BIOLOGICAL HEALTH RISKS
QUALITY OF LABORATORIES

PROFICIENCY TEST
IN VETERINARY DIAGNOSIS

DEFINITIVE GLOBAL REPORT

PT-PROGRAM 2024-3

CAPRIPOX (CAPX)

EURL

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A draft version of this report was submitted to the expert on 30/08/2024.

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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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: PT2024CAPXSER) and/or to assess the ability of the participating laboratories to detect CAPX virus DNA in different matrices (virology component of the PT: PT2024CAPXVIR).

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-one laboratories were provided with the PT2024CAPXSER panel, each comprising ten serum aliquots. Also, thirty-two laboratories were supplied with the PT2024CAPXVIR 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 PT2024CAPXSER PANEL: REFERENCE SERUM SAMPLES

3.2.1.1 Origin of the samples

Replicates of nine reference serum samples, either free from detectable antibodies to capripox viruses (n=2; coded PT2024CAPXSER_N1 and PT2024CAPXSER_N2) or containing detectable antibodies to capripox viruses (n=7; coded PT2024CAPXSER_P1, PT2024CAPXSER_P2, PT2024CAPXSER_P3, PT2024CAPXSERP4, PT2024CAPXSER_P5, PT2024CAPXSER_P6, PT2024CAPXSER_P7) were used. Sample PT2024CAPXSER_P5 was repeated twice in the panel. Therefore, the panel included ten samples in total.

In total, 310 aliquots were distributed to 31 participating laboratories. PT2024CAPXSER_P2 and PTCAPXSER_P3 were dilutions of PTCAPXSER_P1, with dilution factors 1/2 and 1/5 respectively. 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 immunoperoxidase monolayer assay (IPMA) (Haegeman *et al.* 2020) and the virus neutralisation (VN) test with the serum titrated against a constant titer of capripox virus.

Table 1: Origin of the samples in the PTCAPX2024 panel (LSDV = lumpy skin disease virus; SPPV = sheeppox virus; POS = positive; NEG = negative).

Sample ID	Origin	Background	Status
PT2024CAPXSER_P1	Bovine	LSDV infected	POS
PT2024CAPXSER_P2	Bovine	LSDV infected	POS
PT2024CAPXSER_P3	Bovine	LSDV infected	POS
PT2024CAPXSER_P4	Ovine	SPPV infected	POS
PT2024CAPXSER_P5 (repeated twice)	Ovine	SPPV infected	POS
PT2024CAPXSER_P6	Bovine	LSDV vaccinated	POS
PT2024CAPXSER_P7	Bovine	LSDV infected	POS
PT2024CAPXSER_N1	Ovine	Commercial serum	NEG
PT2024CAPXSER_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), IPMA and VN. For all tests, 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), IPMA and VN.

The reference serum samples PT2024CAPXSER_N1 and PT2024CAPXSER_N2 were considered as negative samples in all tests. The reference serum samples, PT2024CAPXSER_P1, PT2024CAPXSER_P2, PT2024CAPXSER_P3, PT2024CAPXSER_P4, PT2024CAPXSER_P5, PT2024CAPXSER_P6 and PT2024CAPXSER_P7 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 PT2024CAPXSER panel (POS = positive; NEG = negative).

Sample ID	ELISA	IPMA	VNT
PT2024CAPXSER_P1	POS	POS	POS
PT2024CAPXSER_P2	POS	POS	POS
PT2024CAPXSER_P3	POS	POS	POS
PT2024CAPXSER_P4	POS	POS	POS
PT2024CAPXSER_P5 (repeated twice)	POS	POS	POS
PT2024CAPXSER_P6	POS	POS	POS
PT2024CAPXSER_P7	POS	POS	POS
PT2024CAPXSER_N1	NEG	NEG	NEG
PT2024CAPXSER_N2	NEG	NEG	NEG

3.2.1.3 Randomisation and panel composition

The samples were randomised uniquely for each laboratory, and the PTCAPXSER2024 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 PT2024CAPXVIR PANEL: REFERENCE BLOOD & TISSUE HOMOGENATE SAMPLES

3.2.2.1 Origin of the samples

Replicates of seven reference samples were tissue homogenate samples. These included samples containing detectable capripox virus DNA (n=7; coded PT2024CAPX_TP1, PT2024CAPX_TP2, PT2024CAPX_TP3, PT2024CAPX_TP4, PT2024CAPX_TP5, and PT2024CAPX_TP6) and one sample free from detectable capripox virus DNA (n=1; coded PT2024CAPX_TN1). Sample PT2024CAPX_TP5 was repeated twice, resulting in a total of eight tissue samples.

The remaining two reference samples were blood samples, either containing detectable capripox virus DNA (n=1; coded PT2024CAPX_BP1) or free from detectable capripox virus DNA (n=1; coded PT2024CAPX_BN1).

In total, 320 aliquots were distributed to 32 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 PTCAPX2024 panel. (GTPV = goatpox virus; LSDV = lumpy skin disease virus; SPPV = sheeppox virus)

Sample ID	Origin	Background	Status
PT2024CAPX_TP1	Goat tissue	GTPV spiked tissue	Capx positive GTPV
PT2024CAPX_TP2	Cattle tissue	LSDV spiked tissue recombinant	Capx positive LSDV Recombinant field strain
PT2024CAPX_TP3	Sheep tissue	SPPV wild tissue	Capx positive SPPV wild-type strain
PT2024CAPX_TP4	Cattle tissue	LSDV vac tissue	Capx positive LSDV vaccine strain
PT2024CAPX_TP5 (repeated twice)	Cattle tissue	LSDV vac tissue	Capx positive LSDV vaccine strain
PT2024CAPX_TP6	Cattle tissue	LSDV wild tissue	Capx positive LSDV classic field (wild) strain
PT2024CAPX_BP1	Cattle blood	LSDV spiked blood wild	Capx positive LSDV classic field (wild) strain
PT2024CAPX_TN1	Cattle tissue	Negative tissue cattle	Doubtful
PT2024CAPX_BN1	Sheep blood	Negative blood sheep	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, sample PT2024CAPXVIR_TN1 showed deviating results in all tests, therefore the status was changed to doubtful. There were no differences detected for the other samples.

For the **detection of capripox virus DNA (primary PCR),** the sample PT2024CAPXVIR_BN1 was considered as capripox negative sample, PT2024CAPXVIR_TN1 was considered as a doubtful sample and the samples PT2024CAPXVIR_TP1, PT2024CAPXVIR_TP2, PT2024CAPXVIR_TP3, PT2024CAPXVIR_TP4, PT2024CAPXVIR_TP5, PT2024CAPXVIR_TP6 and PT2024CAPXVIR_BP1 as positive samples.

For the **capripox virus species differentiation**, the sample PT2024CAPXVIR_BN1 was considered as capripox negative sample, PT2024CAPXVIR_TN1 was considered as a doubtful sample, sample PT2024CAPXVIR_TP3 as SPPV positive samples (where SPPV or SPPV/GTPV results were considered acceptable), the samples PT2024CAPXVIR_TP2, PT2024CAPXVIR_TP4, PT2024CAPXVIR_TP5, PT2024CAPXVIR_TP6 and PT2024CAPXVIR_BP1 as LSDV positive samples (where LSDV was considered acceptable) and sample PT2024CAPXVIR_TP1 as GTPV positive sample (where GTPV or SPPV/GTPV was considered acceptable).

Finally, for the **field or vaccine strain differentiation**, the sample PT2024CAPXVIR_BN1 was considered as capripox negative sample, PT2024CAPXVIR_TN1 was considered as a doubtful sample. PT2024CAPX_TP1 was considered as GTPV. The samples PT2024CAPXVIR_TP6 and PT2024CAPXVIR_BP1 were considered as LSDV classic field strains, while sample PT2024CAPX_TP2 was considered as LSDV recombinant field strain and PT2024CAPX_TP4 and PT2024CAPX_TP5 were considered as LSDV vac strain. Sample PT2024CAPXVIR_TP3 was considered as SPPV field 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 PT2024CAPXVIR panel (GTPV = goatpox virus; LSDV = lumpy skin disease virus; SPPV = sheeppox virus; NEG = negative; NI = not interpretable).

Sample ID	Primary PCR	Species differentiation	DIVA
PT2024CAPX_TP1	POS	GTPV	GTPV
PT2024CAPX_TP2	POS	LSDV	LSDV recombinant field strain
PT2024CAPX_TP3	POS	SPPV	SPPV wild-type strain
PT2024CAPX_TP4	POS	LSDV	LSDV vaccine strain

Sample ID	Primary PCR	Species differentiation	DIVA
PT2024CAPX_TP5 (repeated twice)	POS	LSDV	LSDV vaccine strain
PT2024CAPX_TP6	POS	LSDV	LSDV classic field (wild) strain
PT2024CAPX_BP1	POS	LSDV	LSDV classic field (wild) strain
PT2024CAPX_TN1	NI	NI	NI
PT2024CAPX_BN1	NEG	NEG	NEG

3.2.2.3 Randomisation and panel composition

The samples were randomised uniquely for each laboratory, and the PTCAPXVIR2024 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, with the exception of sample PT2024CAPX_TN1.

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 in previous editions of the PT, all answers (LSDV, LSDV vac, LSDV wild) were accepted in the DIVA part for the sample containing an LSDV recombinant field strain. This year, if an LSDV recombinant field strain was present, it was expected to classify it correctly as LSDV Rec. Other answers were classified as failures.

4 THE PARTICIPANTS

Twenty-four 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
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	Laboratory of the Ministry of Agriculture (LMA) of Georgia: Kutaisi Zonal Diagnostic Laboratory	0	1
Kazakhstan	National Veterinary Reference Centre Astana	1	0
Kazakhstan	National Veterinary Reference Centre Almaty	1	0
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	0	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 NRL to QL: 22/04/2024
Randomisation of the samples by QL: 25/04/2024 (serology) + 26/04/2024 (virology)
Sending samples (frozen at - 20 °C) to participants: 06/05/2024
Deadline for submitting results: 14/06/2024
Extended deadline: 05/07/2024
Individual report: 28/08/2024

6 COMPLIANCE WITH THE PROCEDURE

All participating laboratories provided a duly dated copy of their results, except for laboratory 97624. This laboratory submitted results for the virology part but not for the serology part. An extended deadline (July 5th) was granted to three laboratories (97611, 97623 and 97638) that received the samples later due to transport problems.

7 RESULTS – QUALITATIVE DATA ANALYSIS

7.1 Serology

The PT2024CAPXSER panel was composed of eight positive samples and two negative samples. The sample PT2024CAPSER_P5 was repeated twice in the panel.

7.1.1 ANTIBODY ELISA

In total, 31 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%. Therefore, all laboratories achieved a satisfactory result. Raw data can be found in Annex 1.

Table 7: 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	P2	P3	P4	P5 (1)	P5 (2)	P6	P7	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
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	POS	POS	POS	POS	POS	POS	NEG	NEG	100
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
97628	POS	POS	POS	POS	POS	POS	POS	POS	NEG	NEG	100

Lab number	P1	P2	P3	P4	P5 (1)	P5 (2)	P6	P7	N1	N2	%
	POS								NEG		
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
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)

Four laboratories submitted results for the VN (dilution antibody) tests. 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 8: 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	P2	P3	P4	P5 (1)	P5 (2)	P6	P7	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
97618	POS	POS	POS	POS	POS	POS	POS	POS	NEG	NEG	100

7.1.3 IMMUNOPEROXYDASE MONOLAYER ASSAY (IPMA)

Only two laboratories submitted results for the immunoperoxydase monolayer assay (IPMA). These 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 9: 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	P2	P3	P4	P5 (1)	P5 (2)	P6	P7	N1	N2	%
	POS								NEG		
97506	POS	POS	POS	POS	POS	POS	POS	POS	NEG	NEG	100
97618	POS	POS	POS	POS	POS	POS	POS	POS	NEG	NEG	100

7.2 Virology

The PT2024CAPXVIR panel was composed of eight positive samples and two negative samples. The sample PT2024CAPVIR_TP5 was repeated twice in the panel.

7.2.1 PRIMARY PCR

7.2.1.1 Results per sample

In total, 32 laboratories participated in the primary PCR virology part of the proficiency test. As 30 laboratories submitted one dataset and two laboratories submitted two datasets, the total number of datasets registered is thus 34. Raw data can be found in Annex 1.

For the sample TN1, the expected result is doubtful and thus not interpretable, implying that the result is not conclusively positive or negative. For this sample, NI, POS or NEG are accepted as correct results.

Table 10: Results per sample (REP = repetition; POS = positive; NEG = negative; NI = non-interpretable).

Sample ID PT2024CAPXVIR	REP	Expected results	# of POS	# of NEG	# of NI	Status*
TP1	No	POS	34	0	0	Frequently detected
TP2	No	POS	34	0	0	Frequently detected
TP3	No	POS	34	0	0	Frequently detected
TP4	No	POS	34	0	0	Frequently detected
TP5	Yes	POS	68	0	0	Frequently detected
TP6	No	POS	34	0	0	Frequently detected
BP1	No	POS	34	0	0	Frequently detected
TN1	No	NI	12	22	0	Doubtful
BN1	No	NEG	0	34	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 method. The target gene, if known, is also mentioned.

Table 11: 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	19	190	190	0	0
Haegeman *et al*. (2013)	D5R/E3L	3	30	30	0	0
Babiuk *et al*. (2008)	P32	2	20	20	0	0
Stubbs *et al*. (2012)	P32	1	10	10	0	0
Haegeman *et al*. (2013) & Bowden *et al*. (2008)	D5R/E3L	1	10	10	0	0
Bowden *et al*. (2008) & Stubbs *et al*. (2012)	P32	1	10	10	0	0
ID.VET - ID GENE® CAPRIPOX VIRUS TRIPLEX	/	2	20	20	0	0
In house	/	5	50	50	0	0
TOTAL		**34**	**340**	**340**	**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). Two laboratories (LAB97605 and LAB97608) performed a secondary PCR and provided results that were in full agreement with the assigned status of the reference samples.

Table 12: 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	TP2	TP3	TP4	TP5 (1)	TP5 (2)	TP6	BP1	TN1	BN1	%
	POS								NI	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**
97602	POS	POS	POS	POS	POS	POS	POS	POS	NEG	NEG	**100**
97603	POS	POS	POS	POS	POS	POS	POS	POS	NEG	NEG	**100**
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**

Lab number	TP1	TP2	TP3	TP4	TP5 (1)	TP5 (2)	TP6	BP1	TN1	BN1	%
	POS								NI	NEG	
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	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
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
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 24 laboratories participated in the Species Differentiation virology component of the proficiency test. Nineteen laboratories submitted one dataset each, while five laboratories submitted two datasets. Despite these five laboratories submitting two datasets, two distinct types of specific kits were used: one for detecting LSDV samples and another for GTPV/SPPV samples. As a result, the table below presents these combined results, maintaining the total number of datasets at 24. Raw data can be found in Annex 1.

For the sample TN1, the expected result is doubtful and thus not interpretable, implying that the result is not conclusively positive or negative. For this sample, NI, NEG, LSDV, SPPV and GTPV are accepted as correct results.

Table 16: Results per sample (REP = repetition; GTPV = goatpox virus; LSDV = lumpy skin disease virus; SPPV = sheeppox virus; NEG = negative; NI = non-interpretable; NA= not analysed).

Sample ID PT2024CAPXVIR	REP	Expected results	# of GTPV	# of LSDV	# of SPPV	# of NEG	# of NI/NA	%
TP1	No	GTPV	22	0	0	0	2	92
TP2	No	LSDV	0	22	0	0	2	92
TP3	No	SPPV	0	0	22	0	2	92
TP4	No	LSDV	0	23	0	0	1	96
TP5	Yes	LSDV	0	46	0	0	2	96
TP6	No	LSDV	0	23	0	0	1	96
BP1	No	LSDV	0	23	0	0	1	96
TN1	No	NI	0	5	0	16	3	100
BN1	No	NEG	0	0	0	23	1	96

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 17: 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)	10	100	91	0	9
Agiannioaki *et al*. (2016) & Chibssa *et al.* (2018) & Wolff *et al*. (2021) & Haegeman *et al*. (2016)	1	10	10	0	0
Wolff *et al*. (2021) (Both duplexes)	3	30	29	0	1
Wolff *et al*. (2021) (Both duplexes) & Sprygin *et al.* (2019)	1	10	10	0	0
Galaye *et al.* (2017) & Wolff *et al.* (2021)	1	10	10	0	0
Wolff *et al.* (2021) (both duplexes) & Haegeman *et al.* (2024)	1	10	10	0	0

Kit or reference	N	NR	NCR	F	NI/NA
Lamien *et al*. (2017) & Galaye *et al*. (2017) & IZS TE B456.1 SOP21 (Biosellal)	1	10	10	0	0
Wolf *et al.* (2021) & Vidanovic *et al.* (2021) & Agianniotaki *et al.* (2017)	1	10	10	0	0
Galaye *et al.* (2015)	1	10	10	0	0
Lamien *et al.* (2011) & Chibssa *et al.* (2018)	1	10	10	0	0
ID GENE LSD DIVA Triplex	1	10	7	0	3
Other	2	20	18	0	2
TOTAL	**24**	**240**	**225**	**0**	**15**

7.2.2.3 Results per laboratory

For the part Species Differentiation, 21 out of 24 participating laboratories provided qualitative results that were in full agreement with the assigned status of the ten reference samples (100% of agreement). Three laboratories used a diagnostic kit that could not differentiate each capripox virus species. These were labs 96707, 97621 and 97638, which used kits that could not differentiate respectively GTPV and SPPV; LSDV; GTPV, SPPV and LSDV rec.

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. (REP = repetition; GTPV = goatpox virus; LSDV = lumpy skin disease virus; SPPV = sheeppox virus; NEG = negative; NI = non-interpretable; NA = not analysed).

Lab number	TP1	TP2	TP3	TP4	TP5 (1)	TP5 (2)	TP6	BP1	TN1	BN1	%
	GTPV	LSDV	SPPV	LSDV	LSDV	LSDV	LSDV	LSDV	NI	NEG	
97506	GTPV	LSDV	SPPV	LSDV	LSDV	LSDV	LSDV	LSDV	NEG	NEG	**100**
97600	GTPV	LSDV	SPPV	LSDV	LSDV	LSDV	LSDV	LSDV	NA	NA	**100**
97602	GTPV	LSDV	SPPV	LSDV	LSDV	LSDV	LSDV	LSDV	NEG	NEG	**100**
97603	GTPV	LSDV	SPPV	LSDV	LSDV	LSDV	LSDV	LSDV	NEG	NEG	**100**
97604	GTPV	LSDV	SPPV	LSDV	LSDV	LSDV	LSDV	LSDV	LSDV	NEG	**100**
97607	NA	LSDV	NA	LSDV	LSDV	LSDV	LSDV	LSDV	NI	NEG	**100**
97608	GTPV	LSDV	SPPV	LSDV	LSDV	LSDV	LSDV	LSDV	NEG	NEG	**100**
97609	GTPV	LSDV	SPPV	LSDV	LSDV	LSDV	LSDV	LSDV	NEG	NEG	**100**
97610	GTPV	LSDV	SPPV	LSDV	LSDV	LSDV	LSDV	LSDV	NEG	NEG	**100**
97611	GTPV	LSDV	SPPV	LSDV	LSDV	LSDV	LSDV	LSDV	LSDV	NEG	**100**
97612	GTPV	LSDV	SPPV	LSDV	LSDV	LSDV	LSDV	LSDV	NEG	NEG	**100**
97613	GTPV	LSDV	SPPV	LSDV	LSDV	LSDV	LSDV	LSDV	NEG	NEG	**100**
97614	GTPV	LSDV	SPPV	LSDV	LSDV	LSDV	LSDV	LSDV	NEG	NEG	**100**
97617	GTPV	LSDV	SPPV	LSDV	LSDV	LSDV	LSDV	LSDV	NEG	NEG	**100**
97618	GTPV	LSDV	SPPV	LSDV	LSDV	LSDV	LSDV	LSDV	NEG	NEG	**100**
97619	GTPV	LSDV	SPPV	LSDV	LSDV	LSDV	LSDV	LSDV	NEG	NEG	**100**

Lab number	TP1	TP2	TP3	TP4	TP5 (1)	TP5 (2)	TP6	BP1	TN1	BN1	%
	GTPV	LSDV	SPPV	LSDV	LSDV	LSDV	LSDV	LSDV	NI	NEG	
97620	GTPV	LSDV	SPPV	LSDV	LSDV	LSDV	LSDV	LSDV	NEG	NEG	100
97621	GTPV	NA	SPPV	NA	NA	NA	NA	NA	NA	NEG	100
97630	GTPV	LSDV	SPPV	LSDV	LSDV	LSDV	LSDV	LSDV	LSDV	NEG	100
97631	GTPV	LSDV	SPPV	LSDV	LSDV	LSDV	LSDV	LSDV	LSDV	NEG	100
97632	GTPV	LSDV	SPPV	LSDV	LSDV	LSDV	LSDV	LSDV	LSDV	NEG	100
97634	GTPV	LSDV	SPPV	LSDV	LSDV	LSDV	LSDV	LSDV	NEG	NEG	100
97637	GTPV	LSDV	SPPV	LSDV	LSDV	LSDV	LSDV	LSDV	NEG	NEG	100
97638	NA	NA	NA	LSDV	LSDV	LSDV	LSDV	LSDV	NEG	NEG	100

7.2.3 DIVA PCR

7.2.3.1 Results per sample

In total, 22 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 13: Results per sample (REP = repetition; GTPV = goatpox virus; LSDV = lumpy skin disease virus; SPPV = sheeppox virus; NEG = negative; NI = non-interpretable; NA = not analysed).

Sample ID	REP	Expected results	# of GTPV	# of LSDV			# of SPPV		# of NEG	# of NI/NA
				Classic field	Vac	Rec field	Wild	Vac		
TP1	No	GTPV	15	0	0	0	0	0	1	6
TP2	No	LSDV rec	0	3	4	14	0	0	0	1
TP3	No	SPPV wild	0	1	0	0	13	0	0	8
TP4	No	LSDV vac	0	1	20	1	0	0	0	0
TP5	Yes	LSDV vac	0	1	41	2	0	0	0	0
TP6	No	LSDV wild	0	22	0	0	0	0	0	0
BP1	No	LSDV wild	0	22	0	0	0	0	0	0
TN1	No	NI	0	3	0	0	0	0	17	2
BN1	No	NEG	0	0	0	0	0	0	19	3

7.2.3.2 Results per method

In the table below, results are listed by method. NI/NA results for samples PTCAPXVIR2024_TP1, PTCAPXVIR2024_TN1 and PTCAPXVIR2024_BN1 were not taken into account. No DIVA exists for GTPV samples and negative samples are not taken into account if NI/NA is answered.

Table 14: 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.* 2024	1	10	10	0	0
Haegeman *et al.* 2014 & Haegeman *et al.* 2024	1	10	10	0	0
Haegeman *et al.* 2024 & Chibssa *et al.* 2018	3	30	29	1	0
Agianniotaki *et al.* 2017 & Haegeman *et al.* 2015 & Gelaye *et al.* 2015	1	10	10	0	0
Vidanovic *et al.* 2016	1	10	8	1	1
Agianniotaki *et al.* 2017 & Haegeman *et al.* 2023	1	10	8	1	1
Agianniotaki *et al.* 2017 & Haegeman *et al.* 2015	1	10	10	0	0
Chibssa *et al.* 2018 and Sprygin *et al.* 2022	1	10	9	0	1

Kit or reference	N	NR	NCR	F	NI/NA
Agianniotaki *et al.* 2017 & Chibssa *et al.* 2015 & Gelaye *et al.* 2015	1	10	10	0	0
Gelaye *et al.* 2015	1	10	7	3	0
Menasherow *et al.* 2014	1	10	9	1	0
Agianniotaki *et al.* 2017 & sanger sequencing	1	10	7	3	0
Wolff *et al.* 2021	2	20	17	1	2
Wolff *et al.* 2021 & Gelaye *et al.* 2015	1	10	10	0	0
Haegeman *et al.* 2024 & Chibssa *et al.* 2018 & Vidanovic *et al.* 2021	1	10	10	0	0
Haegeman *et al.* 2024 & Vidanovic *et al.* 2021 & Agianniotaki *et al.* 2017	1	10	9	0	1
ID.VET - ID GENE® LSD DIVA TRIPLEX	1	10	8	0	2
Other	2	20	16	3	1
TOTAL	**22**	**220**	**189**	**14**	**9**

7.2.3.3 Results per laboratory

Eight out of 22 laboratories provided results that were in full agreement with the assigned status of the reference samples (100% agreement). Four laboratories misclassified aliquot PTCAPXVIR2024_TP3. as NA or NI instead of SPPV wild. Three laboratories misclassified sample PTCAPXVIR2024_TP2 as LSDV Vac or LSDV wild instead of LSDV rec. Four labs misclassified the two above mentioned samples. LAB97622 misclassified PTCAPXVIR2024_TP2 as LSDV vac and PTCAPXVIR2024_TP3 as LSDV wild instead of respectively LSDV rec and SPPV wild.

The remaining two laboratories misclassified three samples. LAB97614 misclassified sample PTCAPXVIR2024_TP2, PTCAPXVIR2024_TP4 and PTCAPXVIR2024_TP5 (REP 1) as LSDV wild instead of respectively LSDV rec, LSDV vac and LSDV vac. LAB97618 misclassified the LSDV vac samples (PTCAPXVIR2024_TP4 and PTCAPXVIR2024_TP5 (REP 1 & 2)) as LSDV rec instead of LSDV vac.

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 15: 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; GTPV = goatpox virus; LSDV = lumpy skin disease virus; SPPV = sheeppox virus; NEG = negative; NI = non-interpretable; NA = not analysed; vac = vaccine, rec = recombinant).

Lab number	TP1	TP2	TP3	TP4	TP5 (1)	TP5 (2)	TP6	BP1	TN1	BN1	%
	GTPV	LSDV Rec	SPPV wild	LSDV vac	LSDV vac	LSDV vac	LSDV Wild	LSDV Wild	NI	NEG	
97506	GTPV	LSDV rec	SPPV wild	LSDV vac	LSDV vac	LSDV vac	LSDV wild	LSDV wild	NEG	NEG	**100**
97600	GTPV	LSDV rec	SPPV wild	LSDV vac	LSDV vac	LSDV vac	LSDV wild	LSDV wild	NEG	NEG	**100**
97602	GTPV	LSDV rec	SPPV wild	LSDV vac	LSDV vac	LSDV vac	LSDV wild	LSDV wild	NEG	NEG	**100**
97604	NA	LSDV vac	NA	LSDV vac	LSDV vac	LSDV vac	LSDV wild	LSDV wild	NI	NEG	**88**
97608	GTPV	LSDV rec	SPPV wild	LSDV vac	LSDV vac	LSDV vac	LSDV wild	LSDV wild	NEG	NEG	**100**
97609	GTPV	LSDV rec	SPPV wild	LSDV vac	LSDV vac	LSDV vac	LSDV wild	LSDV wild	NEG	NEG	**100**
97610	NA	LSDV vac	NA	LSDV vac	LSDV vac	LSDV vac	LSDV wild	LSDV wild	NEG	NA	**86**
97611	NA	LSDV rec	NA	LSDV vac	LSDV vac	LSDV vac	LSDV wild	LSDV wild	NEG	NA	**100**
97612	NA	LSDV rec	NA	LSDV vac	LSDV vac	LSDV vac	LSDV wild	LSDV wild	NA	NA	**100**
97613	GTPV	LSDV rec	SPPV wild	LSDV vac	LSDV vac	LSDV vac	LSDV wild	LSDV wild	NEG	NEG	**100**
97614	GTPV	LSDV wild	SPPV wild	LSDV wild	LSDV wild	LSDV vac	LSDV wild	LSDV wild	NEG	NEG	**70**
97617	GTPV	LSDV vac	SPPV wild	LSDV vac	LSDV vac	LSDV vac	LSDV wild	LSDV wild	NEG	NEG	**90**
97618	GTPV	LSDV rec	SPPV wild	LSDV rec	LSDV rec	LSDV rec	LSDV wild	LSDV wild	NEG	NEG	**70**
97620	GTPV	LSDV wild	SPPV wild	LSDV vac	LSDV vac	LSDV vac	LSDV wild	LSDV wild	NEG	NEG	**90**
97621	NA	LSDV vac	SPPV wild	LSDV vac	LSDV vac	LSDV vac	LSDV wild	LSDV wild	NEG	NEG	**89**

Lab number	TP1	TP2	TP3	TP4	TP5 (1)	TP5 (2)	TP6	BP1	TN1	BN1	%
	GTPV	LSDV Rec	SPPV wild	LSDV vac	LSDV vac	LSDV vac	LSDV Wild	LSDV Wild	NI	NEG	
97622	NEG	LSDV vac	LSDV wild	LSDV vac	LSDV vac	LSDV vac	LSDV wild	LSDV wild	NEG	NEG	70
97630	GTPV	LSDV wild	NI	LSDV vac	LSDV vac	LSDV vac	LSDV wild	LSDV wild	LSDV wild	NEG	89
97631	GTPV	LSDV rec	NA	LSDV vac	LSDV vac	LSDV vac	LSDV wild	LSDV wild	LSDV wild	NEG	100
97632	GTPV	LSDV rec	SPPV wild	LSDV vac	LSDV vac	LSDV vac	LSDV wild	LSDV wild	LSDV wild	NEG	100
97634	GTPV	LSDV rec	SPPV wild	LSDV vac	LSDV vac	LSDV vac	LSDV wild	LSDV wild	NEG	NEG	100
97637	GTPV	LSDV rec	NA	LSDV vac	LSDV vac	LSDV vac	LSDV wild	LSDV wild	NEG	NEG	100
97638	NA	NA	NA	LSDV vac	LSDV vac	LSDV vac	LSDV wild	LSDV wild	NEG	NEG	100

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, 31 out of 31 laboratories provided qualitative results that were in full agreement with the assigned status of the reference serum samples.

8.2 Virology component of the PT

The expected result of the sample TN1 is doubtful and thus not interpretable, implying that the result is not conclusively positive or negative. For this sample, NI, NEG, POS (LSDV, SPPV and GTPV) are accepted as correct results.

For the detection of capripox virus **DNA by real-time PCR (primary PCR)** in the PT panel: 32 out of 32 participating laboratories provided qualitative results that were in full agreement with the assigned status of the ten reference samples (100% of agreement). Two 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 24 participating laboratories produced qualitative results that matched the assigned status of the ten reference samples, achieving a 100% agreement rate. An NA result for any of the negative samples was considered correctly as well. One laboratory (LAB97607) used a diagnostic kit designed exclusively for detecting LSDV strains was used, rendering them unable to differentiate between the various species (LSDV, SPPV, and GTPV). As a result, the aliquots containing SPPV or GTPV (PT2024CAPXVIR_TP1 and PT2024CAPXVIR_TP3) were not classified. LAB97638 used a method that can only detect LSDV wild and LSDV vaccine strains. Therefore, PT2024CAPXVIR_TP1, PT2024CAPXVIR_TP2 and PT2024CAPXVIR_TP3 could not be detected, since these contained respectively a GTPV, LSDV field recombinant and SPPV strain. These responses were excluded from the evaluation. Finally, one laboratory (LAB97621) did not analyse the samples containing an LSDV strain, although they used a method that can detect these strains. Therefore, the six containing an LSDV strains are considered as misclassified.

For the **DIVA PCR**, the results of three samples were not taken into account. These were PT2024CAPXVIR_TP1. TP1 is a GTPV strain and there is currently no DIVA available for GTPV. For this sample, all results were considered correct. In addition, NA was also considered correctly for both negative samples. Since there are methods available for the discrimination of LSDV recombinant field strains from other LSDV strains, only LSDV rec was considered as correct for PTCAPXVIR2024_TP2. NA or NI was only accepted if a lab 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. Eight out of 23 laboratories provided results that were in full agreement with the assigned status of the reference samples (100% agreement). Three laboratories (LAB97617, LAB97620 and LAB97621) misclassified sample PTCAPXVIR2024_TP2 as LSDV vaccine or LSDV wild instead of LSDV rec, reaching a level of agreement of 90%. Four laboratories misclassified aliquot PTCAPXVIR2024_TP3. As NA instead of SPPV Wild. These laboratories only used a method for the differentiation of LSDV and could

therefore not make the differentiation between wild type and vaccine SPPV strains. For all these laboratories, all the LSDV samples were classified correctly. Three laboratories (LAB97604, LAB97610 and LAB97630) did not analyse the SPPV sample (PTCAPXVIR2024_TP3) and misclassified the LSDV recombinant (PTCAPXVIR2024_TP2) as well. They all used a method that cannot distinguish wild type and vaccine SPPV strains. LAB97638 did not classify samples PTCAPXVIR2024_TP2 and PTCAPXVIR2024TP3, because no method was in place to classify these samples. The other samples were classified correctly. LAB97622 misclassified PTCAPXVIR2024_TP2 and PTCAPXVIR2024TP3 and reached a level of agreement of 80%. The two remaining labs (LAB97614 and LAB97618) reached a level of agreement of 70%, since both misclassified three samples. LAB97614 misclassified PTCAPXVIR2024TP2 as LSDV wild and both PTCAPXVIR2024_TP4 and PTCAPXVIR2024_TP5 (REP 1) as LSDV wild instead of LSDV vac. Remarkably, the other aliquot of PTCAPXVIR2024_TP5 was correctly classified as LSDV vac. LAB97618 misclassified the three LSDV vac samples (PTCAPXVIR2024_TP4 and PTCAPXVIR2024_TP5 (REP 1 & 2)) as LSDV rec.

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. In the ELISA part, results were submitted by 31 laboratories, all reaching a level of agreement of 100%. This implies that all participating laboratories met the minimum established criteria. In the VNT section, results were submitted by four laboratories, while two laboratories participated in the IPMA component. For both the IPMA and the VNT, each participating lab achieved a maximum score of 100% and thus a satisfactory result.

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, 32 out of 32 laboratories delivered results that entirely matched the expected status of the 10 reference samples, achieving a 100% agreement rate and thus a satisfactory result. Furthermore, two laboratories conducted a secondary PCR analysis. All yielded results that perfectly aligned with the reference sample status. For the species differentiation part, twenty-one out of the 24 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. Additionally, LAB97607 utilised a diagnostic kit designed exclusively for distinguishing LSDV strains, lacking the ability to differentiate SPPV and GTPV. All LSDV samples were correctly classified. LAB97638 used a kit that could only detect LSDV wild and vaccine strains. These aliquots containing these strains were correctly classified. However, the other aliquots were not classified. Since no complete dataset was submitted, a satisfactory result cannot be awarded. LAB97621 did not analyse the aliquots containing an LSDV strain in the panel. However, they used a method that could classify these samples. Therefore, the six aliquots containing an LSDV strain are considered as misclassified, resulting in a level of agreement of

40% and thus an unsatisfactory result. For the DIVA PCR part, eight out of twenty-three laboratories achieved a perfect agreement with the expected status of the reference samples, resulting in a 100% agreement rate and a satisfactory result. LAB97617, LAB97620 and LAB97621 misclassified only aliquot PTCAPXVIR2024_TP2, reaching a level of agreement of 90% and a satisfactory result. Four laboratories incorrectly categorized aliquots PTCAPXVIR2024_TP3 as NA instead of SPPV Wild. Since they used a method that could not differentiate the SPPV strains, they are only evaluated on the results of the LSDV samples. All LSDV samples were correctly classified. LAB97638 used a method that could not differentiate the SPPV strains and the LSDV recombinant. The other samples were correctly classified. LAB97604, LAB97610 and LAB97630 did not classify PTCAPXVIR_TP3 and misclassified sample PTCAPXVIR2024_TP2. These eight laboratories cannot be awarded a satisfactory result, since no complete dataset was submitted. The remaining laboratories achieved an unsatisfactory result since they misclassified two (LAB97622) or three (LAB97614 and LAB97618) aliquots and thus not reached a level of agreement of 90%.

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 are shown in Figure I. Since there was one repetition, only the sample PT2024CAPXSER_P5 was included in the figure down below.

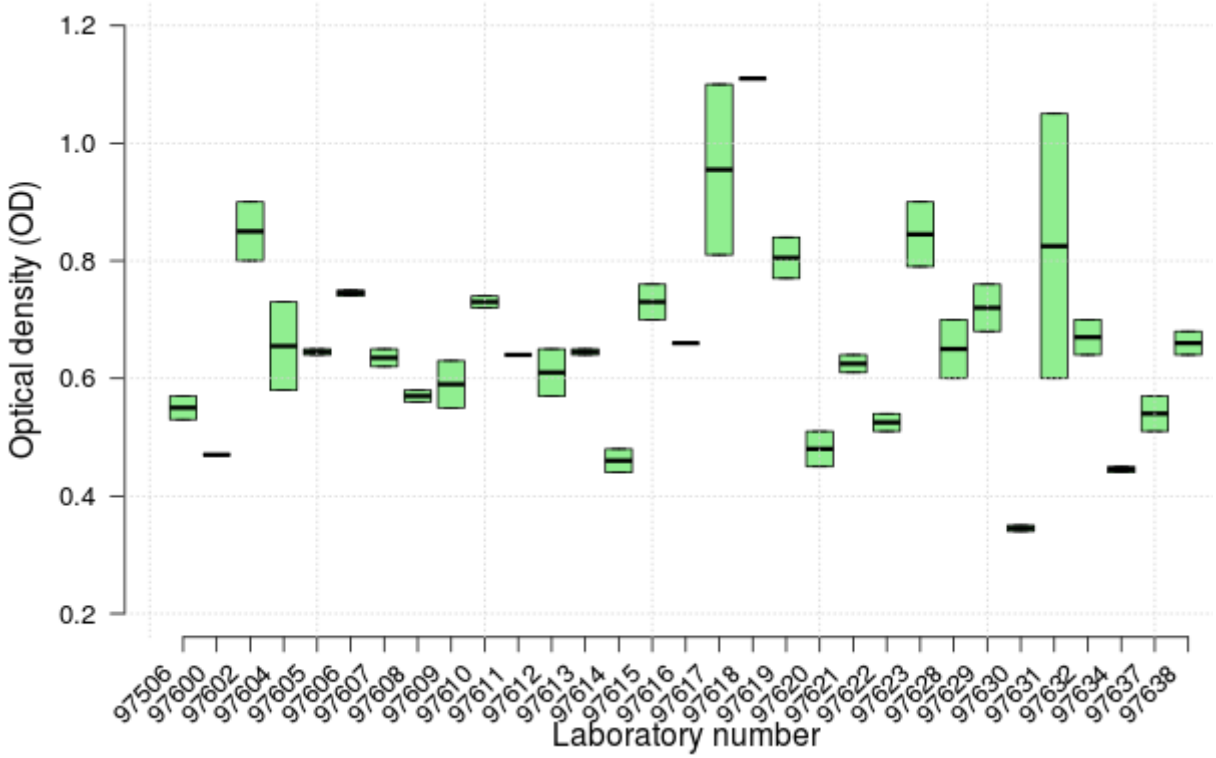


Fig I: Distribution of the optical density (OD) for the PT2024CAPXVIR_P5 samples (LAB97506-97638).

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 are shown in Figure II. This panel included one repetition, therefore only the sample PT2024CAPXVIR_TP5 was included in the figure down below. Laboratories that used two different methods are represented twice in the boxplot.

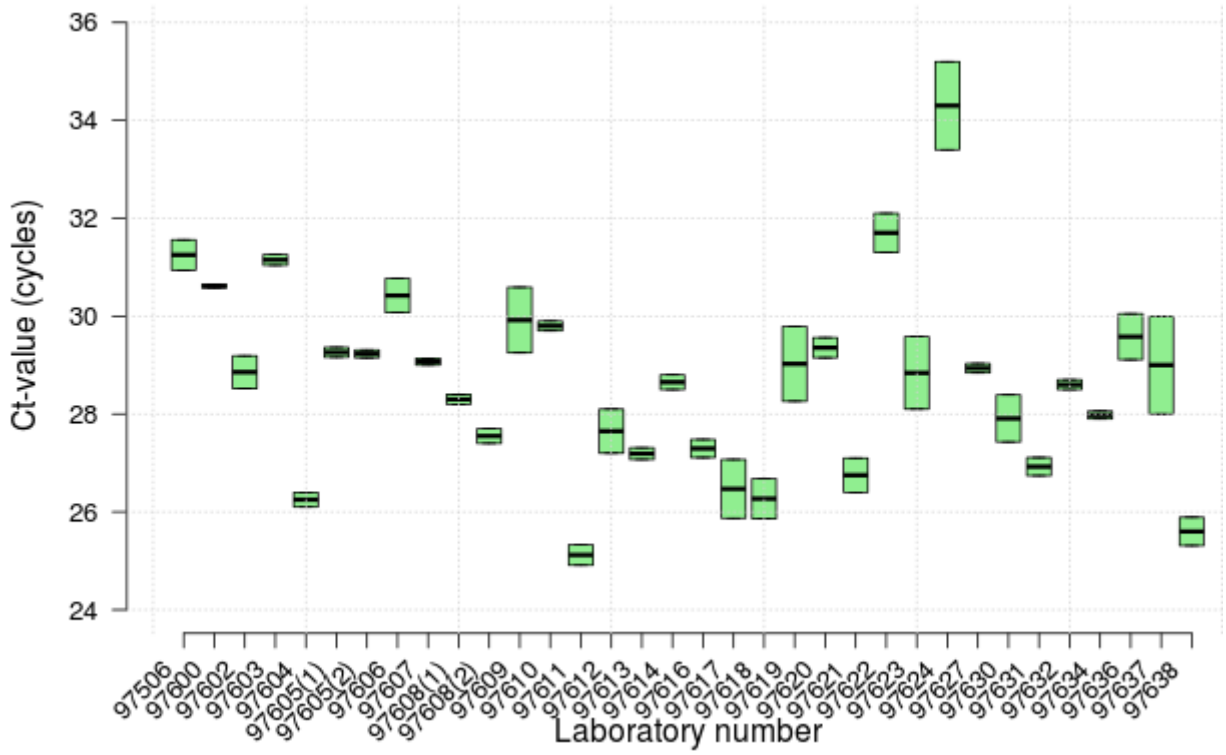


Fig II: Distribution of the Ct-values data for the PT2024CAPXVIR_TP5 samples (LAB97506-97638).

10.2 Annex 2: Quantitative results (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%	0,872	0,052	0,0543	NEG	S/P%= (OD(sample)-OD(NC))/(OD(PC)-OD(NC))*100
	N2					0,0518	NEG	
	P1					1,2791	POS	
	P2					1,0849	POS	
	P3					0,8998	POS	
	P4					0,4631	POS	
	P5					0,5656	POS	
	P5					0,5301	POS	
	P6					0,6418	POS	
	P7					0,7492	POS	
97600	N1	IDVet - ID SCREEN® CAPRIPOX DOUBLE ANTIGEN MULTI-SPECIES	30%	0,822	0,042	0,038	NEG	
	N2					0,039	NEG	
	P1					1,056	POS	
	P2					0,93	POS	
	P3					0,747	POS	
	P4					0,372	POS	
	P5					0,467	POS	
	P5					0,466	POS	
	P6					0,42	POS	
	P7					0,692	POS	
97602	N1	IDVet - ID SCREEN® CAPRIPOX DOUBLE ANTIGEN MULTI-SPECIES	30%	1,031	0,0675	0,0665	NEG	
	N2					0,07	NEG	
	P1					1,5215	POS	
	P2					1,332	POS	
	P3					0,9685	POS	
	P4					0,548	POS	
	P5					0,902	POS	
	P5					0,8045	POS	
	P6					0,787	POS	
	P7					0,9945	POS	
97604	N1	IDVet - ID SCREEN® CAPRIPOX DOUBLE ANTIGEN MULTI-SPECIES	30%	0,79	0,188	0,256	NEG	(Sample-NC)/(PC-NC)*100
	N2					0,246	NEG	
	P1					1,189	POS	
	P2					1,086	POS	
	P3					0,749	POS	

lab ID	Sample ID	Reagens	Cut-off	Pos control	Neg control	OD	Interpretation	Comment
	P4					0,398	POS	
	P5					0,576	POS	
	P5					0,725	POS	
	P6					0,546	POS	
	P7					0,843	POS	
97605	N1	IDVet - ID SCREEN® CAPRIPOX DOUBLE ANTIGEN MULTI-SPECIES	30%	0,92	0,05	0,047	NEG	S/P%=((ODsample-ODnc)/(ODpc-ODnc))*100
	N2					0,052	NEG	
	P1					1,192	POS	
	P2					1,043	POS	
	P3					0,808	POS	
	P4					0,372	POS	
	P5					0,643	POS	
	P5					0,646	POS	
	P6					0,553	POS	
	P7					0,908	POS	
97606	N1	IDVet - ID SCREEN® CAPRIPOX DOUBLE ANTIGEN MULTI-SPECIES	30%	0,9452	0,04635	0,0513	NEG	
	N2					0,049	NEG	
	P1					1,7025	POS	
	P2					1,6448	POS	
	P3					1,3054	POS	
	P4					0,5804	POS	
	P5					0,7462	POS	
	P5					0,7353	POS	
	P6					1,1084	POS	
	P7					1,3355	POS	
97607	N1	IDVet - ID SCREEN® CAPRIPOX DOUBLE ANTIGEN MULTI-SPECIES	30%	0,872	0,047	0,044	NEG	All samples were run in duplicates and the average value is given.
	N2					0,043	NEG	
	P1					1,082	POS	
	P2					0,945	POS	
	P3					0,746	POS	
	P4					0,334	POS	
	P5					0,619	POS	
	P5					0,646	POS	
	P6					0,372	POS	
	P7					0,591	POS	
97608	N1	IDVet - ID SCREEN® CAPRIPOX DOUBLE ANTIGEN MULTI-SPECIES	30%	1,132	0,0495	0,0555	NEG	
	N2					0,0495	NEG	
	P1					1,8885	POS	
	P2					1,5415	POS	

lab ID	Sample ID	Reagens	Cut-off	Pos control	Neg control	OD	Interpretation	Comment
	P3					1,3715	POS	
	P4					0,652	POS	
	P5					0,5765	POS	
	P5					0,5615	POS	
	P6					1,2915	POS	
	P7					1,221	POS	
97609	N1	IDVet - ID SCREEN® CAPRIPOX DOUBLE ANTIGEN MULTI-SPECIES	30%	1,0469	0,0617	0,052	NEG	100x [(ODsample - ODNc)/(ODPc - ODNc)])
	N2					0,06785	NEG	
	P1					1,74485	POS	
	P2					1,5238	POS	
	P3					1,4515	POS	
	P4					0,60965	POS	
	P5					0,631	POS	
	P5					0,55285	POS	
	P6					1,2759	POS	
	P7					1,1063	POS	
97610	N1	IDVet - ID SCREEN® CAPRIPOX DOUBLE ANTIGEN MULTI-SPECIES	30%	0,876	0,045	0,04	NEG	S/P%=(ODsample-ODNC)/(ODPC-ODNC)*100
	N2					0,044	NEG	
	P1					1,34	POS	
	P2					1,14	POS	
	P3					0,93	POS	
	P4					0,51	POS	
	P5					0,74	POS	
	P5					0,72	POS	
	P6					0,5	POS	
	P7					0,78	POS	
97611	N1	IDVet - ID SCREEN® CAPRIPOX DOUBLE ANTIGEN MULTI-SPECIES	30%	0,804	0,05	0,041	NEG	S/P%=((ODSample - OD Neg control)/(OD Positive Control- OD Negative Control)) *100
	N2					0,058	NEG	
	P1					1,189	POS	
	P2					1,024	POS	
	P3					0,871	POS	
	P4					0,324	POS	
	P5					0,643	POS	
	P5					0,639	POS	
	P6					0,451	POS	
	P7					0,689	POS	
97612	N1	IDVet - ID SCREEN® CAPRIPOX DOUBLE ANTIGEN MULTI-SPECIES	30%	0,9615	0,044	0,045	NEG	S/P%=(ODsample-ODneg/ODpos-ODneg)*100
	N2					0,042	NEG	
	P1					1,764	POS	

lab ID	Sample ID	Reagens	Cut-off	Pos control	Neg control	OD	Interpretation	Comment
	P2					1,564	POS	
	P3					1,261	POS	
	P4					0,559	POS	
	P5					0,569	POS	
	P5					0,652	POS	
	P6					1,16	POS	
	P7					1,308	POS	
97613	N1	IDVet - ID SCREEN® CAPRIPOX DOUBLE ANTIGEN MULTI-SPECIES	30%	0,8229	0,0464	0,0488	NEG	S/P%=((sample OD-NC OD)/(PC OD-NC OD))*100
	N2					0,0464	NEG	
	P1					1,3216	POS	
	P2					1,0969	POS	
	P3					0,8522	POS	
	P4					0,444	POS	
	P5					0,6508	POS	
	P5					0,6351	POS	
	P6					0,4938	POS	
	P7					0,8242	POS	
97614	N1	IDVet - ID SCREEN® CAPRIPOX DOUBLE ANTIGEN MULTI-SPECIES	30%	1,191	0,042	0,046	NEG	S/P% = (OD sample - OD NC) / (OD PC - OD NC) x 100
	N2					0,047	NEG	
	P1					1,757	POS	
	P2					1,541	POS	
	P3					1,296	POS	
	P4					0,705	POS	
	P5					0,435	POS	
	P5					0,475	POS	
	P6					1,204	POS	
	P7					1,098	POS	
97615	N1	IDVet - ID SCREEN® CAPRIPOX DOUBLE ANTIGEN MULTI-SPECIES	30%	0,909	0,047	0,05	NEG	S/P % = (OD sample - OD neg control) /((OD Positive COntrol - OD Negative Control) X 100
	N2					0,052	NEG	
	P1					1,252	POS	
	P2					1,055	POS	
	P3					0,83	POS	
	P4					0,313	POS	
	P5					0,759	POS	
	P5					0,704	POS	
	P6					0,478	POS	
	P7					0,784	POS	

lab ID	Sample ID	Reagens	Cut-off	Pos control	Neg control	OD	Interpretation	Comment
97616	N1	IDVet - ID SCREEN® CAPRIPOX DOUBLE ANTIGEN MULTI-SPECIES	30%	0,713	0,0465	0,049	NEG	S/P=sample OD - (-ve contr OD)/(+ contr OD) - (-ve contr OD)x100
	N2					0,051	NEG	
	P1					1,231	POS	
	P2					1,033	POS	
	P3					0,782	POS	
	P4					0,408	POS	
	P5					0,655	POS	
	P5					0,656	POS	
	P6					0,57	POS	
	P7					0,792	POS	
97617	N1	IDVet - ID SCREEN® CAPRIPOX DOUBLE ANTIGEN MULTI-SPECIES	30%	0,959	0,043	0,045	NEG	S/P % = (ODsample-ODnegative)/(ODpositive-ODnegative)x100
	N2					0,046	NEG	
	P1					1,445	POS	
	P2					1,276	POS	
	P3					1,039	POS	
	P4					0,435	POS	
	P5					1,096	POS	
	P5					0,812	POS	
	P6					0,699	POS	
	P7					1,234	POS	
97618	N1	IDVet - ID SCREEN® CAPRIPOX DOUBLE ANTIGEN MULTI-SPECIES	30%	0,72	0,054	0,9	NEG	ELISA Indirect
	N2					0,45	NEG	
	P1					168,16	POS	
	P2					151,05	POS	
	P3					112,61	POS	
	P4					55,7	POS	
	P5					111,41	POS	
	P5					111,86	POS	
	P6					54,05	POS	
	P7					102,85	POS	
97619	N1	IDVet - ID SCREEN® CAPRIPOX DOUBLE ANTIGEN MULTI-SPECIES	30%	0,884	0,047	0,052	NEG	S/P%
	N2					0,052	NEG	
	P1					1,136	POS	
	P2					0,856	POS	
	P3					0,828	POS	
	P4					0,331	POS	
	P5					0,838	POS	
	P5					0,766	POS	
	P6					0,441	POS	

lab ID	Sample ID	Reagens	Cut-off	Pos control	Neg control	OD	Interpretation	Comment
	P7					0,689	POS	
97620	N1	IDVet - ID SCREEN® CAPRIPOX DOUBLE ANTIGEN MULTI-SPECIES	30%	1,119	0,044	0,049	NEG	S/P%=(ODsample-ODnc)/(ODpc-ODnc)*100
	N2					0,049	NEG	
	P1					1,79	POS	
	P2					1,6	POS	
	P3					1,237	POS	
	P4					0,596	POS	
	P5					0,446	POS	
	P5					0,505	POS	
	P6					1,458	POS	
	P7					1,131	POS	
97621	N1	IDVet - ID SCREEN® CAPRIPOX DOUBLE ANTIGEN MULTI-SPECIES	30%	0,79	0,05	0,05	NEG	S/P%= ((OD sample- OD NC)/(OD PC- OD NC))*100
	N2					0,05	NEG	
	P1					1,1	POS	
	P2					0,94	POS	
	P3					0,74	POS	
	P4					0,31	POS	
	P5					0,61	POS	
	P5					0,64	POS	
	P6					0,43	POS	
	P7					0,62	POS	
97622	N1	IDVet - ID SCREEN® CAPRIPOX DOUBLE ANTIGEN MULTI-SPECIES	30%	0,791	0,054	0,056	NEG	
	N2					0,057	NEG	
	P1					1,206	POS	
	P2					1,043	POS	
	P3					0,866	POS	
	P4					0,423	POS	
	P5					0,51	POS	
	P5					0,541	POS	
	P6					0,542	POS	
	P7					0,888	POS	
97623	N1	IDVet - ID SCREEN® CAPRIPOX DOUBLE ANTIGEN MULTI-SPECIES	30%	1,209	0,06	0,06	NEG	All samples were run in duplicates and the average value is given.
	N2					0,053	NEG	
	P1					2,118	POS	
	P2					1,833	POS	
	P3					1,708	POS	
	P4					0,7	POS	
	P5					0,898	POS	

lab ID	Sample ID	Reagens	Cut-off	Pos control	Neg control	OD	Interpretation	Comment
	P5					0,785	POS	
	P6					1,63	POS	
	P7					1,779	POS	
	N1					0,1	NEG	
	N2					0,05	NEG	
	P1					1,1	POS	
	P2					0,9	POS	
97628	P3	IDVet - ID SCREEN® CAPRIPOX DOUBLE ANTIGEN MULTI-SPECIES	30%	0,6	0,05	0,7	POS	S/P%= (ODsample-ODnc)/(ODpc-ODnc)*100
	P4					0,3	POS	
	P5					0,7	POS	
	P5					0,6	POS	
	P6					0,4	POS	
	P7					0,6	POS	
	N1					0,065	NEG	
	N2					0,042	NEG	
	P1					1,103	POS	
	P2					1,014	POS	
97629	P3	IDVet - ID SCREEN® CAPRIPOX DOUBLE ANTIGEN MULTI-SPECIES	450	0,76	0,0895	0,736	POS	S/P%= (ODsample-ODnc)/(ODpc-ODnc)*100
	P4					0,372	POS	
	P5					0,764	POS	
	P5					0,683	POS	
	P6					0,418	POS	
	P7					0,647	POS	
	N1					0,081	NEG	
	N2					0,055	NEG	
	P1					0,644	POS	
	P2					0,554	POS	
97630	P3	IDVet - ID SCREEN® CAPRIPOX DOUBLE ANTIGEN MULTI-SPECIES	30%	0,39	0,05	0,482	POS	S/P%=100*((Odsample-Odnc)/(Odpc-Odnc))
	P4					0,312	POS	
	P5					0,344	POS	
	P5					0,354	POS	
	P6					0,271	POS	
	P7					0,406	POS	
	N1					0,046	NEG	
	N2					0,042	NEG	
97631	P1	IDVet - ID SCREEN® CAPRIPOX DOUBLE ANTIGEN MULTI-SPECIES	30%	0,888	0,042	1,403	POS	s/p%=(od smple-odnc)/(odpc-odnc)x100
	P2					1,047	POS	
	P3					0,9205	POS	
	P4					0,637	POS	

lab ID	Sample ID	Reagens	Cut-off	Pos control	Neg control	OD	Interpretation	Comment
	P5					0,604	POS	
	P5					1,052	POS	
	P6					0,635	POS	
	P7					1,151	POS	
	N1					0,047	NEG	
	N2					0,047	NEG	
	P1					1,212	POS	
	P2					1,035	POS	
97632	P3	IDVet - ID SCREEN® CAPRIPOX DOUBLE ANTIGEN MULTI-SPECIES	30%	0,785	0,045	0,853	POS	S/P%=(ODsample-ODnegctrl)/(ODposctrl-ODnegctrl)x100
	P4					0,365	POS	
	P5					0,637	POS	
	P5					0,697	POS	
	P6					0,467	POS	
	P7					0,725	POS	
	N1					0,05	NEG	
	N2					0,048	NEG	
	P1					1,07	POS	
	P2					0,902	POS	
97634	P3	IDVet - ID SCREEN® CAPRIPOX DOUBLE ANTIGEN MULTI-SPECIES	30%	0,6835	0,047	0,733	POS	
	P4					0,4465	POS	
	P5					0,4475	POS	
	P5					0,436	POS	
	P6					0,528	POS	
	P7					0,6365	POS	
	N1					0,074	NEG	
	N2					0,058	NEG	
	P1					1,31	POS	
	P2					1,148	POS	
97637	P3	IDVet - ID SCREEN® CAPRIPOX DOUBLE ANTIGEN MULTI-SPECIES	30%	0,719	0,054	0,959	POS	S/P%= [(OD sample-ODneg)/(ODpc-ODnc)]*100
	P4					0,421	POS	
	P5					0,569	POS	
	P5					0,508	POS	
	P6					0,955	POS	
	P7					0,967	POS	
	N1					0,046	NEG	
	N2					0,045	NEG	
97638	P1	IDVet - ID SCREEN® CAPRIPOX DOUBLE ANTIGEN MULTI-SPECIES	30%	0,811	0,047	1,418	POS	
	P2					1,121	POS	
	P3					0,868	POS	

lab ID	Sample ID	Reagens	Cut-off	Pos control	Neg control	OD	Interpretation	Comment
	P4					0,61	POS	
	P5					0,638	POS	
	P5					0,683	POS	
	P6					0,59	POS	
	P7					0,929	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	250	=	6	NEG	
	N2												=	2	NEG	
	P1												=	6250	POS	
	P2												=	650	POS	
	P3												=	150	POS	
	P4												=	1250	POS	
	P5												=	250	POS	
	P5												=	6250	POS	
	P6												=	250	POS	
	P7												=	250	POS	
97600	N1	After WAOH terrestrial chapter 3.4.12 LSD 2021	Other	LSDV Neethling	1/5	2	125	IAH Pirbright VN84	480	10	480	10	<	10	NEG	Celltype: SFT-R (CCLV-RIE 43)
	N2												<	10	NEG	
	P1												=	320	POS	
	P2												=	160	POS	
	P3												=	80	POS	
	P4												=	480	POS	
	P5												=	320	POS	
	P5												=	320	POS	
	P6												=	60	POS	
	P7												=	80	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	
	P2												=	320	POS	
	P3												=	160	POS	
	P4												=	320	POS	
	P5												=	320	POS	
	P5												=	320	POS	
	P6												=	40	POS	
	P7												=	160	POS	
97618	N1	Pirbright	OA3.Ts	LSDV Neethling	1/5	0.2-0.0016	100	laboratory reference material	1:20	5	20	5	=	5	NEG	
	N2												<	5	NEG	
	P1												>	640	POS	
	P2												>	640	POS	
	P3												=	320	POS	
	P4												=	640	POS	
	P5												=	320	POS	
	P5												=	320	POS	
	P6												=	640	POS	
	P7												=	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	0,003	Anti-Bovine Ig G Peroxidase antibody produced in rabbit A 5295 SIGMA	0,0001	0,02	300	1	=	1	NEG
	N2														=	1	NEG
	P1														=	300	POS
	P2														=	300	POS
	P3														=	300	POS
	P4														=	300	POS
	P5														=	300	POS
	P5														=	300	POS
	P6														=	300	POS
	P7														=	300	POS
97618	N1	EURL Capripoxviruses Protocol	OA3.Ts	LSDV Neethling	0,02	0,02	100	Antiserum Pirbright	0,003	Anti-Bovine Ig G Peroxidase antibody produced in rabbit A 5295 SIGMA	0,001	0,02	300	0	=	0	NEG
	N2														=	0	NEG
	P1														=	0	POS
	P2														=	300	POS
	P3														=	300	POS
	P4														=	300	POS
	P5														=	300	POS
	P5														=	300	POS
	P6														=	300	POS
	P7														=	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	Macherey Nagel:	DNA nucleospin	Haegeman et al. 2013	D5R	37	29,04	50	=	50	NEG	Internal modifications to extraction protocol/kit: Addition of EC
	BP1									=	29,16	POS	
	TN1									=	50	NEG	
	TP1									=	31,59	POS	
	TP2									=	30,05	POS	
	TP3									=	22,89	POS	
	TP4									=	31,08	POS	
	TP5									=	31,56	POS	
	TP5									=	30,94	POS	
	TP6									=	29,71	POS	
97600	BN1	Bowden et al. 2008 modified by FLI	Biosellal	Biosellal Superball	Bowden et al. 2008	ORF074 (P32)	45	30,39	45	>	45	NEG	Qiagen QuantiTect Multiplex PCR Kit NoRox
	BP1									=	30,48	POS	
	TN1									>	45	NEG	
	TP1									=	30,7	POS	
	TP2									=	32,73	POS	
	TP3									=	22,99	POS	
	TP4									=	30,66	POS	
	TP5									=	30,58	POS	
	TP5									=	30,65	POS	
	TP6									=	29,64	POS	
97602	BN1	Bowden et al	Invitrogen/Thermo Fisher Scientific	MagMAX Core Nucleic Acid Purification Kit	Bowden et al. 2008	ORF074 (P32)	45	19,06	45	>	45	NEG	
	BP1									=	27,63	POS	
	TN1									>	45	NEG	
	TP1									=	26,72	POS	
	TP2									=	28,32	POS	
	TP3									=	26,07	POS	
	TP4									=	29,17	POS	
	TP5									=	28,52	POS	
	TP5									=	29,19	POS	
	TP6									=	29,6	POS	
97603	BN1	Bowden et al. 2008; Babiuk et al. 2008; SOP from Pirbright	Roche	Roche-MP 96/ Viral NA SV	Invitrogen/Thermo Fisher Scientific	ORF074 (P32)	Cq value < 35 for positive results	26,59	0	=	0	NEG	
	BP1									=	28,19	POS	
	TN1									=	39,33	NEG	
	TP1									=	28,5	POS	
	TP2									=	29,62	POS	
	TP3									=	24,46	POS	
	TP4									=	30,75	POS	
	TP5									=	31,04	POS	
	TP5									=	31,26	POS	
	TP6									=	30,27	POS	
97604	BN1	6.3.51	Roche	MagNa Pure 96 DNA (Roche)	Other	ORF074 (P32)	40			=		NEG	
	BP1									=	24,2	POS	
	TN1									=	33,2	POS	
	TP1									=	24,1	POS	
	TP2									=	24,4	POS	
	TP3									=	19	POS	
	TP4									=	25,9	POS	
	TP5									=	26,4	POS	
	TP5									=	26,1	POS	
	TP6									=	24,3	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
97605 (1)	BN1	Bowden et al. 2008	Roche	Roche-MP 96/ Viral NA SV	Bowden et al. 2008	ORF074 (P32)	No cut-off	28,16	45	>	45	NEG	
	BP1									=	27,95	POS	
	TN1									=	36,89	POS	
	TP1									=	28,15	POS	
	TP2									=	29,14	POS	
	TP3									=	23,66	POS	
	TP4									=	29,62	POS	
	TP5									=	29,16	POS	
	TP5									=	29,36	POS	
	TP6									=	27,09	POS	
97605 (2)	BN1	Haegeman et al. 2013	Roche	Roche-MP 96/ Viral NA SV	Haegeman et al. 2013	E3L	No cut-off	28,23	45	>	45	NEG	
	BP1									=	28,2	POS	
	TN1									=	36,99	POS	
	TP1									=	28,42	POS	
	TP2									=	29,35	POS	
	TP3									=	23,63	POS	
	TP4									=	29,44	POS	
	TP5									=	29,15	POS	
	TP5									=	29,31	POS	
	TP6									=	27,35	POS	
97606	BN1	DNA extraction and real time PCR (Bowden et al. 2008)	Qiagen	QIAamp Viral RNA Mini Kit	Bowden et al. 2008	ORF074 (P32)	45	34,67	45	=	0	NEG	
	BP1									=	31,65	POS	
	TN1									=	0	NEG	
	TP1									=	30,75	POS	
	TP2									=	30,39	POS	
	TP3									=	24,01	POS	
	TP4									=	30,46	POS	
	TP5									=	30,77	POS	
	TP5									=	30,07	POS	
	TP6									=	29,37	POS	
97607	BN1	P32	Qiagen	Qiagen DNAesay Blood and tissue	Sso Advanced Universal Probes	ORF074 (P32)		30,83	0	=	0	NEG	
	BP1									=	28,08	POS	
	TN1									=	36	POS	
	TP1									=	27,59	POS	
	TP2									=	27,17	POS	
	TP3									=	23,14	POS	
	TP4									=	28,79	POS	
	TP5									=	29	POS	
	TP5									=	29,14	POS	
	TP6									=	28,88	POS	
97608 (1)	BN1	Capri-p32-Bowden	Macherey-Nagel)	NucleoMagVet extraction kit	Bowden et al. 2008 Perfecta qPCR kit (Quanta Biosciences)	ORF074 (P32)	40	28,8	40	>	40	NEG	
	BP1									=	29	POS	
	TN1									>	40	NEG	
	TP1									=	29	POS	
	TP2									=	28,2	POS	
	TP3									=	22	POS	
	TP4									=	28,1	POS	
	TP5									=	28,4	POS	
	TP5									=	28,2	POS	
	TP6									=	27,2	POS	
	BN1	Capri-Pol	Macherey-Nagel)				40	28,1	40	>	40	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
97608 (2)	BP1 TN1 TP1 TP2 TP3 TP4 TP5 TP5 TP6			NucleoMagVet extraction kit	Perfecta qPCR kit (Quanta Biosciences)	poly (A) polymerase small subunit region (LSDV068 gene)				= > = = = = = = =	28,4 40 28,4 27,6 21 27,3 27,7 27,4 26,5	POS NEG POS POS POS POS POS POS POS	NucleoMagVet (Macherey-Nagel) extraction kit; Perfecta qPCR kit (Quanta Biosciences); Target: poly (A) polymerase small subunit region (LSDV068 gene)
97609	BN1 BP1 TN1 TP1 TP2 TP3 TP4 TP5 TP5 TP6	Bowden et al Generic Capripox virus detection Real Time PCR	Indical Bioscience	Indispin pathogen kit	Bowden et al. 2008 QuantiFast Pathogen+ IC PCR Kit	ORF074 (P32)	37	33,85	45	= = = = = = = = = =	45 29,94 45 30,32 31,74 25,89 30,64 30,59 29,25 31,06	NEG POS NEG POS POS POS POS POS POS POS	
97610	BN1 BP1 TN1 TP1 TP2 TP3 TP4 TP5 TP5 TP6	Bowden et al	Thermofisher Scientific	Kingfisher Flex/Magnifiq Pathogen kit	Bowden et al. 2008		>40	28,3	40	> = > = = = = = = =	40 26,1 40 27,8 27,6 24,9 29,8 29,7 29,9 26,5	NEG POS NEG POS POS POS POS POS POS POS	
97611	BN1 BP1 TN1 TP1 TP2 TP3 TP4 TP5 TP5 TP6	Haegeman et al 2013	Qiagen	QIAamp Viral RNA Mini Kit	Invitrogen/Thermo Fisher Scientific	E3L	CT 38	23,83	38	> = = = = = = = = =	38 23,59 32,58 25,35 25,33 18,67 25,18 25,33 24,91 24,16	NEG POS POS POS POS POS POS POS POS POS	
97612	BN1 BP1 TN1 TP1 TP2 TP3 TP4 TP5 TP5 TP6	IDvet	Roche	High Pure Viral Nucleic Acid Kit	IDVet - ID GENE® CAPRIPOX VIRUS TRIPLEX		38 Cq	27,2	38	> = > = = = = = = =	38 26,9 38 25,6 25,8 19,5 27,1 28,1 27,2 25,9	NEG POS NEG POS POS POS POS POS POS POS	
97613	BN1 BP1	Babiuk et al. 2008	Indical Bioscience	Indispin pathogen kit	Babiuk et al. 2008	ORF074 (P32)	40	23,08	40	< =	40 25,37	NEG 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									=	32,4	POS	
	TP1									=	27,22	POS	
	TP2									=	27,22	POS	
	TP3									=	19,68	POS	
	TP4									=	27,35	POS	
	TP5									=	27,07	POS	
	TP5									=	27,31	POS	
	TP6									=	25,88	POS	
97614	BN1	SOP G.72	Invitrogen/Thermo Fisher Scientific	MagMAX Core Nucleic Acid Purification Kit	Babiuk et al. 2008	ORF074 (P32)	35	26	40	>	40	NEG	
	BP1									=	27,6	POS	
	TN1									=	38	NEG	
	TP1									=	29,6	POS	
	TP2									=	28,7	POS	
	TP3									=	20,6	POS	
	TP4									=	28,7	POS	
	TP5									=	28,8	POS	
	TP5									=	28,5	POS	
	TP6									=	27,9	POS	
97616	BN1	Path-ID qPCR	Qiagen	QIAmp DNA mini kit	Bowden et al. 2008	ORF074 (P32)	35	28,02	0	=	0	NEG	Comment sample TN1: Since Ct value is close to cut off, despite negative interpretation, it is required to test this animal again using a new samples (Ct in second test was 37.39) despite negative interpretation
	BP1									=	25,55	POS	
	TN1									=	36,29	NEG	
	TP1									=	27,2	POS	
	TP2									=	27,81	POS	
	TP3									=	21,05	POS	
	TP4									=	28,3	POS	
	TP5									=	27,48	POS	
	TP5									=	27,11	POS	
	TP6									=	27,92	POS	
97617	BN1	Bowden et al. 2008	Indical Bioscience	Other - Bowden et al. 2008	ORF074 (P32)	38	31,61	40	Bowden et al. 2008	>	40	NEG	
	BP1									=	26,38	POS	
	TN1									>	40	NEG	
	TP1									=	26,75	POS	
	TP2									=	27,43	POS	
	TP3									=	20,2	POS	
	TP4									=	26,48	POS	
	TP5									=	25,87	POS	
	TP5									=	27,07	POS	
	TP6									=	27,91	POS	
97618	BN1	Bowden et al. 2008	Invitrogen/Thermo Fisher Scientific	Pure Link Genomic DNA Mini Kit (Invitrogen)	Invitrogen/Thermo Fisher Scientific - Invitrogen/Thermo Fisher Scientific kit	ORF074 (P32)	23,41	0	Bowden et al. 2008	=	0	NEG	
	BP1									=	24,15	POS	
	TN1									=	0	NEG	
	TP1									=	25,35	POS	
	TP2									=	25,21	POS	
	TP3									=	18,88	POS	
	TP4									=	27,96	POS	
	TP5									=	26,68	POS	
	TP5									=	25,86	POS	
	TP6									=	24,24	POS	
97619	BN1	Stubbs et al. 2012	Other		Path_ID qPCR	ORF074 (P32)	38	33	0	=	0	NEG	Internal modifications to extraction protocol/kit: PerkinELMER chemagic Viral
	BP1									=	28,11	POS	
	TN1									=	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
	TP1									=	28,04	POS	DNA/RNA 300 Kit special H96 using the chemagic 360 instrument
	TP2									=	29,94	POS	
	TP3									=	21,12	POS	
	TP4									=	29,12	POS	
	TP5									=	29,79	POS	
	TP5									=	28,26	POS	
	TP6									=	28,67	POS	
97620	BN1	SOP487 v2	Qiagen	QIAamp Viral RNA Mini Kit	Bowden et al. 2008	ORF074 (P32)	Ct < 45	27,49	-	=	-	NEG	
	BP1									=	28,705	POS	
	TN1									=	35,639	POS	
	TP1									=	29,743	POS	
	TP2									=	30,944	POS	
	TP3									=	21,814	POS	
	TP4									=	29,497	POS	
	TP5									=	29,561	POS	
	TP5									=	29,153	POS	
	TP6									=	28,721	POS	
97621	BN1	Bowden et al. 2008	Qiagen	MagAttract 96 cador Pathogen Kit	Path_ID qPCR	ORF074 (P32)	35	35	40	>	40	NEG	Comment sample TN1: average of the result of two extractions
	BP1									=	24,6	POS	
	TN1									=	34,9	POS	
	TP1									=	24,2	POS	
	TP2									=	23,1	POS	
	TP3									=	17,4	POS	
	TP4									=	26,3	POS	
	TP5									=	26,4	POS	
	TP5									=	27,1	POS	
	TP6									=	24,2	POS	
97622	BN1	In House - AVS-0959	Roche	Roche-MP 96/ Viral NA SV	Other	ORF074 (P32)	Ct 36 - 45 is considered doubtfull	23,8	-	=	-	NEG	
	BP1									=	25,5	POS	
	TN1									=	-	NEG	
	TP1									=	25,9	POS	
	TP2									=	26,6	POS	
	TP3									=	24,6	POS	
	TP4									=	32,1	POS	
	TP5									=	32,1	POS	
	TP5									=	31,3	POS	
	TP6									=	27,9	POS	
97623	BN1	Haegeman et al. 2013 and Bowden et al. 2008	Indical Bioscience	Haegeman et al. 2013	E3L	Positive <37 Doubtful 37-45 Negative >45. IC GAPDH : <30	26	45	Haegeman et al. 2013 and Bowden et al. 2008	>	45	NEG	Internal modifications to extraction protocol/kit: IndiMag Pathogen Kit 200 microL sample + 20 microL Proteinase K. Extraction robot: Maelstrom-9600 TANBead. Results are given as the average of triplicates. Protocols in use were both Haegeman et al. 2013 and Bowden et al. 2008. No species differentiation nor DIVA PCR has been performed.
	BP1									=	29,54	POS	
	TN1									>	45	NEG	
	TP1									=	28,47	POS	
	TP2									=	27,42	POS	
	TP3									=	25	POS	
	TP4									=	27,7	POS	
	TP5									=	29,58	POS	
	TP5									=	28,1	POS	
	TP6									=	28,3	POS	
97624	BN1	SOP 10-BM-001/ Bowden et.al	Other		Invitrogen/Thermo Fisher Scientific	ORF074 (P32)	38	24	0	=	0	NEG	
	BP1									=	34,4	POS	
	TN1									<	40	NEG	
	TP1									=	31,2	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
	TP2									=	30,9	POS	
	TP3									=	28,4	POS	
	TP4									=	34,1	POS	
	TP5									=	35,2	POS	
	TP5									=	33,4	POS	
	TP6									=	33,4	POS	
97627	BN1	SOP	Qiagen	QIAmp DNA mini kit	IDVet - ID GENE® CAPRIPOX VIRUS TRIPLEX	31,96	SOP	Qiagen		=		NEG	
	BP1									=	28,68	POS	
	TN1									=	36,66	POS	
	TP1									=	28,15	POS	
	TP2									=	27,92	POS	
	TP3									=	23,63	POS	
	TP4									=	29,37	POS	
	TP5									=	28,84	POS	
	TP5									=	29,03	POS	
	TP6									=	26,9	POS	
97630	BN1	Bowden	Indical Bioscience	Indispin pathogen kit	Bowden et al. 2008 PerfeCTa qPCR ToughMix (Quanta BioSciences Gaithersburg MD USA)	ORF074 (P32)	45	28,45	45	>	45	NEG	
	BP1									=	26,71	POS	
	TN1									=	37,12	POS	
	TP1									=	27,49	POS	
	TP2									=	28,86	POS	
	TP3									=	20,13	POS	
	TP4									=	26,87	POS	
	TP5									=	27,43	POS	
	TP5									=	28,39	POS	
	TP6									=	25,2	POS	
97631	BN1	Bowden et al	Roche	High Pure Viral Nucleic Acid Kit	Qiagen - MO-TO 46		40	29,03		=		NEG	
	BP1									=	25,04	POS	
	TN1									=	34,15	POS	
	TP1									=	25,04	POS	
	TP2									=	28,28	POS	
	TP3									=	23,45	POS	
	TP4									=	25,62	POS	
	TP5									=	26,74	POS	
	TP5									=	27,11	POS	
	TP6									=	21,99	POS	
97632	BN1	Bowden et al. (2008) & Stubbs et al. (2012)	Sacace Biotechnologies	Sacace-viral Nucleic acid Extraction	Other - Path_ID qPCR			31,5	0	=	0	NEG	Bowden et al. (2008) Capx virus tissue tropism and shedding: A quantitative study in experimentally infected sheep and goats; Stubbs et al. (2012) Validation of a high-throughput real-time PCR assay for the detection of capripoxviral DNA
	BP1									=	29,7	POS	
	TN1									=	36,4	POS	
	TP1									=	21,9	POS	
	TP2									=	28,2	POS	
	TP3									=	23,5	POS	
	TP4									=	28,4	POS	
	TP5									=	28,7	POS	
	TP5									=	28,5	POS	
	TP6									=	28,2	POS	
97634	BN1	Bowden et al. 2008	Biosellal	Biosellal Superball	Bowden et al. 2008		40	30,53	45	>	45	NEG	
	BP1									=	25,51	POS	
	TN1									>	45	NEG	
	TP1									=	26,2	POS	
	TP2									=	26,93	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
	TP3									=	20,54	POS	
	TP4									=	27,97	POS	
	TP5									=	28,06	POS	
	TP5									=	27,9	POS	
	TP6									=	25,73	POS	
97636	BN1	Identification of the genome of Lumpy Skin Diseases virus	Indical Bioscience	Indispin pathogen kit	Invitrogen/Thermo Fisher Scientific			29	0	=	0	NEG	
	BP1									=	28,86	POS	
	TN1									=	0	NEG	
	TP1									=	29,63	POS	
	TP2									=	27,69	POS	
	TP3									=	23,77	POS	
	TP4									=	31,37	POS	
	TP5									=	29,11	POS	
	TP5									=	30,05	POS	
	TP6									=	26,57	POS	
97637	BN1	Bowden et al. 2008	Roche	High Pure Viral Nucleic Acid Kit	Roche - Roche kit	ORF074 (P32)	23	Bowden 2008		=	-	NEG	
	BP1									=	28	POS	
	TN1									=	-	NEG	
	TP1									=	27	POS	
	TP2									=	26	POS	
	TP3									=	21	POS	
	TP4									=	29	POS	
	TP5									=	28	POS	
	TP5									=	30	POS	
	TP6									=	28	POS	
97638	BN1	Bowden et al. 2008	Indical Bioscience	Indispin pathogen kit	Bowden et al. 2008			26,13	0	=	0	NEG	
	BP1									=	25,75	POS	
	TN1									=	0	NEG	
	TP1									=	25,74	POS	
	TP2									=	25,89	POS	
	TP3									=	20,05	POS	
	TP4									=	25,48	POS	
	TP5									=	25,31	POS	
	TP5									=	25,89	POS	
	TP6									=	24,93	POS	

10.2.5 VIRO: SPECIES DIFFERENTIATION

Lab ID	Sample ID	Protocol/ SOP	Producer Extraction protocol / kit	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	Combination of different protocols			Other	Combination of different protocols	45	21,82	50	=	50	NEG	Internal modifications to extraction protocol/kit: Addition of EC For the differentiation between the different strains we used Agiannioaki et al. 2016, Chibssa et al. 2018, Wolff et al. 2021 and Haegeman et al. 2016
	BP1									=	30,05	LSDV	
	TN1									=	50	NEG	
	TP1									=	35,83	GTPV	
	TP2									=	29,83	LSDV	
	TP3									=	37,18	SPPV	
	TP4									=	31,5	LSDV	
	TP5									=	31,51	LSDV	
	TP5									=	30,93	LSDV	
	TP6									=	30,27	LSDV	
97600	BN1	Lamien et al. 2011	Biosellal		Other			53		=		NA	Roche LC480 Mix
	BP1									=	53	LSDV	
	TN1									=		NA	
	TP1									=	62,5	GTPV	
	TP2									=	53	LSDV	
	TP3									=	45,5	SPPV	
	TP4									=	53	LSDV	
	TP5									=	53	LSDV	
	TP5									=	53	LSDV	
	TP6									=	53	LSDV	
97602	BN1	FRET Lamien			Lamien et al. 2011			22,09	45	>	45	NEG	
	BP1									=	31,51	LSDV	
	TN1									>	45	NEG	
	TP1									=	28,78	GTPV	
	TP2									=	32,04	LSDV	
	TP3									=	32,38	SPPV	
	TP4									=	34,52	LSDV	
	TP5									=	33,69	LSDV	
	TP5									=	34,93	LSDV	
	TP6									=	36,06	LSDV	
97603	BN1	Lamien et al. 2011	Roche		Lamien et al. 2011	Classical PCR amplification of a portion of the RP030 gene: conventional		172	0	=	0	NEG	151 bp product for SPPV 172 bp for GTPV and LSDV
	BP1									=	172	LSDV	
	TN1									=	0	NEG	
	TP1									=	172	GTPV	
	TP2									=	172	LSDV	
	TP3									=	151	SPPV	
	TP4									=	172	LSDV	
	TP5									=	172	LSDV	
	TP5									=	172	LSDV	
	TP6									=	172	LSDV	
97604	BN1	6.3.51	Roche		Other					=		NEG	
	BP1									=		LSDV	
	TN1									=		LSDV	
	TP1									=		GTPV	
	TP2									=		LSDV	
	TP3									=		SPPV	
	TP4									=		LSDV	
	TP5									=		LSDV	
	TP5									=		LSDV	
	TP6									=		LSDV	

Lab ID	Sample ID	Protocol/ SOP	Producer Extraction protocol / kit	Extraction protocol / kit	RT-PCR protocol / kit	Methodology	Cut-off	Pos control	Neg control	Operator	Raw data (Ct cp value)	Interpretation	General comments
97607	BN1	GPCR	QIAGEN		Sso Advanced Universal Probes	GPCR		32,15	0	=	0	NEG	
	BP1									=	28,47	LSDV	
	TN1									=	0	NI	
	TP1									=	0	NA	
	TP2									=	27,55	LSDV	
	TP3									=	0	NA	
	TP4									=	30,25	LSDV	
	TP5									=	30,38	LSDV	
	TP5									=	30,82	LSDV	
	TP6									=	29,85	LSDV	
97608 (1)	BN1	Haegeman et al. 2024	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	23,6	40	>	40	NEG	
	BP1									=	29,6	LSDV	
	TN1									>	40	NEG	
	TP1									=	31,9	GTPV	
	TP2									>	40	LSDV	
	TP3									=	23,1	SPPV	
	TP4									=	28,7	LSDV	
	TP5									=	29,1	LSDV	
	TP5									=	28,7	LSDV	
	TP6									=	27,3	LSDV	
97608 (2)	BN1	LSD-WTR-Mix-FAM/LSD-VR-Mix-HEX	Other		Other	LSD-WTR-Mix-FAM/LSD-VR-Mix-HEX	40	26,2	40	>	40	NEG	
	BP1									=	31	LSDV	
	TN1									>	40	NEG	
	TP1									>	40	NI	
	TP2									=	30,2	LSDV	
	TP3									>	40	NI	
	TP4									=	28,8	LSDV	
	TP5									=	28,8	LSDV	
	TP5									=	28,8	LSDV	
	TP6									=	28,4	LSDV	
97609	BN1	Lamien et al 2011 conventional PCR partial GPCR	Indical Bioscience		Lamien et al. 2011 HotStart Taq Polymerase Qiagen				0	=	0	NEG	
	BP1									=	172	LSDV	
	TN1									=	0	NEG	
	TP1									=	172	GTPV	Pos control GTPV: LSDV/GTPV band (172 bp)
	TP2									=	172	LSDV	
	TP3									=	151	SPPV	Pos control SPPV: SPPV band (151 bp)
	TP4									=	172	LSDV	
	TP5									=	172	LSDV	
	TP5									=	172	LSDV	
	TP6									=	172	LSDV	
97610	BN1	Lamien et al 2011	Kingfisher Flex	Magnifiq Pathogen kit	Lamien et al. 2011	Real time PCR+melting	>40	21,12	40	>	40	NEG	
	BP1									=	28,8	LSDV	
	TN1									>	40	NEG	
	TP1									=	30,29	GTPV	
	TP2									=	29,58	LSDV	
	TP3									=	26,81	SPPV	
	TP4									=	31,21	LSDV	
	TP5									=	31,85	LSDV	
	TP5									=	32,26	LSDV	
	TP6									=	29,72	LSDV	
	BN1		QIAGEN						82,2	=	78,6	NEG	

Lab ID	Sample ID	Protocol/ SOP	Producer Extraction protocol / kit	Extraction protocol / kit	RT-PCR protocol / kit	Methodology	Cut-off	Pos control	Neg control	Operator	Raw data (Ct cp value)	Interpretation	General comments
97611 (1)	BP1	HRM Galaye et al 2017			Galaye et al. 2017	In-house Melt curve PCR test using Qiagen Quantitect SYBR Green chemistry				=	75,6	LSDV	LSD positive control: Tm75.8 SPPV ctrl :Tm74.8 GTPV positive control: 74.4 HRM: Sheep: Tm 75.00 Goat: Tm 74.40 LSD: Tm 75.80/76 Neg: <Tm70 or >Tm78 on HRM test or No Cq/> 37 amplification plot
	TN1									=	75,8	LSDV	
	TP1									=	74,4	GTPV	
	TP2									=	75,8	LSDV	
	TP3									=	74,8	SPPV	
	TP4									=	76	LSDV	
	TP5									=	75,8	LSDV	
	TP5									=	75,8	LSDV	
	TP6									=	75,8	LSDV	
97611 (2)	BN1	Wolff et al 2021	QIAGEN		Wolff et al. 2021 (SPPV/GTPV)	In-House Multiplex PCR	38	38	38	>	38	NEG	Positive controls : GTPV CT 33.5; SPPV CT 24.7
	BP1									>	38	NEG	
	TN1									>	38	NEG	
	TP1									=	27,2	GTPV	
	TP2									>	38	NEG	
	TP3									=	19,6	SPPV	
	TP4									>	38	NEG	
	TP5									>	38	NEG	
	TP5									>	38	NEG	
	TP6									>	38	NEG	
97612 (1)	BN1	E. Gelaye and C.E. Lamien et al. 2017	Roche		Galaye et al. 2017	PCR HRM		30	32	=	32,6	NEG	To confirm LSDV positive samples we added method 2: IZS TE B456.1 SOP021
	BP1									=	23,3	LSDV	
	TN1									=	32,2	NEG	
	TP1									=	22,7	GTPV	
	TP2									=	21,9	LSDV	
	TP3									=	21,8	SPPV	
	TP4									=	24,6	LSDV	
	TP5									=	24,2	LSDV	
	TP5									=	24,1	LSDV	
	TP6									=	22,1	LSDV	
97612 (2)	BN1	IZS TE B456.1 SOP021	Roche		Other	Duplex PCR real time	> 38	25,5	38	>	38	NEG	The "IZS TE B456.1 SOP21" uses the commercial kit: Bio-T kit Lumpy Skin Disease - BioSellal. This assay detects only Lumpy Skin Disease Virus.
	BP1									=	27,8	LSDV	
	TN1									>	38	NEG	
	TP1									>	38	NEG	
	TP2									=	26,6	LSDV	
	TP3									>	38	NEG	
	TP4									=	27,4	LSDV	
	TP5									=	28,6	LSDV	
	TP5									=	28	LSDV	
	TP6									=	26,4	LSDV	
97613	BN1	Lamien et al. 2011			Lamien et al. 2011			24,37	0	=	0	NEG	
	BP1									=	26,79	LSDV	
	TN1									=	0	NEG	
	TP1									=	28,06	GTPV	
	TP2									=	28,1	LSDV	
	TP3									=	40,17	SPPV	
	TP4									=	28,81	LSDV	
	TP5									=	29,13	LSDV	
	TP5									=	27,46	LSDV	
	TP6									=	26,18	LSDV	
97614	BN1	SOP G.72		Other - Lamien et al. 2011		37	25	40	SOP G.72	>	40	NEG	
	BP1									=	35	LSDV	

Lab ID	Sample ID	Protocol/ SOP	Producer Extraction protocol / kit	Extraction protocol / kit	RT-PCR protocol / kit	Methodology	Cut-off	Pos control	Neg control	Operator	Raw data (Ct cp value)	Interpretation	General comments
	TN1									>	40	NEG	
	TP1									=	33	GTPV	
	TP2									=	32	LSDV	
	TP3		Invitrogen/ThermoFisher Scientific							=	30	SPPV	
	TP4									=	34	LSDV	
	TP5									=	32	LSDV	
	TP5									=	32	LSDV	
	TP6									=	33	LSDV	
	BN1									=	0	NEG	
	BP1									=	338	LSDV	
	TN1									=	0	NEG	
	TP1									=	302	GTPV	
97617 (1)	TP2	Chibssa et al. 2018	Indical Bioscience		Chibssa et al. 2018	Convencional PCR	N/A	200	0	=	338	LSDV	Test could not between SPPV and GTPV
	TP3									=	302	SPPV	
	TP4									=	338	LSDV	
	TP5									=	338	LSDV	
	TP5									=	338	LSDV	
	TP6									=	338	LSDV	
	BN1									=		NA	
	BP1									=		NA	
	TN1									=		NA	
	TP1									=	172	GTPV	
NA97617 (2)	TP2	Lamien et al. 2011	Indical Bioscience		Lamien et al. 2011	Convencional PCR	N/A			=		NA	Performed to differentiate SPPV from GTPV
	TP3									=	151	SPPV	
	TP4									=		NA	
	TP5									=		NA	
	TP5									=		NA	
	TP6									=		NA	
	BN1									=		NEG	
	BP1									=		LSDV	
	TN1									=		NEG	
	TP1									=		GTPV	
97618	TP2	In house	Invitrogen/ThermoFisher Scientific	Galaye et al. 2015	Galaye E. et al. 2015				In house	=		LSDV	
	TP3									=		SPPV	
	TP4									=		LSDV	
	TP5									=		LSDV	
	TP5									=		LSDV	
	TP6									=		LSDV	
	BN1									=	0	NEG	
	BP1									=	28	LSDV	
	TN1									=	0	NEG	
	TP1									=	28	GTPV	
97619	TP2	Lamien et al 2011	Other		Lamien et al. 2011	SGreen PCR/ Sanger sequencing	no app	33	0	=	29	LSDV	Internal modifications to extraction protocol/kit: PerkinELMER chemagic Viral DNA/RNA 300 Kit special H96 using the chemagic 360 instrument
	TP3									=	21	SPPV	
	TP4									=	29	LSDV	
	TP5									=	29	LSDV	
	TP5									=	28	LSDV	
	TP6									=	28	LSDV	
	BN1									=	-	NEG	
97620	BP1	Lamien et al. 2011	QIAGEN		Lamien et al. 2011	PCR	/	172	-	=	172	LSDV	Conventional PCR for species differentiation based on the PCR product length. Result is expressed as PCR product length in bp.
	TN1									=	-	NEG	
	TP1									=	172	GTPV	

Lab ID	Sample ID	Protocol/ SOP	Producer Extraction protocol / kit	Extraction protocol / kit	RT-PCR protocol / kit	Methodology	Cut-off	Pos control	Neg control	Operator	Raw data (Ct cp value)	Interpretation	General comments
	TP2									=	172	LSDV	
	TP3									=	151	SPPV	
	TP4									=	172	LSDV	
	TP5									=	172	LSDV	
	TP5									=	172	LSDV	
	TP6									=	172	LSDV	
97621	BN1	Lamien et al. 2011	QIAGEN		Lamien et al. 2011	Conventional PCR (differential amplicon by size)				=		NEG	
	BP1									=		NA	
	TN1									=		NA	
	TP1									=		GTPV	
	TP2									=		NA	
	TP3									=		SPPV	
	TP4									=		NA	
	TP5									=		NA	
	TP5									=		NA	
	TP6									=		NA	
97630	BN1	Wolff et al. 2021	Indical Bioscience		Wolff et al. 2021 (Both duplexes)	Real-time PCR	40			>	45	NEG	PerfeCTa qPCR ToughMix (Quanta BioSciences Gaithersburg MD USA)
	BP1									=	28,16	LSDV	
	TN1									=	39,07	LSDV	
	TP1									=	32,08	GTPV	
	TP2									=	39,5	LSDV	
	TP3									=	21,87	SPPV	
	TP4									=	28,81	LSDV	
	TP5									=	28,62	LSDV	
	TP5									=	29,64	LSDV	
	TP6									=	26,54	LSDV	
97631	BN1	Wolff et al	Roche		Qiagen - MO-TO 47		40	29,26		=		NEG	
	BP1									=	26,55	LSDV	
	TN1									=	33,95	LSDV	
	TP1									=	28,88	GTPV	
	TP2									=	28,45	LSDV	
	TP3									=	24,46	SPPV	
	TP4									=	26,27	LSDV	
	TP5									=	28,27	LSDV	
	TP5									=	26,16	LSDV	
	TP6									=	24,74	LSDV	
97632 (1)	BN1	Wolff et al. (2021)	Sacace Biotechnologies		Wolff et al. 2021 (Both duplexes)		32	0	Wolff et al. (2021)	=	0	NEG	Sprygin et al. (2019) A real-time PCR screening assay for the universal detection of lumpy skin disease virus DNA
	BP1									=	31,5	LSDV	
	TN1									=	35,6	LSDV	
	TP1									=	30,1	GTPV	
	TP2									=	39,4	LSDV	
	TP3									=	24,7	SPPV	
	TP4									=	28,7	LSDV	
	TP5									=	29,5	LSDV	
	TP5									=	28,9	LSDV	
	TP6									=	29,5	LSDV	
97632 (2)	BN1	Sprygin et al. (2019)	Sacace Biotechnologies		Other			32,4	0	=	0		
	BP1									=	28,6		
	TN1									=	37,9		
	TP1									=	0		
	TP2									=	27,1		

Lab ID	Sample ID	Protocol/ SOP	Producer Extraction protocol / kit	Extraction protocol / kit	RT-PCR protocol / kit	Methodology	Cut-off	Pos control	Neg control	Operator	Raw data (Ct cp value)	Interpretation	General comments
	TP3									=	0		
	TP4									=	28,1		
	TP5									=	28,3		
	TP5									=	27,4		
	TP6									=	27,2		
97634	BN1	Wolff	Biosellal		Wolff et al. 2021 (Both duplexes)	40			Wolff	>	45	NEG	
	BP1									=	29,52	LSDV	
	TN1									>	45	NEG	
	TP1									=	28,54	GTPV	
	TP2									=	27,15	LSDV	
	TP3									=	21,31	SPPV	
	TP4									=	29,42	LSDV	
	TP5									=	27,75	LSDV	
	TP5									=	29,24	LSDV	
	TP6									=	28,09	LSDV	
97637	BN1	Wolf et al 2021; Vidanovic et al. 2021; Agianniotaki et al. 2017	Roche		Wolff et al. 2021 (SPPV/ GTPV)				Wolf et al 2021; Vidanovic et al. 2021; Agianniotaki et al. 2017	=		NEG	
	BP1									=	29	LSDV	
	TN1									=		NEG	
	TP1									=	32	GTPV	
	TP2									=	27	LSDV	
	TP3									=	23	SPPV	
	TP4									=	33	LSDV	
	TP5									=	26	LSDV	
	TP5									=	30	LSDV	
	TP6									=	27	LSDV	
97638	BN1	Commercial kit	Indical Bioscience		Other			26,51	0	=	0	NEG	Commercial kit: ID GENE LSD DIVA Triplex
	BP1									=	20,73	LSDV	
	TN1									=	0	NEG	
	TP1									=		NA	
	TP2									=		NA	
	TP3									=		NA	
	TP4									=	22,77	LSDV	
	TP5									=	23,29	LSDV	
	TP5									=	23,66	LSDV	
	TP6									=	21,14	LSDV	

10.2.6 VIRO: DIVA PCR

Lab ID	Sample ID	Protocol/ SOP	Producer Extraction protocol / kit	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	Combination of methods	Other		Other	Combination of different methods	45	21,88	50	=	50	NEG	Internal modifications to extraction protocol/kit: Addition of EC For the differentiation between the different strains we used Agiannioaki et al. 2016, Chibssa et al. 2018, Wolff et al. 2021 and Haegeman et al. 2024
	BP1									=	29,75	LSDV wild	
	TN1									=	50	NEG	
	TP1									=	36,03	GTPV	
	TP2									=	30,79	LSDV REC	
	TP3									=	22,95	SPPV wild	
	TP4									=	28,44	LSDV Vac	
	TP5									=	30,68	LSDV Vac	
	TP5									=	29,78	LSDV Vac	
	TP6									=	29,81	LSDV wild	
97600	BN1	Haegeman et al. 2024	Biosellal		Other - Haegeman et al. 2024		50	28,9	50	=		NEG	SPPV Differentiation with classical PCR published by Haegeman et al. 2014
	BP1									=	37,08	LSDV wild	
	TN1									=		NEG	
	TP1									=		GTPV	
	TP2									=	40,59	LSDV REC	
	TP3									=		SPPV wild	
	TP4									=	33,93	LSDV Vac	
	TP5									=	33,51	LSDV Vac	
	TP5									=	33,57	LSDV Vac	
	TP6									=	36,45	LSDV wild	
97602	BN1	Haegeman et al 2024			Other - Haegeman et al. 2024			24,19	45	>	45	NEG	For SPPV and GTPV: Chibbsa et al. 2018, gel-based differentiation
	BP1									=	33,92	LSDV wild	
	TN1									>	45	NEG	
	TP1									=		GTPV	
	TP2									=	33,45	LSDV REC	
	TP3									=		SPPV wild	
	TP4									=	33,01	LSDV Vac	
	TP5									=	32,69	LSDV Vac	
	TP5									=	33,61	LSDV Vac	
	TP6									=	34,56	LSDV wild	
97604	BN1	6.3.51	Roche	Other						=		NEG	
	BP1									=		LSDV wild	
	TN1									=		NI	
	TP1									=		NA	
	TP2									=		LSDV Vac	
	TP3									=		NA	
	TP4									=		LSDV Vac	
	TP5									=		LSDV Vac	
	TP5									=		LSDV Vac	
	TP6									=		LSDV wild	
97608 (1)	BN1	LSD-WTR-Mix-FAM/LSD-VR-Mix-HEX	Other		Other - Haegeman et al. 2024	LSD-WTR-Mix-FAM/LSD-VR-Mix-HEX	40	26,2		>	40	NEG	
	BP1									=	31	LSDV wild	
	TN1									>	40	NEG	
	TP1									>	40	NI	
	TP2									=	30,2	LSDV REC	
	TP3									=	40	NI	
	TP4									=	28,8	LSDV Vac	
	TP5									=	28,8	LSDV Vac	
	TP5									=	28,8	LSDV Vac	

Lab ID	Sample ID	Protocol/ SOP	Producer Extraction protocol / kit	Extraction protocol / kit	RT-PCR protocol / kit	Methodology	Cut-off	Pos control	Neg control	Operator	Raw data (Ct cp value)	Interpretation	General comments
	TP6									=	28,4	LSDV wild	
97608 (2)	BN1	SPPV-DIVA Mix	Other		Chibssa et al. 2018					=		NEG	
	BP1									=		NI	
	TN1									=		NEG	
	TP1									=		GTPV	
	TP2									=		NI	
	TP3									=		SPPV wild	
	TP4									=		NI	
	TP5									=		NI	
	TP5									=		NI	
	TP6									=		NI	
97609 (1)	BN1	DIVA LSDV Real Time PCR Agianniotaki et al 2017	Indical Bioscience	Agianniotaki et al. 2017	Platinum Taq DNA Polymerase Invitrogen.		38	33,91	45	=		NEG	
	BP1									=	29,87	LSDV wild	
	TN1									=		NEG	
	TP1									=		NEG	
	TP2									=	32,38	LSDV Vac	
	TP3									=		NEG	
	TP4									=	30,52	LSDV Vac	
	TP5									=	30,57	LSDV Vac	
	TP5									=	30,52	LSDV Vac	
	TP6									=	31,25	LSDV wild	
97609 (2)	BN1	SPPV WT Sybr Green Real Time PCR / Haegeman et al 2015	Indical Bioscience	Haegeman et al. 2015			38	33,52	45	=		NEG	
	BP1									=		NEG	
	TN1									=		NEG	
	TP1									=		NEG	
	TP2									=		NEG	
	TP3									=	24,73	SPPV wild	
	TP4									=		NEG	
	TP5									=		NEG	
	TP5									=		NEG	
	TP6									=		NEG	
97609 (3)	BN1	97609	Partial GPCR (3F/3R) PCR and Sequencing	Indical Bioscience	HotStart Taq DNA Polymerase kit Qiagen					=		NEG	
	BP1									=		LSDV wild	
	TN1									=		NEG	
	TP1									=		GTPV	
	TP2									=		LSDV REC	
	TP3									=		SPPV wild	
	TP4									=		LSDV Vac	
	TP5									=		LSDV Vac	
	TP5									=		LSDV Vac	
	TP6									=		LSDV wild	
97610	BN1	Vidanovic 2016	Kingfisher Flex/Magnifiq Pathogen kit	Kingfisher Flex/Magnifiq Pathogen kit	Vidanovic et al. 2016		>40	26,52	40	=		NA	
	BP1									=	28,25	LSDV wild	
	TN1									=		NA	
	TP1									=		NA	
	TP2									=		LSDV Vac	
	TP3									=		NA	
	TP4									=		LSDV Vac	
	TP5									=	31,27	LSDV Vac	
	TP5									=	31,14	LSDV Vac	
	TP6									=	29,53	LSDV wild	

Lab ID	Sample ID	Protocol/ SOP	Producer Extraction protocol / kit	Extraction protocol / kit	RT-PCR protocol / kit	Methodology	Cut-off	Pos control	Neg control	Operator	Raw data (Ct cp value)	Interpretation	General comments
97611 (1)	BN1	Agianniotaki et al 2017	QIAGEN		Other - Agianniotaki et al. 2017	Multiplex real time LSDV	37	37	37	=		NEG	Field Positive control: cT 28.83 Vac Positive control: cT 31.04 Negative control: No Cq or >37 GTPV (our lab does not differentiate between Vac and field for goat samples) SPPV only (our lab does not differentiate between Vac and field for sheep samples)
	BP1									=	25,19	LSDV wild	
	TN1									=	36,05	LSDV wild	
	TP1									=		GTPV	
	TP2									=	27,06	LSDV Vac	
	TP3									=		SPPV	
	TP4									=	27,43	LSDV Vac	
	TP5									=	27,3	LSDV Vac	
	TP5									=	27,24	LSDV Vac	
	TP6									=	25,97	LSDV wild	
97611 (2)	BN1	Haegemann et al 2024	QIAGEN		Other - Haegeman et al. 2024		37	37	37	=		NEG	Vac positive: Ct17 (Neethling Vac), 2024 PT Recombinant sample =CT33 on Wild type test (FAM), 2022 PT Recombinant sample=CT35 on Wild type test (FAM), 2022 PT Field sample Ct29 on Wildtype test (FAM)
	BP1									=	27,1	LSDV wild	
	TN1									>	37	NEG	
	TP1									=		GTPV	
	TP2									=	29,2	LSDV REC	
	TP3									=		SPPV	
	TP4									=	26	LSDV Vac	
	TP5									=	26,4	LSDV Vac	
	TP5									=	26,6	LSDV Vac	
	TP6									=	27,8	LSDV wild	
97612 (1)	BN1	T.R. Chibssa and C.E. Lamien et al. 2018	Roche		Chibssa et al. 2018	Gel-based PCR				=		NA	This method is used to differentiate sheeppox virus field isolates from vaccine strains. Therefore we analyzed only the sample CPXVIR24-9 which tested SPPV positive in PCR-HRM Gelaye et al. 2017
	BP1									=		NA	
	TN1									=		NA	
	TP1									=		NA	
	TP2									=		NA	
	TP3									=		SPPV wild	
	TP4									=		NA	
	TP5									=		NA	
	TP5									=		NA	
	TP6									=		NA	
97612 (2)	BN1	A. Krotova and A. Sprygin et al. 2022	Roche		Other - Other	LSDV ORF134 PCR amplification and Sanger Sequencing				=		NA	This method is used to distinguish among recombinant field and vaccine LSDV strains. We tested only the LSDV positive samples in TE IZS B456.1 SOP021
	BP1									=		LSDV wild	
	TN1									=		NA	
	TP1									=		NA	
	TP2									=		LSDV REC	
	TP3									=		NA	
	TP4									=		LSDV Vac	
	TP5									=		LSDV Vac	
	TP5									=		LSDV Vac	
	TP6									=		LSDV wild	
97613 (1)	BN1	Agianniotaki et al. 2017			Other - Agianniotaki et al. 2017			30,87	0	=	0	NEG	
	BP1									=	28,06	LSDV wild	
	TN1									=	37,85	LSDV wild	
	TP1									=	0	NEG	
	TP2									=	29,33	LSDV Vac	
	TP3									=	0	NEG	
	TP4									=	29,14	LSDV Vac	
	TP5									=	29,58	LSDV Vac	
	TP5									=	30,25	LSDV Vac	
	TP6									=	27,28	LSDV wild	
	BN1									=		NEG	

Lab ID	Sample ID	Protocol/ SOP	Producer Extraction protocol / kit	Extraction protocol / kit	RT-PCR protocol / kit	Methodology	Cut-off	Pos control	Neg control	Operator	Raw data (Ct cp value)	Interpretation	General comments
97613 (2)	BP1	Chibssa et al. 2018			Other - Chibssa et al. 2018					=		LSDV wild	
	TN1									=		NEG	
	TP1									=		GTPV	
	TP2									=		LSDV Vac	
	TP3									=		SPPV wild	
	TP4									=		LSDV Vac	
	TP5									=		LSDV Vac	
	TP5									=		LSDV Vac	
	TP6									=		LSDV wild	
97613 (3)	BN1	Gelaye et al. 2015			Other - Gelaye et al. 2015					=		NEG	
	BP1									=		LSDV wild	
	TN1									=		NI	
	TP1									=		NA	
	TP2									=		LSDV REC	
	TP3									=		NA	
	TP4									=		LSDV Vac	
	TP5									=		LSDV Vac	
	TP5									=		LSDV Vac	
	TP6									=		LSDV wild	
97614	BN1	SOP G.72	Invitrogen/ThermoFisher Scientific	Other - Gelaye et al. 2015						=		NEG	
	BP1									=		LSDV wild	
	TN1									=		NEG	
	TP1									=		GTPV	
	TP2									=		LSDV wild	
	TP3									=		SPPV Vac	
	TP4									=		LSDV wild	
	TP5									=		LSDV wild	
	TP5									=		LSDV Vac	
	TP6									=		LSDV wild	
97617	BN1	Menasherow et al. 2014	Indical Bioscience		Menasherow et al. 2014.	Conventional PCR	N/A	57,65	0	=		NEG	
	BP1									=	57	LSDV wild	
	TN1									=		NA	
	TP1									=		GTPV	
	TP2									=	57,65	LSDV Vac	
	TP3									=		SPPV	
	TP4									=	57,65	LSDV Vac	
	TP5									=	57,65	LSDV Vac	
	TP5									=	57,65	LSDV Vac	
	TP6									=	57	LSDV wild	
97618	BN1	DIVA GREECE Agianniotaki et al 2017	Invitrogen/ThermoFisher Scientific		Agianniotaki et al. 2017			32,71	0	=	0	NEG	RT-PCR protocol / kit - Invitrogen/ThermoFisher Scientific/Platinum Taq DNA Polymerase PCR protocol kit - Taq DNA Polymerase (Qiagen)
	BP1									=	30,14	LSDV wild	
	TN1									=	0	NEG	
	TP1									=	0	GTPV	
	TP2									=	30,87	LSDV REC	
	TP3									=	0	SPPV wild	
	TP4									=	32,64	LSDV REC	
	TP5									=	33,54	LSDV REC	
	TP5									=	32,06	LSDV REC	
	TP6									=	30,63	LSDV wild	
97620 (1)	BN1	Haegeman et al ~~2023~~2024	QIAGEN		Haegeman et al. ~~2023~~2024	RT PCR	Ct < 45	30,23	-	=	-	NEG	LSD DIVA PCR
	BP1									=	31,929	LSDV wild	FAM positive is wild type strain

Lab ID	Sample ID	Protocol/ SOP	Producer Extraction protocol / kit	Extraction protocol / kit	RT-PCR protocol / kit	Methodology	Cut-off	Pos control	Neg control	Operator	Raw data (Ct cp value)	Interpretation	General comments
	TN1									=	40,94	LSDV wild	VIC positive is vaccine strain
	TP1									=	37,57	NI	
	TP2									=	31,207	LSDV wild	
	TP3									=	36,786	NI	
	TP4									=	31,701	LSDV Vac	
	TP5									=	31,479	LSDV Vac	
	TP5									=	31,19	LSDV Vac	
	TP6									=	32,62	LSDV wild	
97620 (2)	BN1	Chibssa et al 2018	QIAGEN		Chibssa et al. 2018	PCR	/	218	-	=	-	NEG	Conventional PCR for differentiation of SPPV field and vaccine strains based on the PCR product length. Result is expressed as PCR product length in bp.
	BP1									=	336	NI	
	TN1									=	-	NEG	
	TP1									=	302	GTPV	
	TP2									=	336	NI	
	TP3									=	302	SPPV wild	
	TP4									=	336	NI	
	TP5									=	336	NI	
	TP5									=	336	NI	
	TP6									=	336	NI	
97621 (1)	BN1	Agianniotaki et al. 2017	QIAGEN		Agianniotaki et al. 2017	Duplex PCR (LSDV wild or Vac)	35	35	40	>	40	NEG	
	BP1									=	26,3	LSDV wild	
	TN1									=	37,6	LSDV wild	
	TP1									>	40	NA	
	TP2									=	24,5	LSDV Vac	
	TP3									>	40	NA	
	TP4									=	27,5	LSDV Vac	
	TP5									=	27,9	LSDV Vac	
	TP5									=	28,8	LSDV Vac	
	TP6									=	23,8	LSDV wild	
97621 (2)	BN1	Haegeman et al. 2015	QIAGEN		Other	Conventional PCR (differential amplicon by size)				=		NA	
	BP1									=		NA	
	TN1									=		NA	
	TP1									=		NA	
	TP2									=		NA	
	TP3									=		SPPV wild	
	TP4									=		NA	
	TP5									=		NA	
	TP5									=		NA	
	TP6									=		NA	
97622	BN1	Other	Roche		Other		>36 is considered doubtfull	23,3	-	=	-	NEG	
	BP1									=	25,7	LSDV wild	
	TN1									=	-	NEG	
	TP1									=	-	NEG	
	TP2									=	28,4	LSDV Vac	
	TP3									=	33,2	LSDV wild	
	TP4									=	31,9	LSDV Vac	
	TP5									=	31,4	LSDV Vac	
	TP5									=	30,9	LSDV Vac	
	TP6									=	28,1	LSDV wild	
97630	BN1	Wolff et al. 2021	Indical Bioscience		Wolff et al. 2021	Real-time PCR				>	45	NEG	PerfeCTa qPCR ToughMix (Quanta BioSciences Gaithersburg MD USA)
	BP1									=	28,016	LSDV wild	
	TN1									=	39,07	LSDV wild	

Lab ID	Sample ID	Protocol/ SOP	Producer Extraction protocol / kit	Extraction protocol / kit	RT-PCR protocol / kit	Methodology	Cut-off	Pos control	Neg control	Operator	Raw data (Ct cp value)	Interpretation	General comments
	TP1									=	32,08	GTPV	
	TP2									=	39,5	LSDV wild	
	TP3									=	21,88	NI	
	TP4									=	28,81	LSDV Vac	
	TP5									=	28,62	LSDV Vac	
	TP5									=	29,64	LSDV Vac	
	TP6									=	26,54	LSDV wild	
	BN1									=		NEG	
	BP1									=	26,48	LSDV wild	
	TN1									=	33,32	LSDV wild	
	TP1									=	28,88	GTPV	
97631	TP2	Wolff et al	Roche		Qiagen - MO-TO 47		40	29,26		=	28,45	LSDV REC	
	TP3									=		NA	
	TP4									=	27,83	LSDV Vac	
	TP5									=	26,82	LSDV Vac	
	TP5									=	26,29	LSDV Vac	
	TP6									=	23,42	LSDV wild	
	BN1									=	0	NEG	
	BP1									=	31,5	LSDV wild	
	TN1									=	35,6	LSDV wild	
	TP1									=	31,6	GTPV	
97632 (1)	TP2	Wolff et al. (2021) Probe	Sacace Biotechnologies		Wolff et al. 2021			31,8	0	=	39,4	LSDV REC	
	TP3									=	24,7	SPPV wild	
	TP4									=	28,7	LSDV Vac	
	TP5									=	29,5	LSDV Vac	
	TP5									=	28,9	LSDV Vac	
	TP6									=	29,5	LSDV wild	
	BN1									=		NEG	
	BP1									=		LSDV wild	
	TN1									=		LSDV wild	
	TP1									=		GTPV	
97632 (2)	TP2	Sequencing RPO30	Sacace Biotechnologies		Gelaye et al. 2015					=		LSDV REC	Gelaye et al. (2015) Genetic differences between field isolates and vaccine strain and implications for vaccination failure
	TP3									=		SPPV wild	
	TP4									=		LSDV Vac	
	TP5									=		LSDV Vac	
	TP5									=		LSDV Vac	
	TP6									=		LSDV wild	
	BN1									>	45	NEG	
	BP1									=	26,74	LSDV wild	This protocol does not detect SPPV. Interpretation of SPPV positive sample was made based on other assays.
	TN1									>	45	NEG	
	TP1									>	45	GTPV	
97634 (1)	TP2	Haegeman 2024	Biosellal		Haegeman et al. 2024		40	30,89	45	=	28,37	LSDV REC	This protocol does not detect GTPV. Interpretation of GTPV positive sample were made based on other assays.
	TP3									>	45	SPPV wild	
	TP4									=	28,85	LSDV Vac	Conclusion that this is recombinant strain was based by comparing the results from Vidanovic assay (2021), and also by sequencing.
	TP5									=	29,35	LSDV Vac	
	TP5									=	28,66	LSDV Vac	
	TP6									=	27,12	LSDV wild	
	BN1									>	45	NEG	
97634 (2)	BP1	Vidanovic 2021	Biosellal		Vidanovic et al. 2021		40	30,68	45	=	29,52	LSDV wild	This assay detects this recombinant strain as vaccine strain. Conclusion that this is recombinant strain was based by comparing the results from Haegeman assay (2024), and also by sequencing.
	TN1									>	45	NEG	
	TP1									>	45	NEG	

Lab ID	Sample ID	Protocol/ SOP	Producer Extraction protocol / kit	Extraction protocol / kit	RT-PCR protocol / kit	Methodology	Cut-off	Pos control	Neg control	Operator	Raw data (Ct cp value)	Interpretation	General comments
	TP2									=	27,15	LSDV REC	
	TP3									>	45	NEG	
	TP4									=	29,29	LSDV Vac	
	TP5									=	29,59	LSDV Vac	
	TP5									=	29,48	LSDV Vac	
	TP6									=	27,66	LSDV wild	
97634 (3)	BN1	Chibssa 2018	Biosellal		Chibssa et al. 2018					=		NA	
	BP1									=		NA	
	TN1									=		NA	
	TP1									=		NA	
	TP2									=		NA	
	TP3									=		SPPV wild	
	TP4									=		NA	
	TP5									=		NA	
	TP5									=		NA	
	TP6									=		NA	
97637	BN1	Haegeman et al. 2024; Vidanovic et al. 2021; Agianniotaki et al. 2017	Roche		Haegeman et al. 2024					=		NEG	
	BP1									=	29	LSDV wild	
	TN1									=		NEG	
	TP1									=	32	GTPV	
	TP2									=	26	LSDV REC	
	TP3									=		NA	
	TP4									=	33	LSDV Vac	
	TP5									=	26	LSDV Vac	
	TP5									=	30	LSDV Vac	
	TP6									=	27	LSDV wild	
97638	BN1	Commercial kit	Indical Bioscience		IDVet - ID GENE® LSD DIVA TRIPLEX			26,51	0	=	0	NEG	
	BP1									=	20,73	LSDV wild	
	TN1									=	0	NEG	
	TP1									=		NA	
	TP2									=		NA	
	TP3									=		NA	
	TP4									=	22,77	LSDV Vac	
	TP5									=	23,29	LSDV Vac	
	TP5									=	23,66	LSDV Vac	
	TP6									=	21,14	LSDV wild	

10.3 Annex 3: Additional information

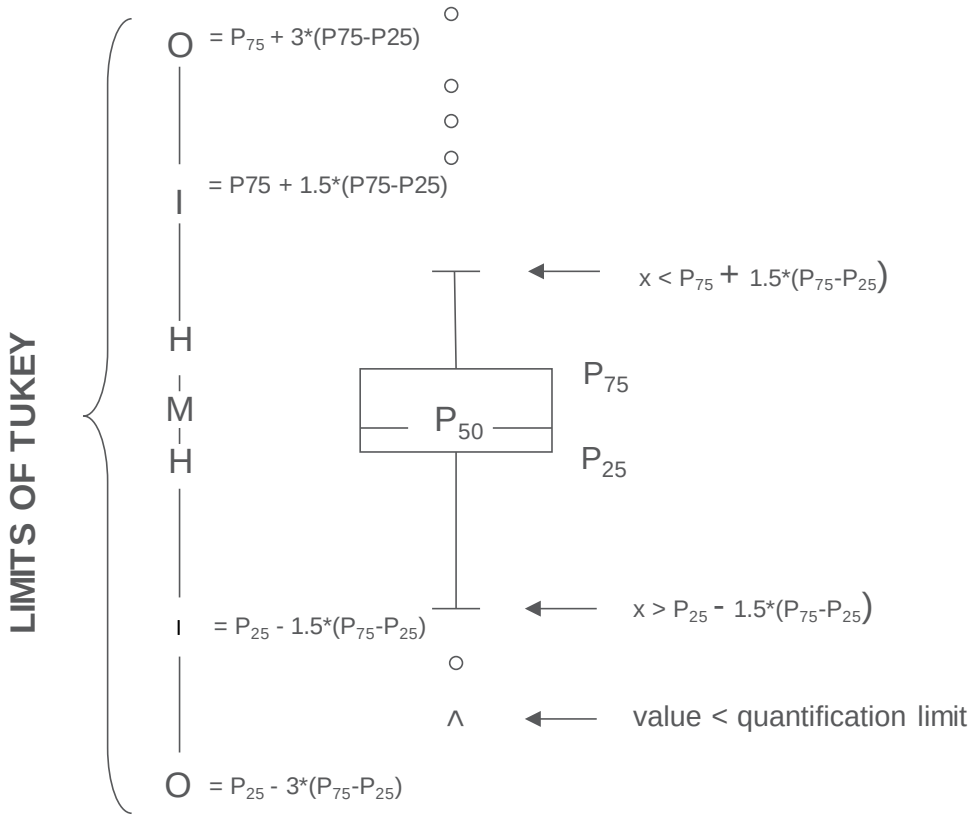
The **calendar** for Proficiency Testing in Veterinary diagnosis is available on our website:

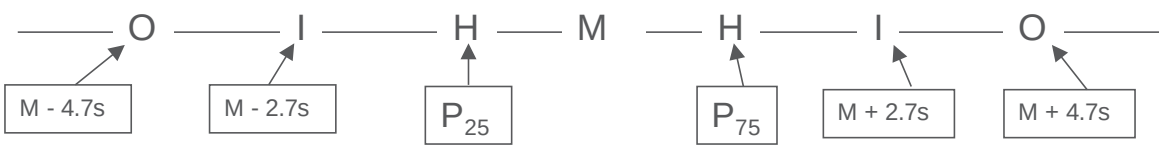
- NL: https://www.sciensano.be/nl/biblio/eke-kalender-2024
- FR: https://www.sciensano.be/fr/biblio/calendrier-eeq-2024
- EN: https://www.sciensano.be/nl/biblio/eqa-calendar-2024

Graphical representation

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

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





Corresponding limits in case of normal distribution

END