEG	
	TP3								>	38	NEG	
	TP4								>	38	NEG	
	TP5								>	38	NEG	
97613	BN1	Lamien et al. 2011	Indical Bioscience	Other - Lamien et al. 2011			27,7	0	=	0	NEG	
	BP1								=	27,8	LSDV	
	BP2								=	37,11	SPPV	
	TN1								=	0	NEG	
	TP1								=	26,83	LSDV	
	TP1								=	27,65	LSDV	
	TP2								=	30,75	SPPV	
	TP3								=	33,77	SPPV	
	TP4								=	24,19	LSDV	
	TP5								=	27,74	LSDV	
97614	BN1	SOP.G.72	QIAGEN	Other - Lamien et al. 2011		38	26	40	=	40	NEG	
	BP1								=	32	LSDV	
	BP2								=	35	SPPV	
	TN1								=	40	NEG	
	TP1								=	30	LSDV	
	TP1								=	30	LSDV	
	TP2								=	26	SPPV	
	TP3								=	27	SPPV	
	TP4								=	27	LSDV	
	TP5								=	30	LSDV	
97617 (1)	BN1	Chibssa et al. 2018	Indical Bioscience	Other - Chibssa et al. 2018	Conventional PCR	N/A	218	0	=	0	NEG	
	BP1								=	338	LSDV	
	BP2								=	218	SPPV	
	TN1								=	0	NEG	
	TP1								=	338	LSDV	
	TP1								=	338	LSDV	
	TP2								=	302	SPPV/GTPV	
	TP3								=	302	SPPV/GTPV	
	TP4								=	338	LSDV	
	TP5								=	338	LSDV	
97617 (2)	BN1	Lamien et al. 2011	Indical Bioscience	Other - Lamien et al. 2011	Conventional PCR	N/A			=		NA	
	BP1								=		NA	
	BP2								=		NA	
	TN1								=		NA	
	TP1								=		NA	
	TP1								=		NA	
	TP2								=	151	SPPV	
	TP3								=	151	SPPV	
	TP4								=		NA	
	TP5								=		NA	
97618	BN1	in house	Thermo Fisher Scientific	Other - Other					=		NEG	This method was not tested as in the previous years by Galaye E. et al. 2015 protocol due to the lack of the reactive and reagents for sequencing as within the NRL this was the single method available for the species differentiation.
	BP1								=		LSDV	
	BP2								=		SPPV	
	TN1								=		NEG	
	TP1								=		LSDV	
	TP1								=		LSDV	
	TP2								=		SPPV	

Lab ID	Sample ID	Protocol/ SOP	Producer Extraction protocol / kit	RT-PCR protocol / kit	Methodology	Cut-off	Pos control	Neg control	Operator	Raw data (Ct cp value)	Interpretation	General comments
	TP3								=		SPPV	
	TP4								=		LSDV	
	TP5								=		LSDV	
97619	BN1	Lamien et al. 2017	Other	Other - Lamien et al. 2017	Sanger sequencing	no app	33	0	=	0	NEG	
	BP1								=	32	LSDV	
	BP2								=	32	SPPV	
	TN1								=	0	NEG	
	TP1								=	27	LSDV	
	TP1								=	28	LSDV	
	TP2								=	24	SPPV	
	TP3								=	26	SPPV	
	TP4								=	24	LSDV	
	TP5								=	28	LSDV	
97620	BN1	Lamien et al 2011	QIAGEN	Other - Lamien et al. 2011	PCR	/	151		=	-	NEG	Conventional PCR for species differentiation based on the PCR product length. Result is expresed as PCR product length in bp.
	BP1								=	172	LSDV	
	BP2								=	151	SPPV	
	TN1								=	-	NEG	
	TP1								=	172	LSDV	
	TP1								=	172	LSDV	
	TP2								=	151	SPPV	
	TP3								=	151	SPPV	
	TP4								=	172	LSDV	
	TP5								=	172	LSDV	
97621	BN1	Lamien et al. 2011	QIAGEN	Other - Lamien et al. 2011	Conventional PCR (differential amplicon by size)				=		NEG	
	BP1								=		LSDV	
	BP2								=		SPPV	
	TN1								=		NEG	
	TP1								=		LSDV	
	TP1								=		LSDV	
	TP2								=		SPPV	
	TP3								=		SPPV	
	TP4								=		LSDV	
	TP5								=		LSDV	
97628	BN1	ST RK 3503-2019. Animals.Methods of laboratory diagnosis of LSDV. ST RK 3904-2023 LAB DIAGNOSTICS OF SHEEP AND GOAT SMALLPOX	IDVet	Other - Other			17,94		=		NEG	RT-PCR protocol/kit - 1)PCR-LSDV-CATTLE-FACTOR test system for detecting DNA of Lumpy Skin Disease virus LSDV by real-time PCR VET-FACTOR LLC Moscow Russia 2) A set of reagents "PCR-SMALLPOX-FACTOR" for detecting sheep and goat smallpox virus (Variola ovium) DNA in biological material by polymerase chain reaction (PCR) with real-time fluorescence detection
	BP1								=	22,21	LSDV	
	BP2								=	25,09	SPPV/GTPV	
	TN1								=		NEG	
	TP1								=	20,33	LSDV	
	TP1								=	20,77	LSDV	
	TP2								=	18,32	SPPV/GTPV	
	TP3								=	18,74	SPPV/GTPV	
	TP4								=	16,47	LSDV	
	TP5								=	21,27	LSDV	
97629	BN1	ST RK 3503-2019. Animals.Methods of laboratory	IDVet	Other - Other			15,9		=		NEG	RT-PCR protocol/kit: 1) PCR-SMALLPOX-FACTOR reagent kit for detecting sheep and goat smallpox virus (Variola ovium) DNA in biological material by polymerase chain reaction (PCR) with real-time fluorescence detection.
	BP1								=	22,72	LSDV	
	BP2								=	24,33	SPPV/GTPV	
	TN1								=		NEG	
	TP1								=	19,75	LSDV	

Lab ID	Sample ID	Protocol/ SOP	Producer Extraction protocol / kit	RT-PCR protocol / kit	Methodology	Cut-off	Pos control	Neg control	Operator	Raw data (Ct cp value)	Interpretation	General comments
	TP1	diagnosis of LSDV. ST RK 3904-2023 LAB DIAGNOSTICS OF SHEEP & GOAT SMALLPOX							=	21,81	LSDV	2)PCR-LSDV-CATTLE-FACTOR test system for detecting DNA of Lumpy Skin Disease virus LSDV by real-time PCR VET-FACTOR LLC Moscow Russia.
	TP2								=	15,79	SPPV/GTPV	
	TP3								=	21,33	SPPV/GTPV	
	TP4								=	16,25	LSDV	
	TP5								=	20,61	LSDV	
97630	BN1	Wolf et al. 2021	Indical Bioscience	Other - Wolff et al. 2021 (Both duplexes)	Wolf et al. 2021	N/A	32,51	45	>	45	NEG	Protocol used: Wolf et al. 2021. Probe-Based Real-Time qPCR Assays for a Reliable Differentiation of Capripox Virus Species. Microorganisms. 2021 Apr 6;9(4):765 Master mix PerfeCTa®qPR ToughMix Low rox (Quantabio) Lot 66259208
	BP1								>	45	NI	
	BP2								=	30,66	SPPV	
	TN1								>	45	NEG	
	TP1								>	45	NI	
	TP1								>	45	NI	
	TP2								=	23,52	SPPV	
	TP3								=	29,88	SPPV	
	TP4								=	24,07	LSDV	
	TP5								=	29,44	LSDV	
97631	BN1	Vidanovic ...	Roche	Qiagen - MO-TO 47		38	28,15		=	-	NEG	
	BP1								=	23,83	LSDV	
	BP2								=	25,15	SPPV	
	TN1								=	-	NEG	
	TP1								=	23,2	LSDV	
	TP1								=	28,3	LSDV	
	TP2								=	22,03	SPPV	
	TP3								=	21,42	SPPV	
	TP4								=	24,63	LSDV	
	TP5								=	26,77	LSDV	
97632 (1)	BN1	Wolf et al 2021	Sacace Biotechnologies	Other - Wolff et al. 2021 (LSDV Vac/WT)		38	30,5	0	=	0	NEG	
	BP1								=	0	NEG	
	BP2								=	0	NEG	
	TN1								=	0	NEG	
	TP1								=	0	NEG	
	TP1								=	0	NEG	
	TP2								=	0	NEG	
	TP3								=	0	NEG	
	TP4								=	21,3	LSDV	
	TP5								=	26,3	LSDV	
97632 (2)	BN1	Wolf et al 2021	Sacace Biotechnologies	Other - Wolff et al. 2021 (SPPV/GTPV)		38	35	0	=	0	NEG	
	BP1								=	0	NEG	
	BP2								=	32,3	SPPV	
	TN1								=	0	NEG	
	TP1								=	0	NEG	
	TP1								=	0	NEG	
	TP2								=	21,7	SPPV	
	TP3								=	21,6	SPPV	
	TP4								=	0	NEG	
	TP5								=	0	NEG	
97632 (3)	BN1	Sprygin et al 2019-Real	Sacace Biotechnologies	Other - Other		38			=	0	NEG	
	BP1								=	29,7	LSDV	

Lab ID	Sample ID	Protocol/ SOP	Producer Extraction protocol / kit	RT-PCR protocol / kit	Methodology	Cut-off	Pos control	Neg control	Operator	Raw data (Ct cp value)	Interpretation	General comments
	BP2	time PCR screening assay for universal detection of LSDV							=	0	NEG	
	TN1								=	0	NEG	
	TP1								=	25	LSDV	
	TP1								=	25,9	LSDV	
	TP2								=	0	NEG	
	TP3								=	0	NEG	
	TP4								=	22,3	LSDV	
	TP5								=	25,7	LSDV	
97634	BN1	Wolff	Biosellal	Other - Wolff et al. 2021 (SPPV/GTPV)		40	30	45	=	45	NEG	
	BP1								>	45	LSDV	
	BP2								=	26,12	SPPV	
	TN1								=	45	NEG	
	TP1								=	45	LSDV	
	TP1								=	45	LSDV	
	TP2								=	21,59	SPPV	
	TP3								=	21,7	SPPV	
	TP4								=	45	LSDV	
	TP5								=	45	LSDV	
97637 (1)	BN1	Wolf et al 2021;	Roche	Other - Wolff et al. 2021 (SPPV/GTPV)					=		NA	
	BP1								=		NA	
	BP2								=	36,7	SPPV	
	TN1								=	-	NEG	
	TP1								=		NA	
	TP1								=		NA	
	TP2								=	23,19	SPPV	
	TP3								=	28,47	SPPV	
	TP4								=		NA	
	TP5								=		NA	
97637 (2)	BN1	Vidanovic et al. 2021	Roche	Other - Vidanovic et al. 2021	Real-time PCR				=	-	NEG	
	BP1								=	26,93	LSDV	
	BP2								=		NA	
	TN1								=		NA	
	TP1								=	26,58	LSDV	
	TP1								=	26,13	LSDV	
	TP2								=		NA	
	TP3								=		NA	
	TP4								=	24,4	LSDV	
	TP5								=	25,42	LSDV	
97638	BN1	Commercial kit	Indical Bioscience	Other - Other			22,99	0	=	0	NEG	
	BP1								=	21,85	LSDV	
	BP2								=		NA	
	TN1								=	0	NEG	
	TP1								=	21,82	LSDV	For species differentiation PCR we used commercial kit: Bio-T Kit Lumpy Skin Disease - DIVA Biosellal
	TP1								=	21,82	LSDV	
	TP2								=		NA	
	TP3								=		NA	
	TP4								=	18,13	LSDV	
	TP5								=	21,47	LSDV	

10.2.6 VIRO: DIVA PCR

Lab ID	Sample	Protocol / SOP	Prod Extrac tion protocol/kit	Extraction protocol/kit	RT-PCR protocol/kit	Methodology	Cut-off	Pos control	Neg control	Operator	Ct cp value	Interpretation	General comments
97506	BN1	Agiannio taki et al. (2016) Chibssa et al. (2018) Wolff et al. (2021) & Haegem an et al. (2023)	Other	Addition of EC	Other - Other	Combiati on of methods	45	29,49	50	=	50	NEG	Extraction kit: Macherey-Nagel Nucleospin Blood kit
	BP1									=	29,19	LSDV Rec (clade 2)	
	BP2									=	30,55	SPPV Vaccine (RM65/Roman ia)	
	TN1									=	50	NEG	
	TP1									=	29,72	LSDV Rec (clade 2)	
	TP1									=	28,21	LSDV Rec (clade 2)	
	TP2									=	25,53	SPPV Wild	
	TP3									=	26,34	SPPV Wild	
	TP4									=	25,76	LSDV Vaccine (clade 1.1)	
	TP5									=	20,78	LSDV Wild (clade 1.2)	
97600	BN1	Haegem an et al. 2023	Biosellal		Other - Haegeman et al. 2023		50	27,8	50	=		NA	SPPV Differentiation with classical PCR published by Haegemann et al. 2014
	BP1									=	32,25	LSDV Rec (clade 2)	
	BP2									=		SPPV Vaccine (RM65/Roman ia)	
	TN1									=		NA	
	TP1									=	34,37	LSDV Rec (clade 2)	
	TP1									=	32,13	LSDV Rec (clade 2)	
	TP2									=		SPPV Wild	
	TP3									=		SPPV Wild	
	TP4									=	27,84	LSDV Vaccine (clade 1.1)	
	TP5									=	31,21	LSDV Wild (clade 1.2)	
97602	BN1	Haegem an et al. 2023	Indical Bioscience		Other - Haegeman et al. 2023			31,84	45	>	45	NEG	
	BP1									=	31,99	LSDV Rec (clade 2)	
	BP2									>	45	SPPV Vaccine (RM65/Roman ia)	
	TN1									>	45	NEG	
	TP1									=	30,13	LSDV Rec (clade 2)	
	TP1									=	30,95	LSDV Rec (clade 2)	
	TP2									=	34,01	SPPV Wild	
	TP3									>	45	SPPV Wild	
	TP4									>	45	LSDV Vaccine (clade 1.1)	
	TP5									=	29,12	LSDV Wild (clade 1.2)	
	BN1		Other				40	26,3	40	>	40	NEG	Extraction kit: NucleoMagVet (Macheray-Nagel)

Lab ID	Sample	Protocol / SOP	Prod Extrac tion protocol/kit	Extraction protocol/kit	RT-PCR protocol/kit	Methodology	Cut-off	Pos control	Neg control	Operator	Ct value cp	Interpretation	General comments
97608 (1)	BP1	LSD-WTR-Mix-FAM/LSD-VR-Mix-HEX			Other - Haegeman et al. 2023					=	29,1	LSDV Rec (clade 2)	qPCR kit: Pefecta (Quanta Biosciences)
	BP2									>	40	SPPV Vaccine (RM65/Romania)	
	TN1									>	40	NEG	
	TP1									=	28,1	LSDV Rec (clade 2)	
	TP1									=	28	LSDV Rec (clade 2)	
	TP2									>	40	SPPV Wild	
	TP3									>	40	SPPV Wild	
	TP4									=	23,6	LSDV Vaccine (clade 1.1)	
	TP5									=	29,4	LSDV Wild (clade 1.2)	
97608 (2)	BN1	SPPV-DIVA Mix	Other		Other - Chibssa et al. 2018	SPPV-DIVA Mix	40			>		NEG	Extraction kit: NucleoMagVet (Macheray-Nagel) qPCR kit: Pefecta (Quanta Biosciences)
	BP1									=		LSDV Rec (clade 2)	
	BP2									>		SPPV Vaccine (RM65/Romania)	
	TN1									=		NEG	
	TP1									=		LSDV Rec (clade 2)	
	TP1									=		LSDV Rec (clade 2)	
	TP2									=		SPPV Wild	
	TP3									=		SPPV Wild	
	TP4									=		LSDV Vaccine (clade 1.1)	
	TP5									=		LSDV Wild (clade 1.2)	
97609 (1)	BN1	DIVA LSDV Real Time PCR Agiannio taki et al 2017	Indical Bioscience		Other - Agianniotaki et al. 2017		38	30,64	45	=	0	NEG	PCR kit used: Platinum Taq DNA Polymerase Invitrogen.
	BP1									=	26,54	LSDV Vaccine (clade 1.1)	
	BP2									=		NA	
	TN1									=		NA	
	TP1									=	26,2	LSDV Vaccine (clade 1.1)	
	TP1									=	25,75	LSDV Vaccine (clade 1.1)	
	TP2									=		NA	
	TP3									=		NA	
	TP4									=	22,91	LSDV Vaccine (clade 1.1)	
	TP5									=	27,11	LSDV Wild (clade 1.2)	
97609 (2)	BN1	Partial GPCR (3F/3R) PCR and Sequencing	Indical Bioscience		Other - Gelaye et al. 2015					=		NA	PCR kit used: HotStart Taq DNA Polymerase kit Qiagen
	BP1									=		LSDV Rec (clade 2)	
	BP2									=		SPPV Vaccine (RM65/Romania)	

Lab ID	Sample	Protocol / SOP	Prod Extrac tion protocol/kit	Extraction protocol/kit	RT-PCR protocol/kit	Method ology	Cut-off	Pos control	Neg control	Operator	Ct value cp	Interpretation	General comments
	TN1									=		NA	
	TP1									=		LSDV Rec (clade 2)	
	TP1									=		LSDV Rec (clade 2)	
	TP2									=		SPPV Wild	
	TP3									=		SPPV Wild	
	TP4									=		LSDV Rec (clade 2)	
	TP5									=		LSDV Wild (clade 1.2)	
	BN1									=		NA	
	BP1									=	31,03	LSDV Vaccine (clade 1.1)	
	BP2									=		NA	
	TN1									=		NA	
	TP1									=	26,75	LSDV Vaccine (clade 1.1)	
97610	TP1	Vidanovic 2021	Other		Other - Vidanovic et al. 2021		>40	29,2	40	=	28,81	LSDV Vaccine (clade 1.1)	Extraction was made with Kingfisher Flex/Magnifiq Pathogen kit
	TP2									=		NA	
	TP3									=		NA	
	TP4									=	24,9	LSDV Vaccine (clade 1.1)	
	TP5									=	29,3	LSDV Wild (clade 1.2)	
	BN1									=		NA	
	BP1									=	24,5	LSDV Rec (clade 2)	
	BP2									=		NA	
	TN1									=		NA	
	TP1									=	24,6	LSDV Rec (clade 2)	
97611 (1)	TP1	Agiannio taki et al 2017	QIAGEN		Other - Agianniotaki et al. 2017	Multiplex real time LSDV	37	37	37	=	24,6	LSDV Rec (clade 2)	Neethling vacc control= No Cq(FAM) HEX=16.9 Recombinant vacc= No Cq (FAM) HEX=26.3 LSD= Cq 26.3 (FAM) HEX =Neg Our Lab does not differentiate between SPPV vaccine and field strain.
	TP2									=		NA	
	TP3									=		NA	
	TP4									=	21,1	LSDV Vaccine (clade 1.1)	
	TP5									=	26,7	LSDV Wild (clade 1.2)	
	BN1									=		NA	
	BP1									=		NA	
	BP2									=		SPPV Vaccine (RM65/Romania)	
	TN1	T.R. Chibssa et al. 2018	Thermo Fisher Scientific		Other - Chibssa et al. 2018	Gel based PCR				=		NA	The method is used to differentiate SPPV Wild from SPPV vaccine. Therefore we analyzed only SPPV positive samples in PCR-HRM Gelaye et al. 2017.
97612 (1)	TP1									=		NA	
	TP1									=		NA	
	TP2									=		SPPV Wild	
	TP3									=		SPPV Wild	
	TP4									=		NA	
	TP5									=		NA	

Lab ID	Sample	Protocol / SOP	Prod Extrac tion protocol/kit	Extraction protocol/kit	RT-PCR protocol/kit	Methodology	Cut-off	Pos control	Neg control	Operator	Ct value	cp	Interpretation	General comments
97612 (2)	BN1	A. Krotova et al. 2023	Thermo Fisher Scientific		Other - Other	LSDV ORF134 PCR Amplification and Sanger sequencing				=			NA	This method is used to differentiate among recombinant field and vaccine LSDV strains. We tested only the LSDV positive samples in TE IZS B456.1 SOP021.
	BP1									=			LSDV Rec (clade 2)	
	BP2									=			NA	
	TN1									=			NA	
	TP1									=			LSDV Rec (clade 2)	
	TP1									=			LSDV Rec (clade 2)	
	TP2									=			NA	
	TP3									=			NA	
	TP4									=			LSDV Vaccine (clade 1.1)	
	TP5									=			LSDV Wild (clade 1.2)	
97613 (1)	BN1	Chibssa et al. 2018	Indical Bioscience	No	Qiagen - MO-TO 47					=			NA	
	BP1									=			NA	
	BP2									=			SPPV Vaccine (RM65/Romania)	
	TN1									=			NA	
	TP1									=			NA	
	TP1									=			NA	
	TP2									=			SPPV Wild	
	TP3									=			SPPV Wild	
	TP4									=			NA	
	TP5									=			NA	
97613 (2)	BN1	Agiannio taki et al. 2017	Indical Bioscience	No	Other - Agianniotaki et al. 2017			28,17	0	=	0		NEG	
	BP1									=	27,23		LSDV Vaccine (clade 1.1)	
	BP2									=	0		NEG	
	TN1									=	0		NEG	
	TP1									=	27,4		LSDV Vaccine (clade 1.1)	
	TP1									=	27,04		LSDV Vaccine (clade 1.1)	
	TP2									=	0		NEG	
	TP3									=	0		NEG	
	TP4									=	23,33		LSDV Vaccine (clade 1.1)	
	TP5									=	27,29		LSDV Wild (clade 1.2)	
97613 (3)	BN1	Haegeman et al. 2023	Indical Bioscience	No	Other - Haegeman et al. 2023					=	0		NEG	
	BP1									=	32,42		LSDV Wild (clade 1.2)	
	BP2									=	0		NEG	
	TN1									=	0		NEG	
	TP1									=	32,98		LSDV Wild (clade 1.2)	
	TP1									=	30,3		LSDV Wild (clade 1.2)	
	TP2									=	0		NEG	
	TP3									=	0		NEG	

Lab ID	Sample	Protocol / SOP	Prod Extrac tion protocol/kit	Extraction protocol/kit	RT-PCR protocol/kit	Method ology	Cut-off	Pos control	Neg control	Operator	Ct value cp	Interpretation	General comments
	TP4									=	24,37	LSDV Vaccine (clade 1.1)	
	TP5									=	31,14	LSDV Wild (clade 1.2)	
	BN1									=		NEG	
	BP1									=		LSDV Rec (clade 2)	
	BP2									=		SPPV Vaccine (RM65/Romania)	
	TN1									=		NEG	
97613 (4)	TP1	Gelaye et al. 2015	Indical Bioscience	No	Qiagen - MO-TO 47					=		LSDV Rec (clade 2)	
	TP1									=		LSDV Rec (clade 2)	
	TP2									=		SPPV Wild	
	TP3									=		SPPV Wild	
	TP4									=		LSDV Vaccine (clade 1.1)	
	TP5									=		LSDV Wild (clade 1.2)	
	BN1									=		NA	
	BP1									=	57,65	LSDV Vaccine (clade 1.1)	
	BP2									=		NA	
	TN1									=		NA	
	TP1									=	57,65	LSDV Vaccine (clade 1.1)	
97617 (1)	TP1	Menasherow et al. 2014	Indical Bioscience		Other - Other			57	0	=	57,65	LSDV Vaccine (clade 1.1)	The method used was that described by Menasherow et al. 2014. Haegemann et al. 2023 was performed to distinguish between recombinant strains.
	TP2									=		NA	
	TP3									=		NA	
	TP4									=	57,65	LSDV Vaccine (clade 1.1)	
	TP5									=	57	LSDV Wild (clade 1.2)	
	BN1									=		NA	
	BP1									=	29,19	LSDV Rec (clade 2)	
	BP2									=		NA	
	TN1									=		NA	
	TP1									=	33,04	LSDV Rec (clade 2)	
97617 (2)	TP1	Haegemann et al. 2023	Indical Bioscience		Other - Haegeman et al. 2023	Real-time PCR	38	27,72	40	=	28,24	LSDV Rec (clade 2)	DIVA discrimination of LSDV strains was performed based on the results obtained in both tests (Menasherow et al. 2014 and Haegemann et al. 2023)
	TP2									=		NA	
	TP3									=		NA	
	TP4									=	24,88	LSDV Vaccine (clade 1.1)	
	TP5									=	28,95	LSDV Wild (clade 1.2)	
97618	BN1	DIVA GREEC E	Thermo Fisher Scientific		Other - Agianniotaki et al. 2017	Real-time PCR		29,99	0	=	0	NEG	RT-PCR protocol / kit - Invitrogen/ThermoFisher Scientific/Platinum Taq DNA Polymerase PCR protocol kit - Taq DNA Polymerase (Qiagen). The
	BP1									=	29,64	LSDV Vaccine (clade 1.1)	

Lab ID	Sample	Protocol / SOP	Prod Extrac tion protocol/kit	Extraction protocol/kit	RT-PCR protocol/kit	Methodology	Cut-off	Pos control	Neg control	Operator	Ct value cp	Interpretation	General comments
	BP2	Agiannio taki et al 2017				method for samples 3 4 5 6 8 9 and 10 PCR method for samples 1 2 and 7				=	0	SPPV Vaccine (RM65/Romania)	protocol implemented within the NRL for Capripox viruses is not able to differentiate between the LSVD Vaccine and LSDV Rec. The results have been interpreted by comparing the presence and aspect of the curves of the LSDV Rec sample from the 2025 PT that have been used as control and those from the present PT. The Romanian NRL for Capripox viruses has to implement the EURL protocol for DIVA testing and species differentiation and will kindly ask for the EURL support by training and guidance providing.
	TN1									=	0	NEG	
	TP1									=	27,72	LSDV Rec (clade 2)	
	TP1									=	27,97	LSDV Rec (clade 2)	
	TP2									=	0	SPPV Wild	
	TP3									=	0	SPPV Wild	
	TP4									=	25,88	LSDV Rec (clade 2)	
	TP5									=	25,44	LSDV Wild (clade 1.2)	
97619	BN1	Haegeman et al. 2023 / Vidanović et al. 2021		Other: Thechemagic 360 instrument Revvity	Other - Haegeman et al. 2023		no app	33	0	=	0	NEG	LSD DIVA PCR. FAM positive is wild type strain (PCR does not differentiate between wild and recombinant) VIC positive is vaccine strain.
	BP1									=	33,55	LSDV Rec (clade 2)	
	BP2									=	33,41	SPPV Vaccine (RM65/Romania)	
	TN1									=	0	NEG	
	TP1									=	27,49	LSDV Rec (clade 2)	
	TP1									=	26,69	LSDV Rec (clade 2)	
	TP2									=	0	SPPV Wild	
	TP3									=	0	SPPV Wild	
	TP4									=	23,73	LSDV Vaccine (clade 1.1)	
	TP5									=	26,16	LSDV Wild (clade 1.2)	
97620 (1)	BN1	Haegeman et al. 2023	QIAGEN		Other - Haegeman et al. 2023	real time PCR	Ct < 45	27,038		=	-	NI	Conventional PCR for differentiation of SPPV field and vaccine strains based on the PCR product length. Result is expresed as PCR product length in bp.
	BP1									=	31,24	LSDV Wild (clade 1.2)	
	BP2									=	-	NI	
	TN1									=	-	NEG	
	TP1									=	31,719	LSDV Wild (clade 1.2)	
	TP1									=	30,706	LSDV Wild (clade 1.2)	
	TP2									=	39,931	NI	
	TP3									=	-	NI	
	TP4									=	26,956	LSDV Vaccine (clade 1.1)	
	TP5									=	30,916	LSDV Wild (clade 1.2)	
97620 (2)	BN1	Chibssa et al. 2018	QIAGEN		Other - Chibssa et al. 2018	PCR	/	302		=	-	NEG	
	BP1									=	336	NI	
	BP2									=	218	SPPV Vaccine (RM65/Romania)	
	TN1									=	-	NEG	
	TP1									=	336	NI	
	TP1									=	336	NI	

Lab ID	Sample	Protocol / SOP	Prod Extrac tion protocol/kit	Extraction protocol/kit	RT-PCR protocol/kit	Methodology	Cut-off	Pos control	Neg control	Operator	Ct value cp	Interpretation	General comments
	TP2									=	302	SPPV Wild	
	TP3									=	302	SPPV Wild	
	TP4									=	336	NI	
	TP5									=	336	NI	
97621 (1)	BN1	Haegeman et al. 2023	QIAGEN		Other - Haegeman et al. 2023	Duplex PCR (LSDV wild or vaccine)	35	35	40	>	40	NEG	This method is DIVA LSDV
	BP1									=	25,64	LSDV Rec (clade 2)	
	BP2									>	40	NEG	
	TN1									>	40	NEG	
	TP1									=	25,77	LSDV Rec (clade 2)	
	TP1									=	24,98	LSDV Rec (clade 2)	
	TP2									>	40	NEG	
	TP3									>	40	NEG	
	TP4									=	21,73	LSDV Vaccine (clade 1.1)	
	TP5									=	26,23	LSDV Wild (clade 1.2)	
97621 (2)	BN1	Haegeman et al. 2015	QIAGEN		Other - Haegeman et al. 2015	Conventional PCR (diferential amplicon by size)				=		NEG	This method is DIVA for SPPV
	BP1									=		NEG	
	BP2									=		SPPV Vaccine (RM65/Romania)	
	TN1									=		NEG	
	TP1									=		NEG	
	TP1									=		NEG	
	TP2									=		SPPV Wild	
	TP3									=		SPPV Wild	
	TP4									=		NEG	
	TP5									=		NEG	
97630	BN1	Wolf et al. 2021	Indical Bioscience		Other - Wolff et al. 2021	real-time PCR	N/A	29,15	45	>	45	NEG	Protocol used: Wolf et al. 2021. Probe-Based Real-Time qPCR Assays for a Reliable Differentiation of Capripox Virus Species. Microorganisms. 2021 Apr 6;9(4):765 Master mix PerfeCTa®qPR ToughMix Low rox (Quantabio) Lot 66259208
	BP1									>	45	NI	
	BP2									=	30,66	NI	
	TN1									>	45	NEG	
	TP1									>	45	NI	
	TP1									>	45	NI	
	TP2									=	23,52	NI	
	TP3									=	29,88	NI	
	TP4									=	24,07	LSDV Vaccine (clade 1.1)	
	TP5									=	29,44	LSDV Wild (clade 1.2)	
97632 (1)	BN1	Haegeman et al.2023	Sacace Biotechnologies		Other - Haegeman et al. 2023		38	32,1	0	=	0	NEG	
	BP1									=	30,8	LSDV Rec (clade 2)	
	BP2									=	0	NEG	
	TN1									=	0	NEG	
	TP1									=	25,4	LSDV Rec (clade 2)	
	TP1									=	26,1	LSDV Rec (clade 2)	
	TP2									=	0	NEG	

Lab ID	Sample	Protocol / SOP	Prod Extrac tion protocol/kit	Extraction protocol/kit	RT-PCR protocol/kit	Method ology	Cut-off	Pos control	Neg control	Operator	Ct value	cp	Interpretation	General comments
	TP3									=	0		NEG	
	TP4									=	21,8		LSDV Vaccine (clade 1.1)	
	TP5									=	27,2		LSDV Wild (clade 1.2)	
	BN1	Haegeman et al 2016- Investigation of a Possible Link Between Vaccination and the 2010 Sheep Pox Epizootic in Morocco	Sacace Biotechnologies		Other - Haegeman et al. 2016		n/a			=			NA	
	BP1									=			NA	
	BP2									=			SPPV Vaccine (RM65/Romania)	
	TN1									=			NA	
	TP1									=			NA	
97632 (2)	TP1									=			NA	
	TP2									=			SPPV Wild	
	TP3									=			SPPV Wild	
	TP4									=			NA	
	TP5									=			NA	
	BN1									=			NEG	
	BP1									=			LSDV Rec (clade 2)	
	BP2									=			SPPV Vaccine (RM65/Romania)	
	TN1									=			NEG	
97632 (3)	TP1	Gelaye et al 2015			Other - Gelaye et al. 2015	Sanger sequencing (RPO30)	n/a			=			LSDV Rec (clade 2)	
	TP1									=			LSDV Rec (clade 2)	
	TP2									=			SPPV Wild	
	TP3									=			SPPV Wild	
	TP4									=			LSDV Vaccine (clade 1.1)	
	TP5									=			LSDV Wild (clade 1.2)	
	BN1									>	45		NEG	
	BP1									=	30,13		LSDV Rec (clade 2)	
	BP2									>	45		NEG	
	TN1									>	45		NEG	
	TP1									=	28,45		LSDV Rec (clade 2)	
97634 (1)	TP1	Haegeman	Biosellal		Other - Haegeman et al. 2023		40	31	45	=	28,37		LSDV Rec (clade 2)	
	TP2									>	45		NEG	
	TP3									>	45		NEG	
	TP4									=	21,66		LSDV Vaccine (clade 1.1)	
	TP5									=	29,88		LSDV Wild (clade 1.2)	
97634	BN1	Chibssa	Biosellal			PCR				=			NI	

Lab ID	Sample	Protocol / SOP	Prod Extrac tion protocol/kit	Extraction protocol/kit	RT-PCR protocol/kit	Method ology	Cut-off	Pos control	Neg control	Operator	Ct value	cp	Interpretation	General comments
(2)	BP1									=			NI	
	BP2									=			SPPV Vaccine (RM65/Romania)	
	TN1				Other - Chibssa et al. 2018					=			NI	
	TP1									=			NI	
	TP1									=			NI	
	TP2									=			SPPV Wild	
	TP3									=			SPPV Wild	
	TP4									=			NI	
	TP5									=			NI	
	BN1									=	-		NEG	
	BP1									=	24,05		LSDV Rec (clade 2)	
	BP2									=			NA	
	TN1									=			NA	
97637	TP1	Haegeman et al. 2023;	Roche		Other - Haegeman et al. 2023	Real time PCR				=	21,17		LSDV Rec (clade 2)	
	TP1									=	29,27		LSDV Rec (clade 2)	
	TP2									=	25,42		NI	
	TP3									=			NA	
	TP4									=	23,69		LSDV Vaccine (clade 1.1)	
	TP5									=			NA	
	BN1									=	0		NEG	
	BP1									=	21,85		LSDV Vaccine (clade 1.1)	
	BP2									=			NA	
	TN1									=	0		NEG	
	TP1									=	21,82		LSDV Vaccine (clade 1.1)	
97638	TP1	Commercial kit	Indical Bioscience		Other - Other			22,99	0	=	21,82		LSDV Vaccine (clade 1.1)	For DIVA PCR we used commercial kit: Bio-T Kit Lumpy Skin Disease - DIVA Biosellal
	TP2									=			NA	
	TP3									=			NA	
	TP4									=	18,13		LSDV Vaccine (clade 1.1)	
	TP5									=	21,47		LSDV Wild (clade 1.2)	

10.3 Annex 3: Additional information

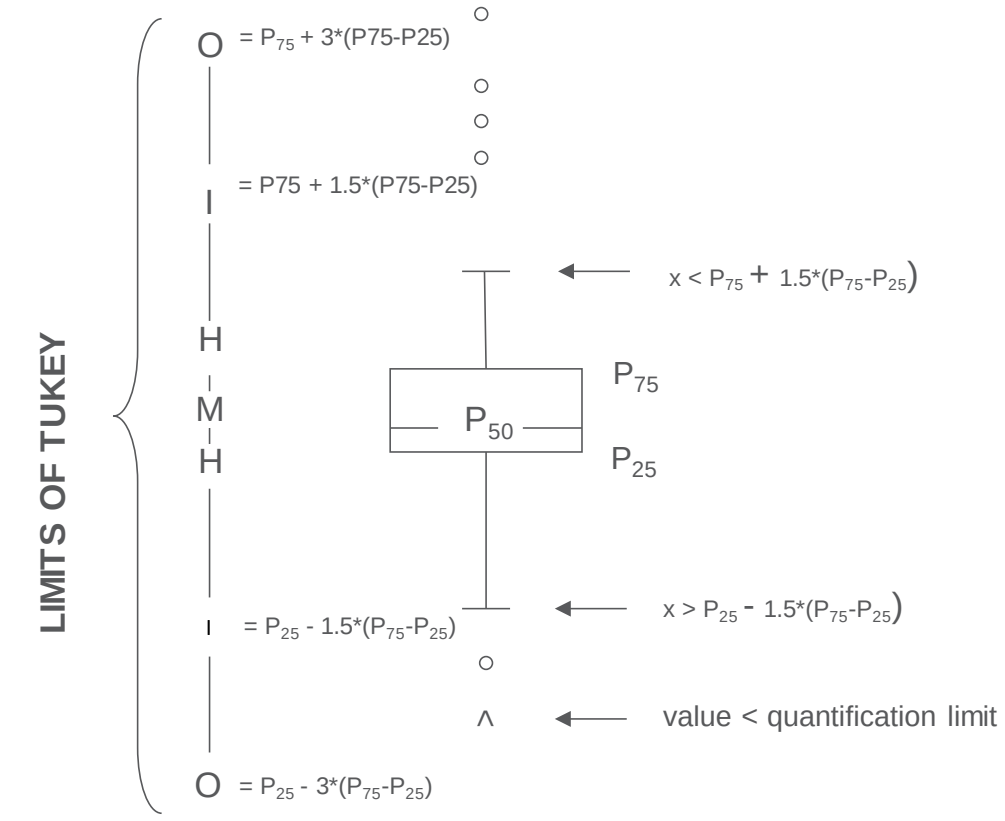
The **calendar** for Proficiency Testing in Veterinary diagnosis is available on our website:

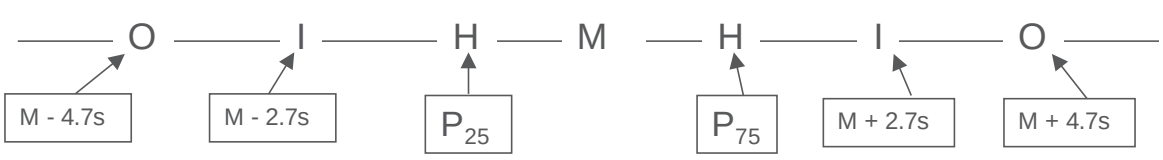
- NL: https://www.sciensano.be/nl/biblio/eke-kalender-2025
- FR: https://www.sciensano.be/fr/biblio/calendrier-eeq-2025
- EN: https://www.sciensano.be/en/biblio/eqa-calendar-2025

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





Corresponding limits in case of normal distribution

END