

**BIOLOGICAL HEALTH RISKS
QUALITY OF LABORATORIES**

COMMITTEE OF EXPERTS

**PROFICIENCY TEST
IN VETERINARY DIAGNOSIS**

DEFINITIVE GLOBAL REPORT

VETERINARY MEDICINE

CAPRIPOX (CAPX)

PROFICIENCY TEST 2023-3

Sciensano/PT VET CAPX/2023-3/E

Biological health risks

Quality of laboratories

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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: PT2023CAPXSER) and/or to assess the ability of the participating laboratories to detect CAPX virus DNA in different matrices (virology component of the PT: PT2023CAPXVIR).

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 containing a background of the samples (species from which the sample is collected and type of sample).

3.2 Reference samples

Thirty-three laboratories were provided with the PT2023CAPXSER panel, each comprising 10 serum aliquots. Also thirty-three laboratories were supplied with the PT2023CAPXVIR panel, consisting of 10 aliquots each of blood and tissue suspension.

The samples were prepared by the European Union Reference Laboratory 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 randomized from 1 to 10, by the Quality of Laboratories, Sciensano.

3.2.1 PT2023CAPXSER PANEL: REFERENCE SERUM SAMPLES

3.2.1.1 Origin of the samples

Replicates of 9 reference serum samples, either free from detectable antibodies to capripox viruses (n=2; coded PT2023CAPXSERN1 and PT2023CAPXSERN2) or containing detectable antibodies to capripox viruses (n=7; coded PT2023CAPXSERP1, PT2023CAPXSERP2, PT2023CAPXSERP3, PT2023CAPXSERP4, PT2023CAPXSERP5, PT2023CAPXSERP6, PT2023CAPXSERP7) were used. However, sample PT2023CAPXSERP6 was repeated twice in the panel.

In total, 330 aliquots were distributed to 33 participating laboratories. These participants received 10 aliquots, however one aliquot was repeated twice. PT2023CAPXSERP2 and PT2023CAPXSERP3 were dilutions of PT2023CAPXSERP1, with dilution factors of 1/2 and 1/5, respectively. The positions of the reference samples were randomized 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 neutralization test with the serum titrated against a constant titer of capripox virus (VNT).

Table 1: Origin of the samples in the PTCAPX2023 panel. (GTPV = goatpox virus; LSDV = lumpy skin disease virus; SPPV = sheeppox virus; POS = positive; NEG = negative).

Sample ID	Origin	Background	Status
PT2023CAPXSERP1	Ovine	SPPV infected	POS
PT2023CAPXSERP2	Ovine	SPPV infected	POS
PT2023CAPXSERP3	Ovine	SPPV infected	POS
PT2023CAPXSERP4	Bovine	LSDV infected	POS
PT2023CAPXSERP5	Bovine	LSDV infected	POS
PT2023CAPXSERP6 (repeated twice)	Bovine	LSDV vaccinated	POS
PT2023CAPXSERP7	Bovine	LSDV infected	POS
PT2023CAPXSERN1	Ovine	Commercial serum	NEG
PT2023CAPXSERN2	Bovine	Commercial serum	NEG

After aliquoting the different reference serum samples, a homogeneity check was performed on 10 aliquots of each sample using the ELISA ID Screen® Capripox Double Antigen Multi-species (ID.Vet), IPMA and VN. For the ELISA, the same qualitative result was obtained for all 10 aliquots of the same reference serum sample. When performing VN with heterologous virus by the EURL, a difference in status was found for samples PT2023CAPXSERP1, PT2023CAPXSERP2 and PT2023CAPXSERP3 compared to the homologous virus. The VN status of these samples was therefore considered as positive or doubtful for these 3 samples. The same holds true for the IPMA results of PT2023CAPXSERP3.

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, 3 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 PT2023CAPXSERN1 and PT2023CAPXSER_SERN2 were considered as negative samples in all tests. In the ELISA ID Screen® Capripox Double Antigen Multi-species (ID.Vet) the reference serum samples, PT2023CAPXSERP1, PT2023CAPXSERP2, PT2023CAPXSERP3, PT2023CAPXSERP4, PT2023CAPXSERP5, PT2023CAPXSERP6 and PT2023CAPXSERP7 as positive samples. In the IPMA, the reference serum PT2023CAPXSERP3 were considered as doubtful, while all other reference serum samples had the same status as in the ELISA. For the VNT, PT2023CAPXSERP1, PTCAPXSERP2 and PTCAPXSERP3 were considered as doubtful, while all other reference serum samples have the same status as in the ELISA.

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 PT2023CAPXSER panel (POS = positive; NEG = negative; NI = non-interpretable).

Sample ID	ELISA	IPMA	VNT
PT2023CAPXSERP1	POS	POS	NI
PT2023CAPXSERP2	POS	POS	NI
PT2023CAPXSERP3	POS	NI	NI
PT2023CAPXSERP4	POS	POS	POS
PT2023CAPXSERP5	POS	POS	POS
PT2023CAPXSERP6 (repeated twice)	POS	POS	POS
PT2023CAPXSERP7	POS	POS	POS
PT2023CAPXSERN1	NEG	NEG	NEG
PT2023CAPXSERN2	NEG	NEG	NEG

3.2.1.3 Randomization and panel composition

The samples were randomized differently for each laboratory, an overview of the randomization can be found in the preliminary report. The PTCAPXSER2023 panel was constituted of 10 samples of 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 PT2023CAPXVIR PANEL: REFERENCE BLOOD & TISSUE HOMOGENATE SAMPLES

3.2.2.1 Origin of the samples

Replicates of 7 reference samples were tissue homogenate samples. On the one hand samples containing detectable capripox virus DNA (n=6; coded PT2023CAPXTP1, PT2023CAPXTP2, PT2023CAPXTP3, PT2023CAPXTP4, PT2023CAPXTP5 and PT2023CAPXTP6) and on the other hand one sample free from detectable capripox virus DNA (n=1; coded PT2023CAPXTN1). The remaining 3 reference samples were blood samples, either free from detectable capripox virus DNA (n=1; coded PT2023CAPXBN1) or containing detectable capripox virus DNA (n=1; coded PT2023CAPXBP1). The latter, sample PT2023CAPXBP1, was repeated twice.

In total, 330 aliquots were distributed to 33 participating laboratories. Each participant received 10 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 PTCAPX2023 panel. (GTPV = goatpox virus; LSDV = lumpy skin disease virus; SPPV = sheeppox virus; NEG = negative)

Sample ID	Origin	Background	Status
PT2023CAPXTP1	Goat tissue	GTPV spiked tissue	Capx positive GTPV
PT2023CAPXTP2	Cattle tissue	LSDV spiked tissue Bulgaria	Capx positive LSDV wild-type strain
PT2023CAPXTP3	Cattle tissue	LSDV spiked tissue recombinant	Capx positive LSDV recombinant strain
PT2023CAPXTP4	Sheep tissue	SPPV wild tissue	Capx positive SPPV wild-type strain
PT2023CAPXTP5	Sheep tissue	SPPV Vaccine tissue	Capx positive SPPV vaccine strain
PT2023CAPXTP6	Cattle tissue	DURCON1 R19	Capx positive LSDV wild-type strain
PT2023CAPXBP1 (repeated twice)	Cattle tissue	Spiked blood Israel	Capx positive LSDV wild-type strain
PT2023CAPXTN1	Cattle tissue	Negative Skin cattle	Capx tissue negative
PT2023CAPXBN1	Cattle blood	Negative blood cattle	Capx blood negative

(GTPV = goatpox virus; LSDV = lumpy skin disease virus; SPPV = sheeppox virus)

After aliquoting the different samples, a homogeneity check was performed on 10 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 10 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).

For the **detection of capripox virus DNA**, the sample PT2023CAPXVIRBN1 and PT2023CAPXVIRTN1 were considered as a capripox virus negative sample and the samples PT2023CAPXVIRTP1, PT2023CAPXVIRTP2, PT2023CAPXVIRTP3, PT2023CAPXVIRTP4, PT2023CAPXVIRTP5, PT2023CAPXVIRTP6 and PT2023CAPXVIRBP1 as positive samples.

For the **capripox virus species differentiation**, the sample PT2023CAPXVIRBN1 and PT2023CAPXVIRTN1 were considered as a negative sample, the samples PT2023CAPXVIRTP4 and PT2023CAPXVIRTP5 as SPPV positive samples (where SPPV or SPPV/GTPV results were considered acceptable), the samples PT2023CAPXVIRTP2, PT2023CAPXVIRTP3, PT2023CAPXVIRTP6 and PT2023CAPXVIRBP1 as LSDV positive samples (where LSDV was considered acceptable) and sample PT2023CAPXVIRTP1 as GTPV positive sample (where GTPV or SPPV/GTPV was considered acceptable).

Finally, for the **field or vaccine strain differentiation**, the sample PT2023CAPXVIRTN1 and PT2023CAPXVIRBN1 considered as negative sample, the samples PT2023CAPXVIRTP2, PT2023CAPXVIRTP6 and PT2023CAPXVIRBP1 as LSDV field strains and PT2023CAPXVIRTP4 as SPPV field strain. PT2023CAPXVIRTP5 was considered as SPPV vaccine strain. For PT2023CAPXVIRTP1 and PT2023CAPXVIRTP3 which contained respectively GTPV DNA and LSDV recombinant DNA, all answers were considered in agreement with the assigned status.

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 PT2023CAPXVIR panel (GTPV = goatpox virus; LSDV = lumpy skin disease virus; SPPV = sheeppox virus; NEG = negative).

Sample ID	RT-PCR	Species differentiation	DIVA
PT2023CAPXTP1	POS	GTPV	GTPV
PT2023CAPXTP2	POS	LSDV	LSDV wild-type strain
PT2023CAPXTP3	POS	LSDV	LSDV recombinant strain
PT2023CAPXTP4	POS	SPPV	SPPV wild-type strain
PT2023CAPXTP5	POS	SPPV	SPPV vaccine strain
PT2023CAPXTP6	POS	LSDV	LSDV wild-type strain
PT2023CAPXBP1 (repeated twice)	POS	LSDV	LSDV wild-type strain

Sample ID	RT-PCR	Species differentiation	DIVA
PT2023CAPXTN1	NEG	NEG	NEG
PT2023CAPXBN1	NEG	NEG	NEG

(GTPV = goatpox virus; LSDV = lumpy skin disease virus; SPPV = sheeppox virus; NEG = negative)

3.2.2.3 Randomization and panel composition

The samples were randomized differently for each laboratory, an overview of the randomization can be found in the preliminary report. The PTCAPXVIR2023 panel was constituted of 10 samples of 600 µl.

3.2.2.4 Stability

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

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

3.3.1 CLASSIFICATION OF RESULTS

Results provided by the participating laboratories are categorized 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.

4 THE PARTICIPANTS

Twenty-three National Reference Laboratories (NRLs) from European Union member states, nine NRLs from non-EU member states, and five private laboratories 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	1	1
Belgium	Sciensano; Department 'Exotic viruses and particular diseases'	1	1
Croatia	Croatian Veterinary Institute	1	1
Cyprus	Laboratory for animal health; Department Virology	0	1
Czech Republic	State Veterinary Institute Prague	1	1
Denmark	Statens Serum Institut; Department Veterinary Virology	1	1
Finland	Finnish Food Authority; Department Virology	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, Athens Veterinary Directorate, Ministry of Rural Development and Food	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 of serology	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
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	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

Table 7: Private laboratories that participated in the PT capripox.

Country	Name of the laboratory	Participation PT serology	Participation PT virology
Australia	Australian Centre for Disease Preparedness (ACDP) CSIRO	1	1
France	Biosellal	0	1
Germany	INDICAL BIOSCIENCE GmbH	1	0
South-Korea	Foreign Animal Disease Division Animal and Plant Quarantine Agency	1	1
United Kingdom	Pirbright institute	1	1

5 SURVEY TIMELINE

Transfer of the samples from NRL to QL: 02/05/2023

Randomization of the samples by QL: 03/05/2023 (serology) + 04/05/2023 (virology)

Sending samples (frozen at - 20 °C) to participants: 15/05/2023

Deadline for submitting results: 16/06/2023.

Preliminary report: 24/07/2023

6 COMPLIANCE WITH THE PROCEDURE

All participating laboratories have provided a duly dated copy of the results. However, laboratory 97532 notified us that they would not be able to submit the results prior to the deadline. This laboratory is working on an ELISA kit prototype but they did not receive all needed raw materials on time. Fortunately, they communicated this issue promptly and were consequently granted an extension. An extended deadline was also granted to four laboratories (97628, 97629, 97630 and 97647) that received the samples later due to transport problems.

7 RESULTS – QUALITATIVE DATA ANALYSIS

7.1 Serology

The PT2023CAPXSER panel was composed of 8 positive samples and 2 negative samples. The sample PT2023CAPSERP6 was repeated twice in the panel.

7.1.1 ANTIBODY ELISA

In total, 33 laboratories participated and submitted their results. The ELISA kits used included the Screen Capripox Double Antigen Multispecies kit from ID.VET, the AsurDx Capripox Antibody Test Kit from BioStone and the Cattletype Capripox Ab prototype kit from INDICAL BIOSCIENCE GmbH. To reach a 100% agreement, the submitted qualitative results must be in full agreement with the assigned status of the reference samples. This maximum score was achieved by 32 laboratories. One laboratory did not fully succeed and obtained a score of 90%. 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, not interpretable answers are showed in orange.

Lab number	P1	P2	P3	P4	P5	P6 Rep 1	P6 Rep 2	P7	N1	N2	%
	POS								NEG		
97506	POS	POS	POS	POS	POS	POS	POS	POS	NEG	NEG	100
97532	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	NEG	POS	POS	POS	POS	POS	NEG	NEG	90
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

Lab number	P1	P2	P3	P4	P5	P6 Rep 1	P6 Rep 2	P7	N1	N2	%
	POS								NEG		
97621	POS	POS	POS	POS	POS	POS	POS	POS	NEG	NEG	100
97622	POS	POS	POS	POS	POS	POS	POS	POS	NEG	NEG	100
97628	POS	POS	POS	POS	POS	POS	POS	POS	NEG	NEG	100
97629	POS	POS	POS	POS	POS	POS	POS	POS	NEG	NEG	100
97630	POS	POS	POS	POS	POS	POS	POS	POS	NEG	NEG	100
97631	POS	POS	POS	POS	POS	POS	POS	POS	NEG	NEG	100
97632	POS	POS	POS	POS	POS	POS	POS	POS	NEG	NEG	100
97634	POS	POS	POS	POS	POS	POS	POS	POS	NEG	NEG	100
97637	POS	POS	POS	POS	POS	POS	POS	POS	NEG	NEG	100
97642	POS	POS	POS	POS	POS	POS	POS	POS	NEG	NEG	100
97643	POS	POS	POS	POS	POS	POS	POS	POS	NEG	NEG	100
97647	POS	POS	POS	POS	POS	POS	POS	POS	NEG	NEG	100

7.1.2 VIRUS NEUTRALIZATION

Five 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 virus neutralization test per participating laboratory. Correct answers are marked in green, while incorrect answers are indicated in red. In addition, not interpretable answers are showed in orange.

Lab number	P1	P2	P3	P4	P5	P6 Rep1	P6 Rep 2	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
97608	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 immunoperoxidase 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, not interpretable answers are showed in orange.

Lab number	P1	P2	P3	P4	P5	P6 Rep1	P6 Rep 2	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 PT2023CAPXVIR panel was composed of 8 positive samples and 2 negative samples. The sample PT2023CAPVIRBP1 was repeated twice in the panel.

7.2.1 RT-PCR

7.2.1.1 Results per sample

In total, 33 laboratories participated in the RT-PCR virology part of the proficiency test. As 29 laboratories submitted 1 dataset, 4 laboratories submitted 2 datasets, the total number of datasets registered is thus 37. Raw data can be found in Annex 1.

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

Sample ID PT2023CAPXVIR	REP	Expected results	# of POS	# of NEG	# of NI	Status*
TP1	1	POS	35	2	0	Frequently detected
TP2	1	POS	37	0	0	Frequently detected
TP3	1	POS	37	0	0	Frequently detected
TP4	1	POS	35	2	0	Frequently detected
TP5	1	POS	36	1	0	Frequently detected
TP6	1	POS	36	1	0	Frequently detected
BP1	2	POS	73	0	1	Frequently detected
TN1	1	NEG	0	37	0	NEG
BN1	1	NEG	0	37	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; number of correct results; FN = false negative; NI = not interpretable).

Kit or reference	Target gene	N	NR	NCR	FN	NI
Haegeman <i>et al.</i> 2013	D5R/E3L	3	30	30	0	0
Bio-T kit® Lumpy Skin Disease	/	1	10	7	3	0
Bowden <i>et al.</i> 2008	P32	22	220	220	0	0
Bowden <i>et al.</i> 2008; Babiuk <i>et al.</i> 2008; SOP from Pirbright	P32	1	10	10	0	0
Bowden <i>et al.</i> 2008/Dietze <i>et al.</i> 2018	P32	1	10	10	0	0
Stubbs <i>et al.</i> 2012	P32	1	10	10	0	0
Perfecta qPCR kit (Quanta Biosciences)	PolymeraseA	1	10	10	0	0
ID.VET - ID GENE® CAPRIPOX VIRUS TRIPLEX	/	1	10	10	0	0
Path_ID qPCR	P32	1	10	10	0	0
Roche kit	P32	1	10	10	0	0
Invitrogen/Thermo Fisher Scientific kit	/	1	10	10	0	0
AgPath-ID One-Step RT-PCR Reagents (by Thermo Fisher Scientific)	/	1	10	6	3	1
Sso Advanced Universal probes	P32/RPO30	2	20	20	0	0
TOTAL		37	370	363	6	1

7.2.1.3 Results per laboratory

For the detection of capripox virus DNA in the PT panel: 32 out of 33 laboratories provided results that were in full agreement with the assigned status of the 10 reference samples (100% agreement). The remaining lab (LAB97514) misclassified 3 aliquots. Four laboratories performed a secondary PCR. Three of them (LAB97605, LAB97607 and LAB 97608) provided results that were in full agreement with the assigned status of the reference samples. LAB97647 misclassified 3 aliquots as NEG and 1 as NI.

Table 12: Results per laboratory. Correct answers are marked in green, while incorrect answers are indicated in red. In addition, not interpretable answers are showed in orange.

Lab number	TP1	TP2	TP3	TP4	TP5	TP6	BP1 Rep 1	BP1 Rep 2	TN1	BN1	%
	POS						NEG				
97506	POS	POS	POS	POS	POS	POS	POS	POS	NEG	NEG	100
97514	NEG	POS	POS	NEG	NEG	POS	POS	POS	NEG	NEG	70
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

Lab number	TP1	TP2	TP3	TP4	TP5	TP6	BP1 Rep 1	BP1 Rep 2	TN1	BN1	%
	POS								NEG		
97604	POS	POS	POS	POS	POS	POS	POS	POS	NEG	NEG	100
97605 (1)	POS	POS	POS	POS	POS	POS	POS	POS	NEG	NEG	100
97605 (2)	POS	POS	POS	POS	POS	POS	POS	POS	NEG	NEG	100
97606	POS	POS	POS	POS	POS	POS	POS	POS	NEG	NEG	100
97607 (1)	POS	POS	POS	POS	POS	POS	POS	POS	NEG	NEG	100
97607 (2)	POS	POS	POS	POS	POS	POS	POS	POS	NEG	NEG	100
97608 (1)	POS	POS	POS	POS	POS	POS	POS	POS	NEG	NEG	100
97608 (2)	POS	POS	POS	POS	POS	POS	POS	POS	NEG	NEG	100
97609	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
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
97624	POS	POS	POS	POS	POS	POS	POS	POS	NEG	NEG	100
97630	POS	POS	POS	POS	POS	POS	POS	POS	NEG	NEG	100
97631	POS	POS	POS	POS	POS	POS	POS	POS	NEG	NEG	100
97632	POS	POS	POS	POS	POS	POS	POS	POS	NEG	NEG	100
97634	POS	POS	POS	POS	POS	POS	POS	POS	NEG	NEG	100
97636	POS	POS	POS	POS	POS	POS	POS	POS	NEG	NEG	100
97637	POS	POS	POS	POS	POS	POS	POS	POS	NEG	NEG	100

Lab number	TP1	TP2	TP3	TP4	TP5	TP6	BP1 Rep 1	BP1 Rep 2	TN1	BN1	%
	POS							NEG			
97642	POS	POS	POS	POS	POS	POS	POS	POS	NEG	NEG	100
97643	POS	POS	POS	POS	POS	POS	POS	POS	NEG	NEG	100
97647 (1)	POS	POS	POS	POS	POS	POS	POS	POS	NEG	NEG	100
97647 (2)	NEG	POS	POS	NEG	POS	NEG	NI	POS	NEG	NEG	60

7.2.2 SPECIES DIFFERENTIATION

7.2.2.1 Results per sample

In total, 24 laboratories participated in the Species Differentiation virology part of the proficiency test. Raw data can be found in Annex 1.

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

Sample ID PT2023CAPXVIR	REP	Expected results	# of GTPV	# of LSDV	# of SPPV	# of NEG	# of NI	%
TP1	1	GTPV	23	0	0	1	0	96
TP2	1	LSDV	0	24	0	0	0	100
TP3	1	LSDV	0	23	0	1	0	96
TP4	1	SPPV	0	0	23	1	0	96
TP5	1	SPPV	0	0	23	1	0	96
TP6	1	LSDV	0	24	0	0	0	100
BP1	2	LSDV	0	47	0	0	1	98
TN1	1	NEG	0	0	0	24	0	100
BN1	1	NEG	0	0	0	24	0	100

7.2.2.2 Results per method

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

Table 17: Results per method (N = number of laboratories; NR = number of results; NCR = number of correct results; F=False; NI = not interpretable).

Kit or reference	N	NR	NCR	F	NI
Wolff <i>et al.</i> 2021	4	40	39	1	0
Lamien <i>et al.</i> 2011	11	110	109	0	1
Sso Advanced Universal probes	1	10	7	3	0
Wolff <i>et al.</i> 2021. and Haegeman <i>et al.</i> 2023	1	10	10	0	0
Lamien <i>et al.</i> 2011 and Galaye <i>et al.</i> 2015	1	10	10	0	0

Kit or reference	N	NR	NCR	F	NI
Galaye <i>et al.</i> 2015	1	10	10	0	0
Galaye <i>et al.</i> 2017 + IZS TE B456.1 SOP21 (Biosellal)	1	10	10	0	0
Galaye <i>et al.</i> 2017	1	10	10	0	0
Lamien <i>et al.</i> 2011 and Chibssa <i>et al.</i> 2018	1	10	10	0	0
Vidanovic <i>et al.</i> 2016 & Lamien <i>et al.</i> 2011	1	10	10	0	0
Vidanovic <i>et al.</i> 2021	1	10	10	0	0
TOTAL	24	240	235	4	1

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 10 reference samples (100% of agreement), whereas LAB97614 and LAB97632 misclassified 1 aliquot (90% of agreement). In addition, in one laboratory (LAB97607), a diagnostic kit with the limitation of differentiating solely among LSDV strains was employed, thus lacking the capability to discriminate between species (SPPV and GTPV).

Table 18: Results per laboratory. Correct answers are marked in green, while incorrect answers are indicated in red. In addition, not interpretable answers are showed in orange.

Lab number	TP1	TP2	TP3	TP4	TP5	TP6	BP1 Rep 1	BP1 Rep 2	TN1	BN1	%
	GTPV	LSDV	LSDV	SPPV	SPPV	LSDV	LSDV	LSDV	NEG	NEG	
97506	GTPV	LSDV	LSDV	SPPV	SPPV	LSDV	LSDV	LSDV	NEG	NEG	100
97600	GTPV	LSDV	LSDV	SPPV	SPPV	LSDV	LSDV	LSDV	NEG	NEG	100
97602	GTPV	LSDV	LSDV	SPPV	SPPV	LSDV	LSDV	LSDV	NEG	NEG	100
97603	GTPV	LSDV	LSDV	SPPV	SPPV	LSDV	LSDV	LSDV	NEG	NEG	100
97604	GTPV	LSDV	LSDV	SPPV	SPPV	LSDV	LSDV	LSDV	NEG	NEG	100
97607	NEG	LSDV	LSDV	NEG	NEG	LSDV	LSDV	LSDV	NEG	NEG	70
97608	GTPV	LSDV	LSDV	SPPV	SPPV	LSDV	LSDV	LSDV	NEG	NEG	100
97609	GTPV	LSDV	LSDV	SPPV	SPPV	LSDV	LSDV	LSDV	NEG	NEG	100
97610	GTPV	LSDV	LSDV	SPPV	SPPV	LSDV	LSDV	LSDV	NEG	NEG	100
97611	GTPV	LSDV	LSDV	SPPV	SPPV	LSDV	LSDV	LSDV	NEG	NEG	100
97612	GTPV	LSDV	LSDV	SPPV	SPPV	LSDV	LSDV	LSDV	NEG	NEG	100
97613	GTPV	LSDV	LSDV	SPPV	SPPV	LSDV	LSDV	LSDV	NEG	NEG	100
97614	GTPV	LSDV	LSDV	SPPV	SPPV	LSDV	NI	LSDV	NEG	NEG	90
97617	GTPV	LSDV	LSDV	SPPV	SPPV	LSDV	LSDV	LSDV	NEG	NEG	100
97618	GTPV	LSDV	LSDV	SPPV	SPPV	LSDV	LSDV	LSDV	NEG	NEG	100
97619	SPPV/ GTPV	LSDV	LSDV	SPPV	SPPV	LSDV	LSDV	LSDV	NEG	NEG	100
97620	GTPV	LSDV	LSDV	SPPV	SPPV	LSDV	LSDV	LSDV	NEG	NEG	100

Lab number	TP1	TP2	TP3	TP4	TP5	TP6	BP1 Rep 1	BP1 Rep 2	TN1	BN1	%
	GTPV	LSDV	LSDV	SPPV	SPPV	LSDV	LSDV	LSDV	NEG	NEG	
97630	GTPV	LSDV	LSDV	SPPV	SPPV	LSDV	LSDV	LSDV	NEG	NEG	100
97631	GTPV	LSDV	LSDV	SPPV	SPPV	LSDV	LSDV	LSDV	NEG	NEG	100
97632	GTPV	LSDV	NEG	SPPV	SPPV	LSDV	LSDV	LSDV	NEG	NEG	90
97634	GTPV	LSDV	LSDV	SPPV	SPPV	LSDV	LSDV	LSDV	NEG	NEG	100
97642	GTPV	LSDV	LSDV	SPPV	SPPV	LSDV	LSDV	LSDV	NEG	NEG	100
97643	GTPV	LSDV	LSDV	SPPV	SPPV	LSDV	LSDV	LSDV	NEG	NEG	100
97647	GTPV	LSDV	LSDV	SPPV	SPPV	LSDV	LSDV	LSDV	NEG	NEG	100

7.2.3 DIVA PCR

7.2.3.1 Results per sample

In total, 23 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 = not interpretable; NA = not analysed).

Sample ID	REP	Expected results	# of GTPV	# of LSDV			# of SPPV		# of NEG	# of NI	# of NA
				Wild	Vac	Rec	Wild	Vac			
TP1	1	GTPV	14	0	0	0	0	0	4	1	4
TP2	1	LSDV wild	0	22	0	0	0	0	0	1	0
TP3	1	LSDV rec	0	3	11	8	0	0	1	0	0
TP4	1	SPPV wild	0	0	0	0	13	1	3	1	5 (2 SPPV)
TP5	1	SPPV Vac	0	0	0	0	0	13	3	2	5 (2 SPPV)
TP6	1	LSDV wild	0	23	0	0	0	0	0	0	0
BP1	2	LSDV wild	0	45	0	0	0	0	0	1	0
TN1	1	NEG	0	0	0	0	0	0	23	0	0
BN1	1	NEG	0	0	0	0	0	0	23	0	0

7.2.3.2 Results per method

In the table below, results are listed by method.

Table 14: Results per method (N = number of laboratories; NR = number of results; number of correct results; F = false; NI = not interpretable; NA = not analysed).

Kit or reference	N	NR	NCR	F	NI	NA
Chibssa <i>et al.</i> 2018 & Agianniotaki <i>et al.</i> 2017 & Haegeman <i>et al.</i> 2023	1	10	10	0	0	0
Bio-T kit® Lumpy Skin Disease (DIVA)	1	10	8	2	0	0
Haegeman <i>et al.</i> 2014 and Haegeman <i>et al.</i> 2023	1	10	10	0	0	0
Haegeman <i>et al.</i> 2023 & Chibssa <i>et al.</i> 2018 & GPCR sequencing	1	10	9	0	0	1
Qiagen MO-TO 47	1	10	8	2	0	0
Wolff <i>et al.</i> 2021 & Chibssa <i>et al.</i> 2018 & Haegeman <i>et al.</i> 2023	1	10	10	0	0	0
Haegeman <i>et al.</i> 2015 & Agianniotaki <i>et al.</i> 2016	1	10	10	0	0	0
Vidanovic <i>et al.</i> 2021	2	20	15	2	0	3
Agianniotaki <i>et al.</i> 2017	1	10	8	2	0	0
Chibssa <i>et al.</i> 2018 & ID GENE LSD DIVA Triplex (ID.VET)	1	10	10	0	0	0
Agianniotaki <i>et al.</i> 2017 & Chibssa <i>et al.</i> 2018	2	20	20	0	0	0
Gelaye <i>et al.</i> 2015	1	10	6	1	3	0
Menasherow <i>et al.</i> 2014	1	10	10	0	0	0
Agianniotaki <i>et al.</i> 2021 and undisclosed assay for ovine samples	1	10	10	0	0	0
Haegeman <i>et al.</i> 2023 & Chibssa <i>et al.</i> 2018	1	10	10	0	0	0
Haegeman <i>et al.</i> 2015 & Agianniotaki <i>et al.</i> 2017	1	10	10	0	0	0
Wolff <i>et al.</i> 2021	2	20	15	3	0	2
Wolff <i>et al.</i> and sequencing	1	10	9	0	0	1
Vidanovic <i>et al.</i> 2016	1	10	7	0	3	0
Sprygin <i>et al.</i> 2018 & Unpublished assay	1	10	7	0	0	3
TOTAL	23	230	203	11	6	10

7.2.3.3 Results per laboratory

Thirteen out of 23 laboratories provided results that were in full agreement with the assigned status of the reference samples (100% agreement). Nine laboratories misclassified aliquots PTCAPXVIR2023TP4 and PTCAPXVIR2023TP5. As NA, NEG or SPPV instead of respectively SPPV Wild and SPPV vaccine. The remaining lab (LAB97614) misclassified four aliquots: PTCAPXVIR2023TP2 and one aliquot of PTCAPXVIR2023BP1 as NI instead of LSDV wild. PTCAPXVIR2023TP4 and PTCAPXVIR2023TP5 were also misclassified as respectively SPPV vaccine and NI.

Table 15: Results per laboratory. Correct answers are marked in green, while incorrect answers are indicated in red. In addition, not interpretable answers are shown in orange.

Lab number	TP1	TP2	TP3	TP4	TP5	TP6	BP1 Rep 1	BP1 Rep 2	TN1	BN1	%
	GTPV	LSDV Wild	LSDV Rec	SPPV Wild	SPPV Vac	LSDV Wild	LSDV Wild	LSDV Wild	NEG	NEG	
97506	GTPV	LSDV Wild	LSDV Rec	SPPV Wild	SPPV Vac	LSDV Wild	LSDV Wild	LSDV Wild	NEG	NEG	100
97514	NEG	LSDV Wild	LSDV Wild	NEG	NEG	LSDV Wild	LSDV Wild	LSDV Wild	NEG	NEG	80
97600	GTPV	LSDV Wild	LSDV Rec	SPPV Wild	SPPV Vac	LSDV Wild	LSDV Wild	LSDV Wild	NEG	NEG	100
97602	NA	LSDV Wild	LSDV Rec	SPPV Wild	SPPV Vac	LSDV Wild	LSDV Wild	LSDV Wild	NEG	NEG	100
97604	GTPV	LSDV Wild	LSDV Wild	SPPV	SPPV	LSDV Wild	LSDV Wild	LSDV Wild	NEG	NEG	80
97608	GTPV	LSDV Wild	LSDV Rec	SPPV Wild	SPPV Vac	LSDV Wild	LSDV Wild	LSDV Wild	NEG	NEG	100
97609	GTPV	LSDV Wild	LSDV Rec	SPPV Wild	SPPV Vac	LSDV Wild	LSDV Wild	LSDV Wild	NEG	NEG	100
97610	NA	LSDV Wild	LSDV Vac	NA	NA	LSDV Wild	LSDV Wild	LSDV Wild	NEG	NEG	80
97611	GTPV	LSDV Wild	LSDV Vac	SPPV	SPPV	LSDV Wild	LSDV Wild	LSDV Wild	NEG	NEG	80
97612	GTPV	LSDV Wild	LSDV Vac	SPPV Wild	SPPV Vac	LSDV Wild	LSDV Wild	LSDV Wild	NEG	NEG	100
97613	NEG	LSDV Wild	LSDV Vac	SPPV Wild	SPPV Vac	LSDV Wild	LSDV Wild	LSDV Wild	NEG	NEG	100
97614	GTPV	NI	LSDV Vac	SPPV Vac	NI	LSDV Wild	NI	LSDV Wild	NEG	NEG	60
97617	GTPV	LSDV Wild	LSDV Vac	SPPV Wild	SPPV Vac	LSDV Wild	LSDV Wild	LSDV Wild	NEG	NEG	100
97618	GTPV	LSDV Wild	LSDV Rec	SPPV Wild	SPPV Vac	LSDV Wild	LSDV Wild	LSDV Wild	NEG	NEG	100

Lab number	TP1	TP2	TP3	TP4	TP5	TP6	BP1 Rep 1	BP1 Rep 2	TN1	BN1	%
	GTPV	LSDV Wild	LSDV Rec	SPPV Wild	SPPV Vac	LSDV Wild	LSDV Wild	LSDV Wild	NEG	NEG	
97620	GTPV	LSDV Wild	LSDV Vac	SPPV Wild	SPPV Vac	LSDV Wild	LSDV Wild	LSDV Wild	NEG	NEG	100
97621	GTPV	LSDV Wild	LSDV Vac	SPPV Wild	SPPV Vac	LSDV Wild	LSDV Wild	LSDV Wild	NEG	NEG	100
97630	GTPV	LSDV Wild	LSDV Wild	NA	NA	LSDV Wild	LSDV Wild	LSDV Wild	NEG	NEG	80
97632	NA	LSDV Wild	LSDV Rec	SPPV Wild	SPPV Vac	LSDV Wild	LSDV Wild	LSDV Wild	NEG	NEG	100
97634	NEG	LSDV Wild	LSDV Vac	NEG	NEG	LSDV Wild	LSDV Wild	LSDV Wild	NEG	NEG	80
97637	NI	LSDV Wild	LSDV Vac	NI	NI	LSDV Wild	LSDV Wild	LSDV Wild	NEG	NEG	80
97642	GTPV	LSDV Wild	LSDV Vac	SPPV Wild	SPPV Vac	LSDV Wild	LSDV Wild	LSDV Wild	NEG	NEG	100
97643	NA	LSDV Wild	LSDV Rec	NA	NA	LSDV Wild	LSDV Wild	LSDV Wild	NEG	NEG	80
97647	NEG	LSDV Wild	NEG	NEG	NEG	LSDV Wild	LSDV Wild	LSDV Wild	NEG	NEG	70

8 DISCUSSION

The purpose of this PT was to assess the performances of the participating laboratories when analyzing reference serum samples of ruminant origin for the detection of antibodies to capripox viruses and/or analyzing 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 neutralization test (VNT) or an immunoperoxidase monolayer assay (IPMA) as well, 32 out of 33 laboratories provided qualitative results that were in full agreement with the assigned status of the reference serum samples. The remaining laboratory misclassified one aliquot, resulting in a level of agreement of 90%.

8.2 Virology component of the PT

For the detection of capripox virus **DNA by real-time PCR (RT-PCR)** in the PT panel: 32 out of 33 participating laboratories provided qualitative results that were in full agreement with the assigned status of the ten reference samples (100% of agreement). One laboratory (LAB97514) misclassified three samples (PT2023CAPXVIRTP1, PT2023CAPXVIRTP4 and PT2023CAPXVIRTP5). Four laboratories added a secondary PCR and three of them provided qualitative results that were in full agreement with the assigned status of the ten reference samples (100% of agreement) The remaining laboratory (LAB97647) that performed a second method misclassified four samples (PT2023CAPXVIRTP1, PT2023CAPXVIRTP4, PT2023CAPXVIRTP6 and PT2023CAPXVIRBP1). The laboratories that misclassified some of the aliquots, use a diagnostic kit that could only detect LSDV strains and it lacked the ability to detect SPPV or GTPV. The misclassified aliquots were the SPPV and GTPV samples. In addition LAB97647 also misclassified one LSDV sample (PT2023CAPXVIRBP1).

For the **differentiation of capripox virus species**, 21 out of the 24 participating laboratories produced qualitative results that matched the assigned status of the 10 reference samples, achieving a 100% agreement rate. However, LAB97614 and LAB97632 incorrectly classified one aliquot respectively PT2023CAPXVIRBP1 and PT2023CAPXVIRTP3, resulting in a 90% agreement rate for that lab. Furthermore, in the remaining laboratory (LAB97607), a diagnostic kit designed exclusively for detecting LSDV strains was used, rendering them unable to differentiate between the various species (SPPV, and GTPV). As a result, the aliquots containing SPPV or GTPV (PT2023CAPXVIRTP1, PT2023CAPXVIRTP4 and PT2023CAPXVIRTP5) were misclassified. These responses were excluded from the evaluation.

For the **DIVA part**, the results of two samples were not taken into account. These were PT2023CAPXVIRTP1 and PT2023CAPXVIRTP3. TP1 is a GTPV strain and there is currently no DIVA available for GTPV. TP3 is a recombinant LSDV strain, a DIVA to recognize these strain has only been made available in march 2023, which is too late to for the NRLs to implement this by the time of the PT. For these two samples, all results were considered correct. Thirteen out of 23 laboratories provided results that were in full agreement with the assigned status of the reference samples (100% agreement). Nine laboratories misclassified aliquots PTCAPXVIR2023TP4 and PTCAPXVIR2023TP5. As NA, NEG or SPPV instead of respectively SPPV Wild and SPPV vaccine. These laboratories only used a method for the differentiation of LSDV and could therefore not make the differentiation of the SPPV strains. For all these laboratories, all the LSDV samples were classified correctly. The remaining laboratory (LAB97614) used a method that can only make the differentiation between vaccine and wild

strains for all capripox viruses. PTCAPXVIR2023TP4 and PTCAPXVIR2023TP5 were misclassified as respectively SPPV vaccine and NI. In addition, two LSDV samples were also misclassified, PT2023CAPXVIRTP2 and PT2023CAPXVIRBP1. For the latter, only one of the two replicates was misclassified, which is surprising.

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 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 analyzed with the used assay. However, a satisfactory performance cannot be awarded, since not all aliquots were analyzed 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 segment, results were submitted by 33 laboratories, with only one of them misclassifying a single aliquot, thereby obtaining a score of 90%. This implies that all participating laboratories met the minimum established criteria. In the VNT section, results were submitted by five 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 RT-PCR, Species Differentiation and DIVA PCR. For the RT-PCR part, 32 out of 33 laboratories delivered results that entirely matched the expected status of the 10 reference samples, achieving a 100% agreement rate and thus a satisfactory result. The one remaining laboratory, LAB97514, misclassified 3 aliquots. This resulted in a level of agreement of 70% and thus an unsatisfactory result. Furthermore, four laboratories conducted a secondary PCR analysis. Among them, LAB97605, LAB97607, and LAB97608 all yielded results that perfectly aligned with the reference sample status. However, LAB97647 incorrectly identified three samples as NEG and one as NI. For the second part (Species Differentiation), 21 out of the 24 participating laboratories delivered qualitative outcomes that were in full agreement with the expected status of the 10 reference samples, achieving a 100% agreement rate. However, LAB97614 and LAB97632 misclassified 1 sample each, resulting in a 90% agreement rate for these two labs. All of these laboratories achieved a satisfactory result. Additionally, LAB97607 utilized a diagnostic kit designed exclusively for distinguishing LSDV strains, lacking the ability to differentiate SPPV and GTPV. All LSDV samples were correctly classified. For the last part (DIVA PCR), thirteen 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. However, nine laboratories incorrectly categorized aliquots PTCAPXVIR2023TP4 and PTCAPXVIR2023TP5 as NA, NEG, or SPPV instead of SPPV Wild and SPPV vaccine, respectively. 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. The remaining laboratory (LAB97614) misclassified four aliquots: PTCAPXVIR2023TP2 and one aliquot of PTCAPXVIR2023BP1 were mistakenly labeled as NI instead of LSDV Wild, and PTCAPXVIR2023TP4 and PTCAPXVIR2023TP5 were misclassified as SPPV vaccine and NI, respectively. Therefore, this lab did not achieve a satisfactory result.

10 ANNEXES (NOT UNDER ACCREDITATION)

10.1 Annex 1: Quantitative results

10.1.1 SERO: ANTIBODY ELISA

lab ID	Random ID	Sample ID	Reagens	Cut-off	Pos control	Neg control	OD	Interpretation	Comment
97506	CAPXSER23-1	P5	ID.VET - ID SCREEN® CAPRIPOX DOUBLE ANTIGEN MULTI-SPECIES	30	0,9312	0,0415	0,97	POS	
	CAPXSER23-2	P2					1,3	POS	
	CAPXSER23-3	P1					1,37	POS	
	CAPXSER23-4	P6					0,67	POS	
	CAPXSER23-5	P6					0,65	POS	
	CAPXSER23-6	N2					0,045	NEG	
	CAPXSER23-7	N1					0,045	NEG	
	CAPXSER23-8	P7					0,87	POS	
	CAPXSER23-9	P4					1,59	POS	
	CAPXSER23-10	P3					1,16	POS	
97532	CAPXSER23-1	P2	INDICAL BIOSCIENCE GmbH - cattletype Capripox Ab prototype	30	0.848	0.058	1,490	POS	S/P% = [(OD sample – ODNC) / (OD PC – OD NC)]* 100
	CAPXSER23-2	P3					1,435	POS	
	CAPXSER23-3	N2					0,063	NEG	
	CAPXSER23-4	N1					0,055	NEG	
	CAPXSER23-5	P4					1,649	POS	
	CAPXSER23-6	P5					1,054	POS	
	CAPXSER23-7	P7					1,003	POS	
	CAPXSER23-8	P6					0,605	POS	
	CAPXSER23-9	P1					1,341	POS	
	CAPXSER23-10	P6					0,582	POS	
97600	CAPXSER23-1	N2	ID.VET - ID SCREEN® CAPRIPOX DOUBLE ANTIGEN MULTI-SPECIES	30	0,868	0,079	0,043	NEG	
	CAPXSER23-2	P6					0,656	POS	
	CAPXSER23-3	P1					1,347	POS	
	CAPXSER23-4	P3					1,276	POS	
	CAPXSER23-5	P5					0,927	POS	
	CAPXSER23-6	P4					1,61	POS	
	CAPXSER23-7	P6					0,641	POS	
	CAPXSER23-8	P7					0,927	POS	
	CAPXSER23-9	P2					1,345	POS	
	CAPXSER23-10	N1					0,044	NEG	
97602	CAPXSER23-1	P1	ID.VET - ID SCREEN® CAPRIPOX DOUBLE ANTIGEN MULTI-SPECIES	30	0,862	0,061	1,204	POS	
	CAPXSER23-2	P4	1,447				POS		

lab ID	Random ID	Sample ID	Reagens	Cut-off	Pos control	Neg control	OD	Interpretation	Comment
	CAPXSER23-3	P3					1,205	POS	
	CAPXSER23-4	N2					0,062	NEG	
	CAPXSER23-5	N1					0,082	NEG	
	CAPXSER23-6	P2					1,362	POS	
	CAPXSER23-7	P6					0,625	POS	
	CAPXSER23-8	P7					0,887	POS	
	CAPXSER23-9	P5					0,9	POS	
	CAPXSER23-10	P6					0,614	POS	
	CAPXSER23-1	P5					0,902	POS	
	CAPXSER23-2	P2					1,238	POS	
	CAPXSER23-3	P6					0,692	POS	
	CAPXSER23-4	N1					0,109	NEG	
97604	CAPXSER23-5	P4	ID.VET - ID SCREEN® CAPRIPOX DOUBLE ANTIGEN MULTI-SPECIES	30	1,054	0,097	1,55	POS	(Sample-NC)/(PC-NC)
	CAPXSER23-6	P1					1,204	POS	
	CAPXSER23-7	P7					0,893	POS	
	CAPXSER23-8	P3					1,092	NEG	
	CAPXSER23-9	P6					0,663	POS	
	CAPXSER23-10	N2					0,088	NEG	
	CAPXSER23-1	P2					1,458	POS	
	CAPXSER23-2	P1					1,367	POS	
	CAPXSER23-3	P4					1,582	POS	
	CAPXSER23-4	P7					1,007	POS	
97605	CAPXSER23-5	N1	ID.VET - ID SCREEN® CAPRIPOX DOUBLE ANTIGEN MULTI-SPECIES	30	1,002	0,051	0,048	NEG	S/P%=((ODsample-ODnc)/(ODpc- ODnc))*100
	CAPXSER23-6	P6					0,699	POS	
	CAPXSER23-7	P5					0,971	POS	
	CAPXSER23-8	P3					1,334	POS	
	CAPXSER23-9	N2					0,047	NEG	
	CAPXSER23-10	P6					0,719	POS	
	CAPXSER23-1	P4					1,489	POS	
	CAPXSER23-2	P6					0,517	POS	
	CAPXSER23-3	P2					1,2531	POS	
	CAPXSER23-4	P6					0,489	POS	
97606	CAPXSER23-5	N1	ID.VET - ID SCREEN® CAPRIPOX DOUBLE ANTIGEN MULTI-SPECIES	30	0,7976	0,0504	0,0521	NEG	
	CAPXSER23-6	P5					0,9079	POS	
	CAPXSER23-7	N2					0,0611	NEG	
	CAPXSER23-8	P7					0,8245	POS	
	CAPXSER23-9	P3					1,0569	POS	
	CAPXSER23-10	P1					1,1432	POS	
97607	CAPXSER23-1	P3	ID.VET - ID SCREEN® CAPRIPOX DOUBLE ANTIGEN MULTI-SPECIES	30	0,986	0,041	1,378	POS	
	CAPXSER23-2	P6					0,618	POS	

lab ID	Random ID	Sample ID	Reagens	Cut-off	Pos control	Neg control	OD	Interpretation	Comment
	CAPXSER23-3	P1					1,371	POS	
	CAPXSER23-4	P5					0,949	POS	
	CAPXSER23-5	P7					1,011	POS	
	CAPXSER23-6	N1					0,041	NEG	
	CAPXSER23-7	P6					0,655	POS	
	CAPXSER23-8	P4					1,671	POS	
	CAPXSER23-9	N2					0,045	NEG	
	CAPXSER23-10	P2					1,422	POS	
	CAPXSER23-1	P6					0,5	POS	
	CAPXSER23-2	P6					0,49	POS	
	CAPXSER23-3	N2					0,06	NEG	
	CAPXSER23-4	N1					0,07	NEG	
97608	CAPXSER23-5	P2	ID.VET - ID SCREEN® CAPRIPOX DOUBLE ANTIGEN MULTI-SPECIES	30	0,88	0,06	1,12	POS	an lab internal positive standard dilution was also use integration of these data in the submission form is not possible
	CAPXSER23-6	P4					1,37	POS	
	CAPXSER23-7	P7					0,71	POS	
	CAPXSER23-8	P5					0,75	POS	
	CAPXSER23-9	P3					1,09	POS	
	CAPXSER23-10	P1					1,09	POS	
	CAPXSER23-1	N1					0,054	NEG	
	CAPXSER23-2	P4					1,2	POS	
	CAPXSER23-3	P3					0,948	POS	
	CAPXSER23-4	P7					0,627	POS	
97609	CAPXSER23-5	P5	ID.VET - ID SCREEN® CAPRIPOX DOUBLE ANTIGEN MULTI-SPECIES	30	0,714	0,052	0,64	POS	S/P%=(ODsample- ODnc)x100/(ODpc-ODnc)
	CAPXSER23-6	P6					0,387	POS	
	CAPXSER23-7	N2					0,06	NEG	
	CAPXSER23-8	P2					0,954	POS	
	CAPXSER23-9	P1					0,891	POS	
	CAPXSER23-10	P6					0,401	POS	
	CAPXSER23-1	N2					0,044	NEG	
	CAPXSER23-2	P6					0,61	POS	
	CAPXSER23-3	P3					1,214	POS	
	CAPXSER23-4	P1					1,378	POS	
97610	CAPXSER23-5	N1	ID.VET - ID SCREEN® CAPRIPOX DOUBLE ANTIGEN MULTI-SPECIES	30	0,949	0,044	0,044	NEG	
	CAPXSER23-6	P4					1,736	POS	
	CAPXSER23-7	P5					0,949	POS	
	CAPXSER23-8	P6					0,593	POS	
	CAPXSER23-9	P7					0,908	POS	
	CAPXSER23-10	P2					1,407	POS	
97611	CAPXSER23-1	P7	ID.VET - ID SCREEN® CAPRIPOX DOUBLE ANTIGEN MULTI-SPECIES	30	0,826	0,0485	0,687	POS	OD Sample-OD Neg / OD Pos- OD Neg *100
	CAPXSER23-2	N2					0,0465	NEG	

lab ID	Random ID	Sample ID	Reagens	Cut-off	Pos control	Neg control	OD	Interpretation	Comment
	CAPXSER23-3	P3					1,146	POS	
	CAPXSER23-4	P6					0,4445	POS	
	CAPXSER23-5	P6					0,421	POS	
	CAPXSER23-6	P1					1,0805	POS	
	CAPXSER23-7	P4					1,218	POS	
	CAPXSER23-8	P2					1,1475	POS	
	CAPXSER23-9	P5					0,688	POS	
	CAPXSER23-10	N1					0,047	NEG	
	CAPXSER23-1	P5					0,609	POS	
	CAPXSER23-2	P6					0,403	POS	
	CAPXSER23-3	N1					0,049	NEG	
	CAPXSER23-4	P4					1,225	POS	
97612	CAPXSER23-5	P1	ID.VET - ID SCREEN® CAPRIPOX DOUBLE ANTIGEN MULTI-SPECIES	30	0,642	0,06	0,958	POS	S/P%=(ODsample-ODneg/ODpos- ODneg)*100
	CAPXSER23-6	P2					0,968	POS	
	CAPXSER23-7	P3					0,884	POS	
	CAPXSER23-8	N2					0,051	NEG	
	CAPXSER23-9	P7					0,659	POS	
	CAPXSER23-10	P6					0,405	POS	
	CAPXSER23-1	P6					0,556	POS	
	CAPXSER23-2	P7					0,849	POS	
	CAPXSER23-3	P5					0,805	POS	
	CAPXSER23-4	N2					0,051	NEG	
97613	CAPXSER23-5	P1	ID.VET - ID SCREEN® CAPRIPOX DOUBLE ANTIGEN MULTI-SPECIES	30	0,749	0,042	1,143	POS	S/P%=((sample OD-NC OD)/(PC OD-NC OD))*100
	CAPXSER23-6	P6					0,543	POS	
	CAPXSER23-7	P3					0,969	POS	
	CAPXSER23-8	P4					1,429	POS	
	CAPXSER23-9	N1					0,051	NEG	
	CAPXSER23-10	P2					1,117	POS	
	CAPXSER23-1	P4					1,26	POS	
	CAPXSER23-2	P6					0,414	POS	
	CAPXSER23-3	P7					0,717	POS	
	CAPXSER23-4	P1					1,13	POS	
97614	CAPXSER23-5	N2	ID.VET - ID SCREEN® CAPRIPOX DOUBLE ANTIGEN MULTI-SPECIES	30	0,8265	0,0445	0,04	NEG	S/P% = (OD sample - OD NC) / (OD PC - OD NC) x 100
	CAPXSER23-6	N1					0,041	NEG	
	CAPXSER23-7	P5					0,773	POS	
	CAPXSER23-8	P3					1,017	POS	
	CAPXSER23-9	P2					1,065	POS	
	CAPXSER23-10	P6					0,419	POS	

lab ID	Random ID	Sample ID	Reagens	Cut-off	Pos control	Neg control	OD	Interpretation	Comment
97615	CAPXSER23-1	P5	ID.VET - ID SCREEN® CAPRIPOX DOUBLE ANTIGEN MULTI-SPECIES	30	0,793	0,058	0,727	POS	S/P % - 100 x (OD Sample - OD Neg Ctrl)/ (OD Pos Ctrl -OD Neg Ctrl)
	CAPXSER23-2	P1					1,056	POS	
	CAPXSER23-3	P4					1,281	POS	
	CAPXSER23-4	P7					0,734	POS	
	CAPXSER23-5	N2					0,059	NEG	
	CAPXSER23-6	P3					1,101	POS	
	CAPXSER23-7	P6					0,535	POS	
	CAPXSER23-8	P6					0,543	POS	
	CAPXSER23-9	P2					1,096	POS	
	CAPXSER23-10	N1					0,057	NEG	
97616	CAPXSER23-1	P5	ID.VET - ID SCREEN® CAPRIPOX DOUBLE ANTIGEN MULTI-SPECIES	30	0,84	0,043	0,737	POS	antibody ELISA column provides S/P values in %
	CAPXSER23-2	P2					1,117	POS	
	CAPXSER23-3	P4					1,406	POS	
	CAPXSER23-4	P3					1,083	POS	
	CAPXSER23-5	N1					0,046	NEG	
	CAPXSER23-6	N2					0,047	NEG	
	CAPXSER23-7	P7					0,793	POS	
	CAPXSER23-8	P1					1,085	POS	
	CAPXSER23-9	P6					0,53	POS	
	CAPXSER23-10	P6					0,577	POS	
97617	CAPXSER23-1	P2	ID.VET - ID SCREEN® CAPRIPOX DOUBLE ANTIGEN MULTI-SPECIES	30	0,749	0,061	1,165	POS	S/P % = (ODsample- ODnegative)/(ODpositive- ODnegative)x100
	CAPXSER23-2	P4					1,441	POS	
	CAPXSER23-3	P3					1,082	POS	
	CAPXSER23-4	P7					0,758	POS	
	CAPXSER23-5	P6					0,487	POS	
	CAPXSER23-6	N2					0,058	NEG	
	CAPXSER23-7	P5					0,778	POS	
	CAPXSER23-8	P1					1,223	POS	
	CAPXSER23-9	P6					0,471	POS	
	CAPXSER23-10	N1					0,062	NEG	
97618	CAPXSER23-1	P1	ID.VET - ID SCREEN® CAPRIPOX DOUBLE ANTIGEN MULTI-SPECIES	30	0,54	0,056	271,13	POS	ELISA Indirect
	CAPXSER23-2	P6					74,22	POS	
	CAPXSER23-3	N1					0	NEG	
	CAPXSER23-4	P7					144,74	POS	
	CAPXSER23-5	N2					0	NEG	
	CAPXSER23-6	P5					143,71	POS	
	CAPXSER23-7	P2					265,56	POS	
	CAPXSER23-8	P4					264,94	POS	

lab ID	Random ID	Sample ID	Reagens	Cut-off	Pos control	Neg control	OD	Interpretation	Comment
	CAPXSER23-9	P6					88,45	POS	
	CAPXSER23-10	P3					219,58	POS	
97619	CAPXSER23-1	P4	ID.VET - ID SCREEN® CAPRIPOX DOUBLE ANTIGEN MULTI-SPECIES	30	1,014	0,045	1,402	POS	
	CAPXSER23-2	P6					0,408	POS	
	CAPXSER23-3	N2					0,041	NEG	
	CAPXSER23-4	P5					0,813	POS	
	CAPXSER23-5	P6					0,434	POS	
	CAPXSER23-6	P2					1,641	POS	
	CAPXSER23-7	P1					1,53	POS	
	CAPXSER23-8	N1					0,048	NEG	
	CAPXSER23-9	P7					0,757	POS	
	CAPXSER23-10	P3					1,594	POS	
97620	CAPXSER23-1	P4	ID.VET - ID SCREEN® CAPRIPOX DOUBLE ANTIGEN MULTI-SPECIES	30	0,813	0,0405	1,414	POS	S/P%=(ODsample-ODnc)/(ODpc-ODnc)*100
	CAPXSER23-2	N2					0,045	NEG	
	CAPXSER23-3	P6					0,513	POS	
	CAPXSER23-4	P7					0,728	POS	
	CAPXSER23-5	P2					0,977	POS	
	CAPXSER23-6	P1					0,982	POS	
	CAPXSER23-7	N1					0,047	NEG	
	CAPXSER23-8	P3					0,903	POS	
	CAPXSER23-9	P6					0,5	POS	
	CAPXSER23-10	P5					0,726	POS	
97621	CAPXSER23-1	P6	ID.VET - ID SCREEN® CAPRIPOX DOUBLE ANTIGEN MULTI-SPECIES	30	0,924	0,045	0,61	POS	S/P%=((OD sample - OD NC)/(OD PC - OD NC)) *100
	CAPXSER23-2	P7					0,88	POS	
	CAPXSER23-3	P2					1,31	POS	
	CAPXSER23-4	P6					0,58	POS	
	CAPXSER23-5	P5					0,76	POS	
	CAPXSER23-6	N2					0,04	NEG	
	CAPXSER23-7	N1					0,05	NEG	
	CAPXSER23-8	P4					1,54	POS	
	CAPXSER23-9	P1					1,27	POS	
	CAPXSER23-10	P3					1,22	POS	
97622	CAPXSER23-1	P5	ID.VET - ID SCREEN® CAPRIPOX DOUBLE ANTIGEN MULTI-SPECIES	30	0,988	0,052	0,757	POS	Procedure has been performed according to the booklet of the kit. No longer or shorter incubation periods are described in there. S/P% = (ODsample-ODnc)/(ODpc-ODnc)*100
	CAPXSER23-2	P2					1,189	POS	
	CAPXSER23-3	N1					0,049	NEG	
	CAPXSER23-4	P1					1,132	POS	
	CAPXSER23-5	P6					0,525	POS	
	CAPXSER23-6	P4					1,397	POS	
	CAPXSER23-7	P3					1,081	POS	

lab ID	Random ID	Sample ID	Reagens	Cut-off	Pos control	Neg control	OD	Interpretation	Comment
	CAPXSER23-8	P6					0,56	POS	
	CAPXSER23-9	P7					0,817	POS	
	CAPXSER23-10	N2					0,048	NEG	
97628	CAPXSER23-1	P5	BioStone - AsurDx Capripox Antibody Test Kit	40	0	0	2,7	POS	PP = (OD450 test sample - NC)/(PC - NC) * 100%
	CAPXSER23-2	P2					4,1	POS	
	CAPXSER23-3	P6					1,4	POS	
	CAPXSER23-4	P3					2,6	POS	
	CAPXSER23-5	P7					2,6	POS	
	CAPXSER23-6	P4					1,4	POS	
	CAPXSER23-7	N1					0,5	NEG	
	CAPXSER23-8	P1					4,4	POS	
	CAPXSER23-9	P6					1,2	POS	
	CAPXSER23-10	N2					0,3	NEG	
	97629	CAPXSER23-1					N2	BioStone - AsurDx Capripox Antibody Test Kit	
CAPXSER23-2		P1	4,5	POS					
CAPXSER23-3		P5	2,5	POS					
CAPXSER23-4		P2	3,8	POS					
CAPXSER23-5		P6	1,2	POS					
CAPXSER23-6		P7	1,2	POS					
CAPXSER23-7		P3	2,9	POS					
CAPXSER23-8		P4	1,8	POS					
CAPXSER23-9		N1	0,5	NEG					
CAPXSER23-10		P6	1,3	POS					
97630	CAPXSER23-1	P6	ID.VET - ID SCREEN® CAPRIPOX DOUBLE ANTIGEN MULTI-SPECIES	30	0,565	0,056	0,405	POS	S/P%=100*((Odsample- Odc)/(Odc-Odc))
	CAPXSER23-2	P5					0,62	POS	
	CAPXSER23-3	P7					0,571	POS	
	CAPXSER23-4	P3					0,899	POS	
	CAPXSER23-5	P1					0,935	POS	
	CAPXSER23-6	N2					0,065	NEG	
	CAPXSER23-7	N1					0,058	NEG	
	CAPXSER23-8	P6					0,419	POS	
	CAPXSER23-9	P2					0,985	POS	
	CAPXSER23-10	P4					0,974	POS	
97631	CAPXSER23-1	N1	ID.VET - ID SCREEN® CAPRIPOX DOUBLE ANTIGEN MULTI-SPECIES	30	1,066	0,0465	0,05	NEG	S/P%= (OD sample- OD NC)/ (OD PC- OD NC)x100
	CAPXSER23-2	P5					1,08	POS	
	CAPXSER23-3	P1					1,587	POS	
	CAPXSER23-4	P4					1,872	POS	
	CAPXSER23-5	P3					1,42	POS	
	CAPXSER23-6	P7					1,081	POS	

lab ID	Random ID	Sample ID	Reagens	Cut-off	Pos control	Neg control	OD	Interpretation	Comment
	CAPXSER23-7	P6					0,696	POS	
	CAPXSER23-8	P2					1,624	POS	
	CAPXSER23-9	N2					0,046	NEG	
	CAPXSER23-10	P6					0,747	POS	
97632	CAPXSER23-1	P3	ID.VET - ID SCREEN® CAPRIPOX DOUBLE ANTIGEN MULTI-SPECIES	30	0,883	0,047	1,257	POS	S/P%=(ODsample- ODnegctrl)/(ODposctrl- ODnegctrl)x100
	CAPXSER23-2	P6					0,618	POS	
	CAPXSER23-3	N1					0,048	NEG	
	CAPXSER23-4	P6					0,62	POS	
	CAPXSER23-5	P7					0,934	POS	
	CAPXSER23-6	P4					1,547	POS	
	CAPXSER23-7	P2					1,437	POS	
	CAPXSER23-8	P1					1,407	POS	
	CAPXSER23-9	N2					0,047	NEG	
	CAPXSER23-10	P5					0,928	POS	
97634	CAPXSER23-1	P3	ID.VET - ID SCREEN® CAPRIPOX DOUBLE ANTIGEN MULTI-SPECIES	30	0,923	0,047	1,067	POS	
	CAPXSER23-2	N1					0,047	NEG	
	CAPXSER23-3	P6					0,72	POS	
	CAPXSER23-4	N2					0,051	NEG	
	CAPXSER23-5	P5					0,823	POS	
	CAPXSER23-6	P6					0,657	POS	
	CAPXSER23-7	P7					0,777	POS	
	CAPXSER23-8	P2					1,121	POS	
	CAPXSER23-9	P4					1,479	POS	
	CAPXSER23-10	P1					1,057	POS	
97637	CAPXSER23-1	P6	ID.VET - ID SCREEN® CAPRIPOX DOUBLE ANTIGEN MULTI-SPECIES	30	0,7	0,07	0,434	POS	
	CAPXSER23-2	P3					0,899	POS	
	CAPXSER23-3	P2					0,955	POS	
	CAPXSER23-4	P7					0,645	POS	
	CAPXSER23-5	P1					1,136	POS	
	CAPXSER23-6	N1					0,057	NEG	
	CAPXSER23-7	P5					0,683	POS	
	CAPXSER23-8	P6					0,437	POS	
	CAPXSER23-9	P4					1,267	POS	
	CAPXSER23-10	N2					0,056	NEG	
97642	CAPXSER23-1	N1	ID.VET - ID SCREEN® CAPRIPOX DOUBLE ANTIGEN MULTI-SPECIES	30	0,819	0,047	0,05	NEG	
	CAPXSER23-2	P3					1,239	POS	
	CAPXSER23-3	P2					1,26	POS	
	CAPXSER23-4	P6					0,657	POS	
	CAPXSER23-5	P7					0,918	POS	
	CAPXSER23-6	P4					1,533	POS	

lab ID	Random ID	Sample ID	Reagens	Cut-off	Pos control	Neg control	OD	Interpretation	Comment
	CAPXSER23-7	P1					1,209	POS	
	CAPXSER23-8	P5					0,878	POS	
	CAPXSER23-9	N2					0,049	NEG	
	CAPXSER23-10	P6					0,645	POS	
97643	CAPXSER23-1	P1	ID.VET - ID SCREEN® CAPRIPOX DOUBLE ANTIGEN MULTI-SPECIES	30	0,8434	0,1067	1,4117	POS	S/P%=(?OD?_sample- ODNC)/(?OD?PC-?OD?NC)x100
	CAPXSER23-2	P6					0,5277	POS	
	CAPXSER23-3	P4					1,4826	POS	
	CAPXSER23-4	P7					0,9753	POS	
	CAPXSER23-5	P3					1,315	POS	
	CAPXSER23-6	N2					0,1282	NEG	
	CAPXSER23-7	P2					1,5124	POS	
	CAPXSER23-8	P5					1,0049	POS	
	CAPXSER23-9	P6					0,5381	POS	
	CAPXSER23-10	N1					0,1127	NEG	
	CAPXSER23-1	N1					0,0445	NEG	S/P% results are reported under Antibody ELISA column.
	CAPXSER23-2	N2					0,0441	NEG	
	CAPXSER23-3	P6					0,5156	POS	SPPV surveillance samples: results for CAPXSER23-8 CAPXSER23-7 and CAPXSER23- 4 are consistent with prior infection; results for CAPXSER23- 1 provide no evidence of prior infection.
	CAPXSER23-4	P3					1,481	POS	
	CAPXSER23-5	P7					0,8355	POS	
	CAPXSER23-6	P5					0,8941	POS	
	CAPXSER23-7	P2					1,596	POS	
97647	CAPXSER23-8	P1	ID.VET - ID SCREEN® CAPRIPOX DOUBLE ANTIGEN MULTI-SPECIES	30	1,101	0,0432	1,497	POS	LSDV surveillance samples: results for CAPXSER23-10 CAPXSER23-6 CAPXSER23-3 CAPXSER23-9 and CAPXSER23- 5 are consistent with prior infection; results for CAPXSER23- 2 provide no evidence of prior infection.
	CAPXSER23-9	P6					0,5101	POS	
	CAPXSER23-10	P4					1,523	POS	

10.1.2 SERO: VIRUS NEUTRALIZATION

Lab ID	Random 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		
97506	CAPXSER23-1	P5	In house	OA3.Ts	LSDV Neethling	1/2	1/50	100	R6F 45dpi	1/400	1/50	400	2	=	750	POS		
	CAPXSER23-2	P2												=	250	POS		
	CAPXSER23-3	P1												=	150	POS		
	CAPXSER23-4	P6												=	50	POS		
	CAPXSER23-5	P6												=	50	POS		
	CAPXSER23-6	N2												=	2	NEG		
	CAPXSER23-7	N1												=	2	NEG		
	CAPXSER23-8	P7												=	250	POS		
	CAPXSER23-9	P4												=	250	POS		
	CAPXSER23-10	P3												=	150	POS		
97600	CAPXSER23-1	N2	After WAOH terrestrial manual Chapter 3.4.12 LSD 2021	OA3.Ts	LSDV Neethling	1:5	2	68	IAH VN 84 Days 37 pi (from Pirbright)	640	10	640	10	<	10	NEG		
	CAPXSER23-2	P6												=	60	POS		
	CAPXSER23-3	P1												=	320	POS		
	CAPXSER23-4	P3												=	120	POS		
	CAPXSER23-5	P5												=	320	POS		
	CAPXSER23-6	P4												=	640	POS		
	CAPXSER23-7	P6												=	80	POS		
	CAPXSER23-8	P7												=	120	POS		
	CAPXSER23-9	P2												=	240	POS		
	CAPXSER23-10	N1												<	10	NEG		
97608	CAPXSER23-1	P6												=	160	POS		
	CAPXSER23-2	P6												=	200	POS		
	CAPXSER23-3	N2												<	10	NEG		
	CAPXSER23-4	N1												<	10	NEG		
	CAPXSER23-5	P2												=	256	POS		
	CAPXSER23-6	P4												640	10	=	1600	POS
	CAPXSER23-7	P7												=	400	POS		
	CAPXSER23-8	P5												>	2560	POS		
	CAPXSER23-9	P3												=	200	POS		
	CAPXSER23-10	P1												=	800	POS		
97612	CAPXSER23-1	P5	internal protocol	MDBK	LSDV Neethling	1:5	from 1:5 to 1:640	100	laboratory reference material	1:80	>=1:10	80	10	=	320	POS		
	CAPXSER23-2	P6												=	40	POS		
	CAPXSER23-3	N1												<	10	NEG		
	CAPXSER23-4	P4												=	160	POS		
	CAPXSER23-5	P1												=	320	POS		
	CAPXSER23-6	P2												=	80	POS		
	CAPXSER23-7	P3												=	80	POS		
	CAPXSER23-8	N2												<	10	NEG		
	CAPXSER23-9	P7												=	80	POS		
	CAPXSER23-10	P6												=	40	POS		
97618	CAPXSER23-1	P1	Pirbright	OA3.Ts		0,2	0.2-0.0016	100		20	5	20	5	=	1280	POS		

Lab ID	Random 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
	CAPXSER23-2	P6												=	320	POS
	CAPXSER23-3	N1												=	5	NEG
	CAPXSER23-4	P7												=	640	POS
	CAPXSER23-5	N2												=	5	NEG
	CAPXSER23-6	P5			LSDV				Antiserum					=	640	POS
	CAPXSER23-7	P2			Neethling				Pirbright					=	1280	POS
	CAPXSER23-8	P4												=	1280	POS
	CAPXSER23-9	P6												=	320	POS
	CAPXSER23-10	P3												=	640	POS

10.1.3 SERO: IPMA

lab ID	Random 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	Secondary antibody (+ reference)	Dilution of secondary antibody	Cut-off	Pos control	Neg control	Operator	Raw data (Ct cp value)	Interpretation
97506	CAPXSER23-1	P5	In house	OA3.Ts	LSDV Neethling	0,02	0,02	100	R6F 45dpi	0,003	Anti bovine IgG (g) whole molecule - peroxidase produced in rabbit (Sigma A5295)	0,0001	0,02	300	1	=	300	POS
	CAPXSER23-2	P2														=	300	POS
	CAPXSER23-3	P1														=	300	POS
	CAPXSER23-4	P6														=	300	POS
	CAPXSER23-5	P6														=	300	POS
	CAPXSER23-6	N2														=	1	NEG
	CAPXSER23-7	N1														=	1	NEG
	CAPXSER23-8	P7														=	300	POS
	CAPXSER23-9	P4														=	300	POS
	CAPXSER23-10	P3														=	300	POS
97618	CAPXSER23-1	P1	EURL Capripox viruses Protocol	OA3.Ts	LSDV Neethling	0,02	50	100	Antiserum Pirbright	0,0025	Anti-Bovine Ig G Peroxidase antibody produced in rabbit A 5295 SIGMA	0,001	50	300	0	=	300	POS
	CAPXSER23-2	P6														=	300	POS
	CAPXSER23-3	N1														=	0	NEG
	CAPXSER23-4	P7														=	300	POS
	CAPXSER23-5	N2														=	0	NEG
	CAPXSER23-6	P5														=	300	POS
	CAPXSER23-7	P2														=	300	POS
	CAPXSER23-8	P4														=	300	POS
	CAPXSER23-9	P6														=	300	POS
	CAPXSER23-10	P3														=	300	POS

10.1.4 VIRO: RT-QPCR

lab ID	Random ID	Sample ID	Protocol ref / SOP	Producer Extraction protocol / kit	Name Extraction protocol / kit	Internal modifications to extraction protocol/kit	RT-PCR protocol / kit	Target of RT primer	Cut-off	Positive control	Negative control	Operator	Raw data (Ct cp value)	Interpretation	Comments per sample	General comments
97506	CAPXVIR23-1	BN1										=	50	NEG		
	CAPXVIR23-2	TP2										=	30,61	POS		
	CAPXVIR23-3	TP4										=	30,07	POS		
	CAPXVIR23-4	TN1										=	50	NEG		
	CAPXVIR23-5	TP3	Haegeman et al. (2013)	Other		Addition of EC to buffer B3	Haegeman et al. 2013	D5R	37	28,77	50	=	28,86	POS		Producer extraction kit: Machenerey nagel
	CAPXVIR23-6	TP1										=	30,67	POS		
	CAPXVIR23-7	BP1										=	28,29	POS		
	CAPXVIR23-8	BP1										=	29,18	POS		
	CAPXVIR23-9	TP6										=	32,94	POS		
	CAPXVIR23-10	TP5										=	29,84	POS		
97514	CAPXVIR23-1	TP3										=	26,3	POS		
	CAPXVIR23-2	TP4										=	0	NEG		Le Bio-T kit® Lumpy Skin Disease cible uniquement LSDV et non l'ensemble des Capripox virus. Les résultats de positivité et négativité sont donc uniquement pour LSDV.
	CAPXVIR23-3	TN1										=	0	NEG		
	CAPXVIR23-4	TP2										=	24,77	POS		
	CAPXVIR23-5	BN1	Bio-T kit® Lumpy Skin Disease	Biosellal	BioExtract Colum		Other	Other		25,16	0	=	0	NEG		
	CAPXVIR23-6	TP5										=	0	NEG		
	CAPXVIR23-7	BP1										=	26,82	POS		
	CAPXVIR23-8	BP1										=	27,41	POS		
CAPXVIR23-9	TP1										=	0	NEG			
CAPXVIR23-10	TP6										=	32,67	POS			
97600	CAPXVIR23-1	BP1										=	28,85	POS	Blood / cattle with skin lesions	
	CAPXVIR23-2	TP6										=	32,71	POS	skin lesions / cattle	
	CAPXVIR23-3	TP1										=	27,64	POS	skin lesions / goat	
	CAPXVIR23-4	TP3										=	32,48	POS	skin lesions / cattle	
	CAPXVIR23-5	TP2										=	32,05	POS	skin lesions /cattle	
	CAPXVIR23-6	BN1	Bowden et al. 2008 modified by FLI	Biosellal	Biosellal Superball	No	Other	ORF074 (P32)	45	32,22	45	>	45	NEG	blood / cattle with skin lesions	RT-PCR protocol / kit: Qiagen . Quanti Tect Multiplex PCR Kit NoRox
	CAPXVIR23-7	TP4										=	29,8	POS	skin lesions /sheep country with vaccine	
	CAPXVIR23-8	TN1										>	45	NEG	skin lesions / cattle	
	CAPXVIR23-9	BP1										=	28,74	POS	blood / cattle with skin lesions	
	CAPXVIR23-10	TP5										=	25,09	POS	skin lesions /sheep country with vaccine	
97602	CAPXVIR23-1	TP3	Bowden	Invitrogen/Thermo Fisher Scientific			Qiagen - MO-TO 45	ORF074 (P32)		22,4	0	=	29,74	POS		

lab ID	Random ID	Sample ID	Protocol ref / SOP	Producer Extraction protocol / kit	Name Extraction protocol / kit	Internal modifications to extraction protocol/kit	RT-PCR protocol / kit	Target of RT primer	Cut-off	Positive control	Negative control	Operator	Raw data (Ct cp value)	Interpretation	Comments per sample	General comments
	CAPXVIR23-2	TP6										=	32,05	POS		
	CAPXVIR23-3	TP4										=	29,45	POS		
	CAPXVIR23-4	BP1			MagMAX Core Nucleic Acid Purification Kit							=	28,88	POS		
	CAPXVIR23-5	BN1										=	0	NEG		
	CAPXVIR23-6	BP1										=	29,91	POS		
	CAPXVIR23-7	TP5										=	28,6	POS		
	CAPXVIR23-8	TN1										=	0	NEG		
	CAPXVIR23-9	TP2										=	28,83	POS		
	CAPXVIR23-10	TP1										=	29,36	POS		
	CAPXVIR23-1	BN1										=	0	NEG		
	CAPXVIR23-2	BP1										=	30,93	POS		
	CAPXVIR23-3	TP4										=	29,39	POS		
97603	CAPXVIR23-4	BP1	Bowden et al. 2008; Babiuk et al. 2008; SOP from Pirbright	Roche	MagNa Pure 96 DNA (Roche)	No	Invitrogen/Thermo Fisher Scientific -	ORF074 (P32)	Cq value<35 indicates a positive amplification result	25,35	0	=	29,65	POS		
	CAPXVIR23-5	TP3										=	27,46	POS		
	CAPXVIR23-6	TP6										=	33,39	POS		
	CAPXVIR23-7	TN1										=	0	NEG		
	CAPXVIR23-8	TP5										=	26,86	POS		
	CAPXVIR23-9	TP1										=	29,35	POS		
	CAPXVIR23-10	TP2										=	28,4	POS		
	CAPXVIR23-1	TP5										=		POS		
	CAPXVIR23-2	TP2										=		POS		
	CAPXVIR23-3	BN1										=		NEG		
	CAPXVIR23-4	TP4										=		POS		
97604	CAPXVIR23-5	TP3	6.3.51	Roche	MagNa Pure 96 DNA (Roche)		Qiagen - MO-TO 46	ORF074 (P32)	37			=		POS		
	CAPXVIR23-6	TN1										=		NEG		
	CAPXVIR23-7	TP1										=		POS		
	CAPXVIR23-8	BP1										=		POS		
	CAPXVIR23-9	TP6										=		POS		
	CAPXVIR23-10	BP1										=		POS		
	CAPXVIR23-1	TP1										=	28,59	POS		
	CAPXVIR23-2	BN1										>	45	NEG		
	CAPXVIR23-3	TN1										>	45	NEG		
	CAPXVIR23-4	BP1										=	27,78	POS		
97605 (1)	CAPXVIR23-5	TP4	Bowden et al. 2008	Roche	Roche-MP 96/ Viral NA SV	No	Other	ORF074 (P32)	No cut-off	27,64	45	=	29,93	POS		
	CAPXVIR23-6	TP3										=	30,03	POS		
	CAPXVIR23-7	TP2										=	30,57	POS		
	CAPXVIR23-8	TP6										=	33,26	POS		
	CAPXVIR23-9	TP5										=	25,68	POS		
	CAPXVIR23-10	BP1										=	27,33	POS		
	CAPXVIR23-1	TP1										=	29,45	POS		
	CAPXVIR23-2	BN1										>	45	NEG		
	CAPXVIR23-3	TN1										>	45	NEG		
	CAPXVIR23-4	BP1										=	28,47	POS		
97605 (2)	CAPXVIR23-5	TP4	Haegeman et al. 2013	Roche	Roche-MP 96/ Viral NA SV	No	Other	E3L	No cut-off	28,48	45	=	30,64	POS		
	CAPXVIR23-6	TP3										=	31,22	POS		
	CAPXVIR23-7	TP2										=	31,43	POS		
	CAPXVIR23-8	TP6										=	33,9	POS		
	CAPXVIR23-9	TP5										=	26,52	POS		
	CAPXVIR23-10	BP1										=	28,27	POS		
97606	CAPXVIR23-1	TP6		Qiagen						37,47	45	=	32,53	POS		

lab ID	Random ID	Sample ID	Protocol ref / SOP	Producer Extraction protocol / kit	Name Extraction protocol / kit	Internal modifications to extraction protocol/kit	RT-PCR protocol / kit	Target of RT primer	Cut-off	Positive control	Negative control	Operator	Raw data (Ct cp value)	Interpretation	Comments per sample	General comments	
	CAPXVIR23-2	TP3	DNA extraction and real time PCR (Bowden et al. 2008)		QIAamp 96 Virus QIAcube HT Kit		Bowden et al. 2008	ORF074 (P32)					=	29,94	POS		
	CAPXVIR23-3	TP5											=	26,29	POS		
	CAPXVIR23-4	BP1											=	28,14	POS		
	CAPXVIR23-5	TP1											=	30,26	POS		
	CAPXVIR23-6	TP4											=	29,18	POS		
	CAPXVIR23-7	TP2											=	31,89	POS		
	CAPXVIR23-8	TN1											=	0	NEG		
	CAPXVIR23-9	BP1											=	29,13	POS		
	CAPXVIR23-10	BN1											=	0	NEG		
97607 (1)	CAPXVIR23-1	TP1											P32	Qiagen	Qiagen DNAeasy Blood and tissue		
	CAPXVIR23-2	BP1	=	28,74	POS												
	CAPXVIR23-3	TP4	=	27,84	POS												
	CAPXVIR23-4	TP5	=	25,22	POS												
	CAPXVIR23-5	TP6	=	29,42	POS												
	CAPXVIR23-6	TP2	=	27,96	POS												
	CAPXVIR23-7	BN1	=	0	NEG												
	CAPXVIR23-8	BP1	=	27,64	POS												
	CAPXVIR23-9	TN1	=	0	NEG	0 = "No Ct"											
	CAPXVIR23-10	TP3	=	27,51	POS												
97607 (2)	CAPXVIR23-1	TP1	RPO-30	Qiagen	Qiagen DNAeasy Blood and tissue		Other	RPO30		30,31	0		=	27,12	POS	RT-PCR kit: Sso Advanced Universal probes	
	CAPXVIR23-2	BP1											=	28,46	POS		
	CAPXVIR23-3	TP4											=	28,33	POS		
	CAPXVIR23-4	TP5											=	25,98	POS		
	CAPXVIR23-5	TP6											=	29,42	POS		
	CAPXVIR23-6	TP2											=	27,89	POS		
	CAPXVIR23-7	BN1											=	0	NEG		0 = "No Ct"
	CAPXVIR23-8	BP1											=	27,67	POS		
	CAPXVIR23-9	TN1											=	0	NEG		0 = "No Ct"
	CAPXVIR23-10	TP3											=	27,54	POS		
97608 (1)	CAPXVIR23-1	BP1	Bowden et al 2008/Dietze et al 2018	Other			Bowden et al. 2008	ORF074 (P32)	40	28,8	40		=	26,8	POS	Extraction: Machery-Nagel - NucleoMagVet kit	
	CAPXVIR23-2	TP6											=	30,5	POS		
	CAPXVIR23-3	TP1											=	28,7	POS		
	CAPXVIR23-4	BN1											>	40	NEG		
	CAPXVIR23-5	BP1											=	27,2	POS		
	CAPXVIR23-6	TP5											=	27,3	POS		
	CAPXVIR23-7	TP4											=	28,5	POS		
	CAPXVIR23-8	TP2											=	29,3	POS		
	CAPXVIR23-9	TP3											=	28,8	POS		
	CAPXVIR23-10	TN1											>	40	NEG		
97608 (2)	CAPXVIR23-1	BP1	In house	Other			Other	Other	40	25,5	40		=	25,5	POS	Extraction: Machery-Nagel - NucleoMagVet kit	
	CAPXVIR23-2	TP6											=	30,1	POS		
	CAPXVIR23-3	TP1											=	27,7	POS		
	CAPXVIR23-4	BN1											>	40	NEG		
	CAPXVIR23-5	BP1											=	25,8	POS		
	CAPXVIR23-6	TP5											=	25,7	POS		
	CAPXVIR23-7	TP4											=	26,9	POS		
	CAPXVIR23-8	TP2											=	27,8	POS		
	CAPXVIR23-9	TP3											=	28,3	POS		

lab ID	Random ID	Sample ID	Protocol ref / SOP	Producer Extraction protocol / kit	Name Extraction protocol / kit	Internal modifications to extraction protocol/kit	RT-PCR protocol / kit	Target of RT primer	Cut-off	Positive control	Negative control	Operator	Raw data (Ct cp value)	Interpretation	Comments per sample	General comments
97609	CAPXVIR23-10	TN1	Bowden et al. 2008	Indical Bioscience	Indispin pathogen kit		Bowden et al. 2008	ORF074 (P32)	35	26,28	0		>	40	NEG	Real-Time PCR was performed using Quantifast Pathogen plus IC kit.
	CAPXVIR23-1	TP5											=	25,42	POS	
	CAPXVIR23-2	TP4											=	27,75	POS	
	CAPXVIR23-3	TP6											=	30,16	POS	
	CAPXVIR23-4	BP1											=	25,92	POS	
	CAPXVIR23-5	TP3											=	26,28	POS	
	CAPXVIR23-6	TP2											=	27,51	POS	
	CAPXVIR23-7	TP1											=	27,62	POS	
	CAPXVIR23-8	BN1											=	0	NEG	
	CAPXVIR23-9	TN1											=	0	NEG	
CAPXVIR23-10	BP1	=	25,29	POS												
97610	CAPXVIR23-1	TP3	Bowden et al. 2008	Indical Bioscience			Bowden et al. 2008	ORF074 (P32)	35	25,2	0		=	28,94	POS	
	CAPXVIR23-2	TN1											=	0	NEG	
	CAPXVIR23-3	BN1											=	0	NEG	
	CAPXVIR23-4	TP2											=	28,8	POS	
	CAPXVIR23-5	TP5											=	27,8	POS	
	CAPXVIR23-6	BP1											=	27,7	POS	
	CAPXVIR23-7	BP1											=	26,9	POS	
	CAPXVIR23-8	TP6											=	31,7	POS	
	CAPXVIR23-9	TP1											=	27,8	POS	
	CAPXVIR23-10	TP4											=	27,4	POS	
97611	CAPXVIR23-1	BP1	Haegeman et al. 2013	Qiagen	QIAamp Viral RNA Mini Kit		Other	E3L	CT 38	23,64	38		=	25,42	POS	Producer RT-PCR protocol / kit: Applied Biosystems Name RT-PCR protocol / kit: TaqMan Fast Virus 1-step Master Mix Lot:01271240 Exp: 30-11-23
	CAPXVIR23-2	TP5											=	20,95	POS	
	CAPXVIR23-3	TP3											=	26,69	POS	
	CAPXVIR23-4	TN1											>	38	NEG	
	CAPXVIR23-5	TP4											=	25,7	POS	
	CAPXVIR23-6	BP1											=	24,66	POS	
	CAPXVIR23-7	TP1											=	24,79	POS	
	CAPXVIR23-8	TP2											=	25,96	POS	
	CAPXVIR23-9	BN1											>	38	NEG	
	CAPXVIR23-10	TP6											=	26,62	POS	
97612	CAPXVIR23-1	TP3	ID.VET	Roche	High Pure Viral Nucleic Acid Kit		ID.VET - ID GENE® CAPRIPOX VIRUS TRIPLEX		38 Ct	29	39		=	25,8	POS	
	CAPXVIR23-2	TP1											=	25,3	POS	
	CAPXVIR23-3	TP5											=	24,7	POS	
	CAPXVIR23-4	BN1											>	39	NEG	
	CAPXVIR23-5	BP1											=	27,1	POS	
	CAPXVIR23-6	TP2											=	27,5	POS	
	CAPXVIR23-7	TP4											=	27	POS	
	CAPXVIR23-8	TN1											>	39	NEG	
	CAPXVIR23-9	TP6											=	31,1	POS	
	CAPXVIR23-10	BP1											=	27,9	POS	
97613	CAPXVIR23-1	TP6	Bowden et al. 2008				Other - Bowden et al. 2008		40	21,97	40		=	27,7	POS	
	CAPXVIR23-2	TP3											=	25,93	POS	
	CAPXVIR23-3	BP1											=	25,51	POS	
	CAPXVIR23-4	TN1											>	40	NEG	
	CAPXVIR23-5	TP4											=	24,63	POS	
	CAPXVIR23-6	TP1											=	25,56	POS	
	CAPXVIR23-7	BP1											=	24,61	POS	
	CAPXVIR23-8	TP2											=	25,77	POS	
	CAPXVIR23-9	TP5											=	22,91	POS	
	CAPXVIR23-10	BN1											>	40	NEG	

lab ID	Random ID	Sample ID	Protocol ref / SOP	Producer Extraction protocol / kit	Name Extraction protocol / kit	Internal modifications to extraction protocol/kit	RT-PCR protocol / kit	Target of RT primer	Cut-off	Positive control	Negative control	Operator	Raw data (Ct cp value)	Interpretation	Comments per sample	General comments
97614	CAPXVIR23-1	BP1	SOP G.72	Qiagen	QIAamp Viral RNA Mini Kit		Other - Bowden et al. 2008	RPO30		24	40		=	27	POS	
	CAPXVIR23-2	TP1											=	29,1	POS	
	CAPXVIR23-3	TP2											=	31	POS	
	CAPXVIR23-4	TP4											=	29,5	POS	
	CAPXVIR23-5	TP5											=	26,2	POS	
	CAPXVIR23-6	BP1											=	28,4	POS	
	CAPXVIR23-7	TP6											=	31,5	POS	
	CAPXVIR23-8	TN1											>	40	NEG	
	CAPXVIR23-9	TP3											=	29,5	POS	
	CAPXVIR23-10	BN1											>	40	NEG	
97616	CAPXVIR23-1	TP6	LSD PCR	Qiagen	QIAamp DNA mini kit		Path_ID qPCR	ORF074 (P32)	35	27,52	0		=	30,37	POS	
	CAPXVIR23-2	TP1											=	26,95	POS	
	CAPXVIR23-3	TP4											=	26,38	POS	
	CAPXVIR23-4	BP1											=	26,31	POS	
	CAPXVIR23-5	TN1											=	0	NEG	
	CAPXVIR23-6	BP1											=	26,68	POS	
	CAPXVIR23-7	TP2											=	25,26	POS	
	CAPXVIR23-8	BN1											=	0	NEG	
	CAPXVIR23-9	TP3											=	25,38	POS	
	CAPXVIR23-10	TP5											=	24,12	POS	
97617	CAPXVIR23-1	BP1	Bowden et al. 2008	Indical Bioscience		No	Other - Bowden et al. 2008	ORF074 (P32)	38	30,5	40		=	28,09	POS	
	CAPXVIR23-2	BP1											=	27,62	POS	
	CAPXVIR23-3	TP3											=	27,97	POS	
	CAPXVIR23-4	TP5											=	26,56	POS	
	CAPXVIR23-5	TN1											>	40	NEG	
	CAPXVIR23-6	TP2											=	28,99	POS	
	CAPXVIR23-7	TP6											=	34,65	POS	
	CAPXVIR23-8	TP1											=	33,11	POS	
	CAPXVIR23-9	BN1											>	40	NEG	
	CAPXVIR23-10	TP4											=	27,83	POS	
97618	CAPXVIR23-1	TP2	Bowden et al. 2008	Invitrogen/Thermo Fisher Scientific	Pure Link Genomic DNA Mini Kit (Invitrogen)		Other	ORF074 (P32)		16,88	0		=	27,81	POS	RT-PCR protocol/kit - Invitrogen by life technologies/Platinum Quantitative PCR SuperMix-UDG with ROX
	CAPXVIR23-2	BP1											=	26,63	POS	
	CAPXVIR23-3	BN1											=	0	NEG	
	CAPXVIR23-4	BP1											=	26,78	POS	
	CAPXVIR23-5	TN1											=	0	NEG	
	CAPXVIR23-6	TP3											=	26,71	POS	
	CAPXVIR23-7	TP4											=	27,58	POS	
	CAPXVIR23-8	TP5											=	26,91	POS	
	CAPXVIR23-9	TP1											=	26,3	POS	
	CAPXVIR23-10	TP6											=	31,67	POS	
97619	CAPXVIR23-1	TP4	Stubbs et al. 2012	Other	PerkinELMER chemagic Viral DNA/RNA 300 Kit special H96 using the chemagic 360 instrument using		Path_ID qPCR	ORF074 (P32)	38	33	38		=	29	POS	
	CAPXVIR23-2	TP2											=	29	POS	
	CAPXVIR23-3	TP6											=	31	POS	
	CAPXVIR23-4	TN1											<	38	NEG	
	CAPXVIR23-5	TP3											=	29	POS	
	CAPXVIR23-6	BP1											=	31	POS	
	CAPXVIR23-7	TP5											=	25	POS	
	CAPXVIR23-8	BN1											<	38	NEG	
	CAPXVIR23-9	TP1											=	29	POS	
	CAPXVIR23-10	BP1											=	31	POS	
97620	CAPXVIR23-1	TP3			KingFisher Flex					23,6	-	=	30,75	POS		

lab ID	Random ID	Sample ID	Protocol ref / SOP	Producer Extraction protocol / kit	Name Extraction protocol / kit	Internal modifications to extraction protocol/kit	RT-PCR protocol / kit	Target of RT primer	Cut-off	Positive control	Negative control	Operator	Raw data (Ct cp value)	Interpretation	Comments per sample	General comments
	CAPXVIR23-2	BP1										=	30,29	POS		
	CAPXVIR23-3	TP6	Bowden TR									=	33,17	POS		
	CAPXVIR23-4	TP4	Babiuk SL Parkyn GR Copps JS Boyle DB									=	32,96	POS		
	CAPXVIR23-5	TP2	(2008): Capripoxvirus tissue tropism and shedding: A quantitative study in experimentally infected sheep and goats.	Invitrogen/Thermo Fisher Scientific			Qiagen - MO-TO 45	ORF074 (P32)				=	31,48	POS		
	CAPXVIR23-6	TN1										=	-	NEG		result in Ct value (for positive samples)
	CAPXVIR23-7	BN1										=	-	NEG		
	CAPXVIR23-8	TP1										=	31,32	POS		
	CAPXVIR23-9	BP1	Virology 371									=	29,43	POS		
	CAPXVIR23-10	TP5	(2008) 380–393									=	27,79	POS		
	CAPXVIR23-1	TP3										=	23	POS		
	CAPXVIR23-2	TP6										=	27,4	POS		
97621	CAPXVIR23-3	TP5			Qiagen/Magattract cador Pathogen Kit							=	23,4	POS		
	CAPXVIR23-4	TN1	Bowden et al. 2008	Qiagen			Path_ID qPCR	ORF074 (P32)	35	35	40	>	40	NEG		
	CAPXVIR23-5	BP1										=	23,7	POS		
	CAPXVIR23-6	BN1										>	40	NEG		
	CAPXVIR23-7	BP1										=	24,2	POS		
	CAPXVIR23-8	TP1										=	25,3	POS		
	CAPXVIR23-9	TP4										=	24	POS		
	CAPXVIR23-10	TP2										=	23,8	POS		
	CAPXVIR23-1	BP1										=	27,7	POS		
	CAPXVIR23-2	BP1										=	28,06	POS		
	CAPXVIR23-3	TP3										=	27,64	POS		
	CAPXVIR23-4	TN1										=	-	NEG		
97622	CAPXVIR23-5	BN1	In House	Roche	Roche-MP 96/ Viral NA SV		Roche - Roche kit	ORF074 (P32)	Ct 36 - 45 is considered doubtfull	27,45	-	=	-	NEG		An in-house developed qPCR was performed.
	CAPXVIR23-6	TP5										=	28,71	POS		
	CAPXVIR23-7	TP4										=	28,58	POS		
	CAPXVIR23-8	TP1										=	27,11	POS		
	CAPXVIR23-9	TP2										=	30,17	POS		
	CAPXVIR23-10	TP6										=	38,65	POS	Doubtfull	
	CAPXVIR23-1	BN1										=	0	NEG		
	CAPXVIR23-2	TP1										=	29	POS		
	CAPXVIR23-3	BP1										=	28,6	POS		
97624	CAPXVIR23-4	TP5			Pure Link Genomic DNA Mini Kit (Invitrogen)		Invitrogen/Thermo Fisher Scientific					=	26,4	POS		
	CAPXVIR23-5	TN1	IAEA Bowden et.al	Invitrogen/Thermo Fisher Scientific								=	0	NEG		
	CAPXVIR23-6	TP3					Invitrogen/Thermo Fisher Scientific kit	ORF074 (P32)	38	21	0	=	38,3	POS		
	CAPXVIR23-7	TP4										=	27,6	POS		
	CAPXVIR23-8	BP1										=	30	POS		
	CAPXVIR23-9	TP6										=	34,5	POS		
	CAPXVIR23-10	TP2										=	25,5	POS		
97630	CAPXVIR23-1	TP6			ID Gene Spin							=	34,033	POS		PerfeCTa® qPCR
	CAPXVIR23-2	BP1	Bowden et al. 2008	ID VET	Universal Extraction Kit		Other			40	29,62	45	=	31,167	POS	ToughMix®
	CAPXVIR23-3	BN1										>	45	NEG		QuantaBio

lab ID	Random ID	Sample ID	Protocol ref / SOP	Producer Extraction protocol / kit	Name Extraction protocol / kit	Internal modifications to extraction protocol/kit	RT-PCR protocol / kit	Target of RT primer	Cut-off	Positive control	Negative control	Operator	Raw data (Ct cp value)	Interpretation	Comments per sample	General comments
	CAPXVIR23-4	TP2										=	31,309	POS		
	CAPXVIR23-5	TP4										=	31,267	POS		
	CAPXVIR23-6	TP3										=	31,942	POS		
	CAPXVIR23-7	TP5										=	26,761	POS		
	CAPXVIR23-8	TP1										=	30,803	POS		
	CAPXVIR23-9	BP1										=	30,38	POS		
	CAPXVIR23-10	TN1										>	45	NEG		
97631	CAPXVIR23-1	TP6										=	29,97	POS		
	CAPXVIR23-2	TP2										=	26,94	POS		
	CAPXVIR23-3	TP4										=	24,54	POS		
	CAPXVIR23-4	TP3										=	27,44	POS		
	CAPXVIR23-5	TP1	Bowden et al	Roche	High Pure Viral Nucleic Acid Kit		Qiagen - MO-TO 46		38	28,92	-	=	26,4	POS		
	CAPXVIR23-6	BP1										=	26,71	POS		
	CAPXVIR23-7	BN1										=		NEG		
	CAPXVIR23-8	BP1										=	24,84	POS		
	CAPXVIR23-9	TP5										=	22,99	POS		
	CAPXVIR23-10	TN1										=		NEG		
97632	CAPXVIR23-1	BP1										=	29,4	POS		
	CAPXVIR23-2	TP4										=	25,9	POS		
	CAPXVIR23-3	TN1	Detection of Capripox viral DNA by real time PCR based on Bowden et al 2008	Sacace Biotechnologies	Sacace-viral Nucleic acid Extraction	N/A	Path_ID qPCR	ORF074 (P32)	38	26,4	0	=	0	NEG		
	CAPXVIR23-4	TP3										=	28,3	POS		
	CAPXVIR23-5	TP2										=	27,8	POS		
	CAPXVIR23-6	BP1										=	30,4	POS		
	CAPXVIR23-7	TP5										=	23,6	POS		
	CAPXVIR23-8	BN1										=	0	NEG		
	CAPXVIR23-9	TP1										=	26,3	POS		
	CAPXVIR23-10	TP6										=	32,8	POS		
97634	CAPXVIR23-1	TP5										=	25,89	POS		
	CAPXVIR23-2	TP6										=	33,22	POS		
	CAPXVIR23-3	BN1										>	45	NEG		
	CAPXVIR23-4	TP2										=	30,43	POS		
	CAPXVIR23-5	TP3	Bowden	Biosellal	Biosellal Superball	No	Bowden et al. 2008		40	27,83	45	=	30,26	POS		
	CAPXVIR23-6	TP4										=	29	POS		
	CAPXVIR23-7	TP1										=	27,32	POS		
	CAPXVIR23-8	BP1										=	27,5	POS		
	CAPXVIR23-9	TN1										>	45	NEG		
	CAPXVIR23-10	BP1										=	27,58	POS		
97636	CAPXVIR23-1	TP3										=	27	POS		
	CAPXVIR23-2	TP6										=	31	POS		
	CAPXVIR23-3	TP5										=	28	POS		
	CAPXVIR23-4	BP1	identification of the genome of Lumpy Skin Diseases virus	Indical Bioscience	Indispin pathogen kit		Invitrogen/Thermo Fisher Scientific					=	30	POS		
	CAPXVIR23-5	TP1								28	0	=	29	POS		
	CAPXVIR23-6	TN1					Invitrogen/Thermo Fisher Scientific kit					=	0	NEG		
	CAPXVIR23-7	BP1										=	30	POS		
	CAPXVIR23-8	TP2										=	28	POS		
	CAPXVIR23-9	BN1										=	0	NEG		
	CAPXVIR23-10	TP4										=	28	POS		
97637	CAPXVIR23-1	BN1										=	-	NEG		
	CAPXVIR23-2	TP4	Bowden et. al. 2008	Roche	High Pure Viral Nucleic Acid Kit		Roche - Roche kit	ORF074 (P32)	38	17,87	-	=	29,42	POS		
	CAPXVIR23-3	TN1										=	-	NEG		
	CAPXVIR23-4	TP1										=	28,35	POS		

lab ID	Random ID	Sample ID	Protocol ref / SOP	Producer Extraction protocol / kit	Name Extraction protocol / kit	Internal modifications to extraction protocol/kit	RT-PCR protocol / kit	Target of RT primer	Cut-off	Positive control	Negative control	Operator	Raw data (Ct cp value)	Interpretation	Comments per sample	General comments
	CAPXVIR23-5	BP1										=	31,17	POS		
	CAPXVIR23-6	TP3										=	30,35	POS		
	CAPXVIR23-7	TP6										=	33,49	POS		
	CAPXVIR23-8	TP5										=	28,45	POS		
	CAPXVIR23-9	BP1										=	29,28	POS		
	CAPXVIR23-10	TP2										=	30,24	POS		
	CAPXVIR23-1	TP5										=	30,41	POS		
	CAPXVIR23-2	TP3										=	28,18	POS		
	CAPXVIR23-3	TP1										=	28,55	POS		
	CAPXVIR23-4	TN1										=	0	NEG		
97642	CAPXVIR23-5	TP2	Timothy R.Bowden (2008)	Other			Bowden et al. 2008	ORF074 (P32)	40	25,9	40	=	27,76	POS		
	CAPXVIR23-6	TP4										=	29,94	POS		
	CAPXVIR23-7	BN1										=	0	NEG		
	CAPXVIR23-8	TP6										=	29,03	POS		
	CAPXVIR23-9	BP1										=	27,26	POS		
	CAPXVIR23-10	BP1										=	26,64	POS		
	CAPXVIR23-1	TP3										=	27,498	POS		
	CAPXVIR23-2	TP6										=	30,673	POS		
	CAPXVIR23-3	TP2										=	27,29	POS		
	CAPXVIR23-4	TP5										=	26,962	POS		
97643	CAPXVIR23-5	BP1	NVR-SOP-20 Bowden et al 2008	Invitrogen/Thermo Fisher Scientific	MagMAX Core Nucleic Acid Purification Kit		Bowden et al. 2008	ORF074 (P32)	NA	21,03	45	=	26,547	POS		
	CAPXVIR23-6	BP1										=	29,083	POS		
	CAPXVIR23-7	BN1										>	45	NEG		
	CAPXVIR23-8	TP4										=	28,41	POS		
	CAPXVIR23-9	TP1										=	26,002	POS		
	CAPXVIR23-10	TN1										>	45	NEG		
	CAPXVIR23-1	TP6										=	36,6	POS	Positive for capripoxvirus DNA	
	CAPXVIR23-2	BN1										>	45	NEG	Negative for capripoxvirus DNA	
	CAPXVIR23-3	TP4										=	31,8	POS	Positive for capripoxvirus DNA	
	CAPXVIR23-4	TP2			Ambion MagMAX-96 Viral RNA Isolation Kit							=	30,5	POS	Positive for capripoxvirus DNA	
97647 (1)	CAPXVIR23-5	TP1	TM-039	Thermo Fisher Scientific		N/A	Bowden et al. 2008	ORF074 (P32)	Ct values: <40 POS 40-45 IND >45 NEG	20,3	45	=	29,9	POS	Positive for capripoxvirus DNA	RT-PCR kit: AgPath-ID One-Step RT-PCR Reagents (by Thermo Fisher Scientific). The Bowden et al. 2008 qPCR is a pan-capripoxvirus assay that does not differentiate between SPPV/ GTPV and LSDV DNA (wild type or vaccine).
	CAPXVIR23-6	BP1										=	31,6	POS	Positive for capripoxvirus DNA	
	CAPXVIR23-7	BP1										=	30,5	POS	Positive for capripoxvirus DNA	
	CAPXVIR23-8	TN1										>	45	NEG	Negative for capripoxvirus DNA	
	CAPXVIR23-9	TP5										=	28	POS	Positive for capripoxvirus DNA	

lab ID	Random ID	Sample ID	Protocol ref / SOP	Producer Extraction protocol / kit	Name Extraction protocol / kit	Internal modifications to extraction protocol/kit	RT-PCR protocol / kit	Target of RT primer	Cut-off	Positive control	Negative control	Operator	Raw data (Ct cp value)	Interpretation	Comments per sample	General comments	
	CAPXVIR23-10	TP3											=	30,1	POS	Positive for capripoxvirus DNA	
	CAPXVIR23-1	TP6											>	45	NEG	Negative for LSDV DNA	Additional screening assay used: Alexander Sprygin et al. BMC Res Notes (2019) 12:371 (a pan-LSDV assay targeting LSDV ORF044 that does not differentiate between WT and vaccine LSDV strains). CAPXVIR23-6 tested as indeterminate for LSDV DNA in the Alexander Sprygin assay. This sample tested positive in the Bowden et al. 2008 pan-capripoxvirus assay and positive for LSDV (WT) DNA in the Wolff et al. 2021 LSDV duplex (Vac/WT) assay.
	CAPXVIR23-2	BN1											>	45	NEG	Negative for LSDV DNA	
	CAPXVIR23-3	TP4											>	45	NEG	Negative for LSDV DNA	
97647 (2)	CAPXVIR23-4	TP2	TM-039	Thermo Fisher Scientific	Ambion MagMAX-96 Viral RNA Isolation Kit	N/A	AgPath-ID One-Step RT-PCR Reagents (by Thermo Fisher Scientific)		Ct values: <40 POS 40-45 IND >45 NEG	31,3	45	=	34,6	POS	Positive for LSDV DNA		
	CAPXVIR23-5	TP1											>	45	NEG	Negative for LSDV DNA	
	CAPXVIR23-6	BP1											=	42,3	NI (not interpretable)	Indeterminate result	
	CAPXVIR23-7	BP1											=	38,4	POS	Positive for LSDV DNA	
	CAPXVIR23-8	TN1											>	45	NEG		
	CAPXVIR23-9	TP5											=	37,5	POS	Positive for LSDV DNA	

lab ID	Random ID	Sample ID	Protocol ref / SOP	Producer Extraction protocol / kit	Name Extraction protocol / kit	Internal modifications to extraction protocol/kit	RT-PCR protocol / kit	Target of RT primer	Cut-off	Positive control	Negative control	Operator	Raw data (Ct cp value)	Interpretation	Comments per sample	General comments
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CAPXVIR23-10 TP3

=

33,5

POS

Positive for LSDV DNA

positive in the Alexander Sprygin assay this is considered a false positive (cross reactive) result for LSDV. Based on the qPCR methods used (including the Wolff et al. 2021 duplex SPPV/GTPV assay) and limited sequence data generated from ORF074 this sample is instead considered to be positive for SPPV DNA (noting that differentiation between WT and vaccine virus was not undertaken).

10.1.5 VIRO: SPECIES DIFFERENTIATION

lab ID	Random ID	Sample ID	Protocol ref / SOP	Producer Extraction protocol / kit	Name Extraction protocol / kit	Internal modifications to the extraction protocol/kit	RT-PCR protocol / kit	Methodology	Cut-off	Positive control	Negative control	Operator	Raw data (Ct cp value)	Interpretation	Comments per sample	General comments		
97506	CAPXVIR2 3-1	BN1	Wolff et al. 2021	Other			Other - Wolff et al. 2021 (Both duplexes)		40	22,09	50	=	50	NEG				
	CAPXVIR2 3-2	TP2											=	30,54			LSDV	
	CAPXVIR2 3-3	TP4											=	30,55			SPPV	
	CAPXVIR2 3-4	TN1											=	50			NEG	
	CAPXVIR2 3-5	TP3											=	50			LSDV	Possible recombinant
	CAPXVIR2 3-6	TP1											=	36,52			GTPV	See Primary PCR
	CAPXVIR2 3-7	BP1											=	28,7			LSDV	
	CAPXVIR2 3-8	BP1											=	29,13			LSDV	
	CAPXVIR2 3-9	TP6											=	32,67			LSDV	
	CAPXVIR2 3-10	TP5											=	30,23			SPPV	
97600	CAPXVIR2 3-1	BP1	Lamien et al. 2011	Biosellal	Biosellal Superball	no	Other - Lamien et al. 2017	Roche - 2 probes system	14,38	45	=	18,33	LSDV	cattle pos.				
	CAPXVIR2 3-2	TP6										=	19,9	LSDV			cattle pos.	
	CAPXVIR2 3-3	TP1										=	17,43	GTPV			goat pos.	
	CAPXVIR2 3-4	TP3										=	19,62	LSDV			cattle pos.	
	CAPXVIR2 3-5	TP2										=	19,99	LSDV			cattle pos.	
	CAPXVIR2 3-6	BN1										>	45	NEG			cattle neg.	
	CAPXVIR2 3-7	TP4										=	28,67	SPPV			sheep pos.	
	CAPXVIR2 3-8	TN1										>	45	NEG			cattle neg.	
	CAPXVIR2 3-9	BP1										=	18,08	LSDV			cattle pos.	
	CAPXVIR2 3-10	TP5										=	25,3	SPPV			sheep pos	
97602	CAPXVIR2 3-1	TP3	Lamien	Invitrogen/ThermoFisher Scientific			Qiagen - MO-TO 47		30,23	0	=	30,05	LSDV					
	CAPXVIR2 3-2	TP6										=	35,43			LSDV		
	CAPXVIR2 3-3	TP4										=	32,14			SPPV		
	CAPXVIR2 3-4	BP1										=	30,39			LSDV		
	CAPXVIR2 3-5	BN1										=	0			NEG		
	CAPXVIR2 3-6	BP1										=	30,28			LSDV		
	CAPXVIR2 3-7	TP5										=	30,55			SPPV		
	CAPXVIR2 3-8	TN1										=	0			NEG		

lab ID	Random ID	Sample ID	Protocol ref / SOP	Producer Extraction protocol / kit	Name Extraction protocol / kit	Internal modifications to the extraction protocol/kit	RT-PCR protocol / kit	Methodology	Cut-off	Positive control	Negative control	Operator	Raw data (Ct cp value)	Interpretation	Comments per sample	General comments											
	CAPXVIR2 3-9	TP2										=	33,61	LSDV													
	CAPXVIR2 3-10	TP1										=	30,08	GTPV													
97603	CAPXVIR2 3-1	BN1	Lamien et al. 2011	Roche		no	Other - Lamien et al. 2011	Classical PCR amplification of a portion of the RP030 gene: conventional	151 bp product for SPPV 172 bp for GTPV and LSDV	172	0		=	0	NEG												
	CAPXVIR2 3-2	BP1											=	172	LSDV												
	CAPXVIR2 3-3	TP4											=	151	SPPV												
	CAPXVIR2 3-4	BP1											=	172	LSDV												
	CAPXVIR2 3-5	TP3											=	172	LSDV												
	CAPXVIR2 3-6	TP6											=	172	LSDV												
	CAPXVIR2 3-7	TN1											=	0	NEG												
	CAPXVIR2 3-8	TP5											=	151	SPPV												
	CAPXVIR2 3-9	TP1											=	172	GTPV												
	CAPXVIR2 3-10	TP2											=	172	LSDV												
	CAPXVIR2 3-1	TP5											=		SPPV												
	CAPXVIR2 3-2	TP2											=		LSDV												
	CAPXVIR2 3-3	BN1											=		NEG												
CAPXVIR2 3-4	TP4	=		SPPV																							
CAPXVIR2 3-5	TP3	6.3.51	Roche			Qiagen - MO-TO 47							=		LSDV												
CAPXVIR2 3-6	TN1												=		NEG												
CAPXVIR2 3-7	TP1												=		GTPV												
CAPXVIR2 3-8	BP1												=		LSDV												
CAPXVIR2 3-9	TP6												=		LSDV												
CAPXVIR2 3-10	BP1												=		LSDV												
CAPXVIR2 3-1	TP1												GPCR	QIAGEN	Qiagen DNAeasy Blood and tissue		Other	GPCR		31,87	0			=	0	NEG	(0 and NEG)=("No Ct" and "LSDV negative") respectively
CAPXVIR2 3-2	BP1																							=	29,32	LSDV	
CAPXVIR2 3-3	TP4																							=	0	NEG	(0 and NEG)=("No Ct" and "LSDV negative") respectively
CAPXVIR2 3-4	TP5																							=	0	NEG	(0 and NEG)=("No Ct" and "LSDV negative") respectively
CAPXVIR2 3-5	TP6	=	30,71	LSDV																							
CAPXVIR2 3-6	TP2	=	28,36	LSDV																							

lab ID	Random ID	Sample ID	Protocol ref / SOP	Producer Extraction protocol / kit	Name Extraction protocol / kit	Internal modifications to the extraction protocol/kit	RT-PCR protocol / kit	Methodology	Cut-off	Positive control	Negative control	Operator	Raw data (Ct cp value)	Interpretation	Comments per sample	General comments
	CAPXVIR2 3-7	BN1										=	0	NEG	(0 and NEG)=("No Ct" and "LSDV negative") respectively	
	CAPXVIR2 3-8	BP1										=	28,77	LSDV		
	CAPXVIR2 3-9	TN1										=	0	NEG	(0 and NEG)=("No Ct" and "LSDV negative") respectively	
	CAPXVIR2 3-10	TP3										=	27,43	LSDV		
	CAPXVIR2 3-1	BP1										=	27,4	LSDV		
	CAPXVIR2 3-2	TP6										=	30,7	LSDV		
	CAPXVIR2 3-3	TP1										=	31,1	GTPV		
	CAPXVIR2 3-4	BN1										>	40	NEG		
	CAPXVIR2 3-5	BP1										=	27,3	LSDV		Extraction: Machery-Nagel - NucleoMagVet kit
9760 8 (1)	CAPXVIR2 3-6	TP5	Wolff et al 2021	Other			Other - Wolff et al. 2021 (Both duplexes)	Wolff et al 2021	40	28,7	40	=	27,6	SPPV		
	CAPXVIR2 3-7	TP4			=	29,4						SPPV				
	CAPXVIR2 3-8	TP2			=	29,7						LSDV				
	CAPXVIR2 3-9	TP3			>	40						LSDV		LSDV-Recombinant because cattle sample and both LSDV assays (field and vaccine assay) reacted negative		
	CAPXVIR2 3-10	TN1										>	40	NEG		
	CAPXVIR2 3-1	BP1										=	28,3	LSDV		
	CAPXVIR2 3-2	TP6										=	31,6	LSDV		
	CAPXVIR2 3-3	TP1										>	40	NEG		
	CAPXVIR2 3-4	BN1										>	40	NEG		
9760 8 (2)	CAPXVIR2 3-5	BP1	Haegeman et al 2023	Other			Other - Haegeman et al. 2016	Haegeman et al 2023	40	26,7	40	=	27,6	LSDV		Extraction: Machery-Nagel - NucleoMagVet kit
	CAPXVIR2 3-6	TP5			>	40						NEG				
	CAPXVIR2 3-7	TP4			>	40						NEG				
	CAPXVIR2 3-8	TP2			=	30,9						LSDV				
	CAPXVIR2 3-9	TP3			=	31						LSDV				
	CAPXVIR2 3-10	TN1			>	40						NEG				
9760 9 (1)	CAPXVIR2 3-1	TP5	Lamien et al 2011	Indical Bioscience			Other - Lamien et al. 2011			0	0	=	0	SPPV	SPPV amplicon length	Extraction was performed manually using Indispin Pathogen kit.
	CAPXVIR2 3-2	TP4			=	0		SPPV	SPPV amplicon length							

lab ID	Random ID	Sample ID	Protocol ref / SOP	Producer Extraction protocol / kit	Name Extraction protocol / kit	Internal modifications to the extraction protocol/kit	RT-PCR protocol / kit	Methodology	Cut-off	Positive control	Negative control	Operator	Raw data (Ct cp value)	Interpretation	Comments per sample	General comments												
97609 (2)	CAPXVIR2 3-3	TP6	Partial GPCR (2F/3R) PCR and Sequencing	Indical Bioscience			Other - Galaye et al. 2015			0	0		0	LSDV	LSDV/GTPV amplicon length	Two positive controls were used (LSDV/GTPV and SPPV).												
	CAPXVIR2 3-4	BP1												LSDV	LSDV/GTPV amplicon length													
	CAPXVIR2 3-5	TP3												LSDV	LSDV/GTPV amplicon length		PCR kit: Invitrogen Platinum polymerase											
	CAPXVIR2 3-6	TP2												LSDV	LSDV/GTPV amplicon length													
	CAPXVIR2 3-7	TP1												GTPV	LSDV/GTPV amplicon length													
	CAPXVIR2 3-8	BN1												NEG														
	CAPXVIR2 3-9	TN1												NEG														
	CAPXVIR2 3-10	BP1												LSDV	LSDV/GTPV amplicon length													
	CAPXVIR2 3-1	TP5												SPPV	LSDV/GTPV amplicon length 100% similarity to Sheeppox virus isolate Saudi Arabia vaccine - MN072627.1 "99.89% similarity to Sheeppox virus strain SPPV/SA5/2016-MG232392.1 99.89% similarity to Sheeppox virus isolate Pendik -MN072629.1"													
	CAPXVIR2 3-2	TP4												SPPV	100% similarity to Lumpy skin disease virus isolate LSDV/02/KASH/IND/2022-OQ588787.1 100% similarity Lumpy skin disease virus isolate pendik (2014) - MN995838.1													Extraction was performed manually using Indispin Pathogen kit. Conventional PCR targeting GPCR gene. PCR kit: Invitrogen Platinum polymerase
	CAPXVIR2 3-3	TP6												LSDV	100% similarity to Lumpy skin disease virus isolate 155920/2012-KX894508.1													
CAPXVIR2 3-4	BP1	LSDV	100% similarity to Lumpy skin disease virus isolate LSDV/02/KASH/IND/2022-OQ588787.1 100% similarity Lumpy skin disease virus isolate pendik (2014) - MN995838.1 100% similarity to Lumpy skin disease virus isolate 155920/2012-KX894508.1																									

lab ID	Random ID	Sample ID	Protocol ref / SOP	Producer Extraction protocol / kit	Name Extraction protocol / kit	Internal modifications to the extraction protocol/kit	RT-PCR protocol / kit	Methodology	Cut-off	Positive control	Negative control	Operator	Raw data (Ct cp value)	Interpretation	Comments per sample	General comments
	CAPXVIR2-3-5	TP3										=	0	LSDV	Among others 100% similarity to Lumpy skin disease virus strain LSDV/HongKong/2020-MW732649.1 (recombinant strain)	
	CAPXVIR2-3-6	TP2										=	0	LSDV	00% similarity to Lumpy skin disease virus isolate LSDV/02/KASH/IND/2022-OQ588787.1 100% similarity Lumpy skin disease virus isolate pendik (2014) - MN995838.1 100% similarity to Lumpy skin disease virus isolate 155920/2012-KX894508.1	
	CAPXVIR2-3-7	TP1										=	0	GTPV	100% similarity to Goatpox virus strain Gorgan -KX576657.1 100% similarity to Goatpox virus G20-LKV-AY077836.1	

lab ID	Random ID	Sample ID	Protocol ref / SOP	Producer Extraction protocol / kit	Name Extraction protocol / kit	Internal modifications to the extraction protocol/kit	RT-PCR protocol / kit	Methodology	Cut-off	Positive control	Negative control	Operator	Raw data (Ct cp value)	Interpretation	Comments per sample	General comments
	CAPXVIR2 3-8	BN1										=	0	NEG		
	CAPXVIR2 3-9	TN1										=	0	NEG		
	CAPXVIR2 3-10	BP1										=	0	LSDV		
	CAPXVIR2 3-1	TP3										=		LSDV		
	CAPXVIR2 3-2	TN1										=		NEG		100% similarity to Lumpy skin disease virus isolate
	CAPXVIR2 3-3	BN1										=		NEG		LSDV/02/KASH/IND/2023-OQ588787.1
	CAPXVIR2 3-4	TP2										=		LSDV		
97610	CAPXVIR2 3-5	TP5	Lamien CE et al	Indical Bioscience			Other - Lamien et al. 2011		35			=		SPPV		100% similarity Lumpy skin disease virus isolate
	CAPXVIR2 3-6	BP1										=		LSDV		pendik (2014) - MN995838.1
	CAPXVIR2 3-7	BP1										=		LSDV		100% similarity to Lumpy skin disease virus isolate
	CAPXVIR2 3-8	TP6										=		LSDV		155920/2012-KX894508.1
	CAPXVIR2 3-9	TP1										=		GTPV		
	CAPXVIR2 3-10	TP4										=		SPPV		
	CAPXVIR2 3-1	BP1										=	75,8	LSDV		
	CAPXVIR2 3-2	TP5										=	74,8	SPPV		HRM Reagent: Quantitect SYBR Green PCR Kit
	CAPXVIR2 3-3	TP3										=	75,6	LSDV		
	CAPXVIR2 3-4	TN1										=		NEG		Methodology: Melt curve using SYBR Green chemistry
97611	CAPXVIR2 3-5	TP4	Galaye et al 2017	QIAGEN			Other	Melt curve using SYBR green chemistry		75,73	70	=	74,8	SPPV		Ref Methodology: Esayas Galaye et al 2017
	CAPXVIR2 3-6	BP1										=	75,8	LSDV		
	CAPXVIR2 3-7	TP1										=	74,4	GTPV		
	CAPXVIR2 3-8	TP2										=	75,8	LSDV		The target of the RT primer: RPO147
	CAPXVIR2 3-9	BN1										=		NEG		
	CAPXVIR2 3-10	TP6										=	76	LSDV		
	CAPXVIR2 3-1	TP3										=	26,2	LSDV		
97612 (1)	CAPXVIR2 3-2	TP1	Galaye et al. 2017	Roche			Other - Galaye et al. 2017	PCR HRM		30	32	=	24,04	GTPV		To confirm LSDV positive samples we added method 2: IZS TE B456.1 SOP021
	CAPXVIR2 3-3	TP5										=	20,9	SPPV		

lab ID	Random ID	Sample ID	Protocol ref / SOP	Producer Extraction protocol / kit	Name Extraction protocol / kit	Internal modifications to the extraction protocol/kit	RT-PCR protocol / kit	Methodology	Cut-off	Positive control	Negative control	Operator	Raw data (Ct cp value)	Interpretation	Comments per sample	General comments
	CAPXVIR2 3-4	BN1										=	32,6	NEG		
	CAPXVIR2 3-5	BP1										=	23,01	LSDV		
	CAPXVIR2 3-6	TP2										=	26,14	LSDV		
	CAPXVIR2 3-7	TP4										=	23,56	SPPV		
	CAPXVIR2 3-8	TN1										=	33,48	NEG		
	CAPXVIR2 3-9	TP6										=	25,56	LSDV		
	CAPXVIR2 3-10	BP1										=	23,3	LSDV		
	CAPXVIR2 3-1	TP3										=	27,7	LSDV		
	CAPXVIR2 3-2	TP1										=	38	NEG	This assay is specific only to LSDV	
	CAPXVIR2 3-3	TP5										=	38	NEG	This assay is specific only to LSDV	
	CAPXVIR2 3-4	BN1										=	38	NEG		IZS TE B456.1 SOP021.
9761 2 (2)	CAPXVIR2 3-5	BP1	IZS TE B456.1 SOP021				Other - Other			26,1	38	=	27,7	LSDV		This SOP uses Bio-T kit Lumpy Skin Disease PCR real time kit (Biosellal) to confirm LSDV positive samples.
	CAPXVIR2 3-6	TP2										=	28,68	LSDV		
	CAPXVIR2 3-7	TP4										=	38	NEG	This assay is specific only to LSDV	
	CAPXVIR2 3-8	TN1										=	38	NEG		
	CAPXVIR2 3-9	TP6										=	34	LSDV		
	CAPXVIR2 3-10	BP1										=	26,7	LSDV		
	CAPXVIR2 3-1	TP6										=	31,47	LSDV		
	CAPXVIR2 3-2	TP3										=	29,86	LSDV		
	CAPXVIR2 3-3	BP1										=	29,11	LSDV		
	CAPXVIR2 3-4	TN1										>	40	NEG		
9761 3	CAPXVIR2 3-5	TP4	Lamien et al. 2011				Lamien et al. 2011			27,72	40	=	35,51	SPPV		
	CAPXVIR2 3-6	TP1										=	29,39	GTPV		
	CAPXVIR2 3-7	BP1										=	28,13	LSDV		
	CAPXVIR2 3-8	TP2										=	29,69	LSDV		
	CAPXVIR2 3-9	TP5										=	36,13	SPPV		
	CAPXVIR2 3-10	BN1										>	40	NEG		
9761 4	CAPXVIR2 3-1	BP1	SOP G.72	QIAGEN			Other - Lamien et al. 2011			25		=	35	LSDV		
	CAPXVIR2 3-2	TP1										=	33	GTPV		

lab ID	Random ID	Sample ID	Protocol ref / SOP	Producer Extraction protocol / kit	Name Extraction protocol / kit	Internal modifications to the extraction protocol/kit	RT-PCR protocol / kit	Methodology	Cut-off	Positive control	Negative control	Operator	Raw data (Ct cp value)	Interpretation	Comments per sample	General comments	
	CAPXVIR2 3-3	TP2										=	32	LSDV			
	CAPXVIR2 3-4	TP4										=	34	SPPV			
	CAPXVIR2 3-5	TP5										=	32	SPPV			
	CAPXVIR2 3-6	BP1										=	33	NI (not interpretable)			
	CAPXVIR2 3-7	TP6										=	32	LSDV			
	CAPXVIR2 3-8	TN1										>	40	NEG			
	CAPXVIR2 3-9	TP3										=	30	LSDV			
	CAPXVIR2 3-10	BN1										>	40	NEG			
97617 (1)	CAPXVIR2 3-1	BP1	Chibssa et al. 2018	Indical Bioscience		No	Other - Chibssa et al. 2018	PCR	N/A	200	0	=	338	LSDV	LSDV		
	CAPXVIR2 3-2	BP1											=	338	LSDV	LSDV	
	CAPXVIR2 3-3	TP3											=	338	LSDV	LSDV	
	CAPXVIR2 3-4	TP5											=	218	SPPV	SPPV vaccine	
	CAPXVIR2 3-5	TN1											=	0	NEG	NEG	
	CAPXVIR2 3-6	TP2											=	338	LSDV	LSDV	
	CAPXVIR2 3-7	TP6											=	338	LSDV	LSDV	
	CAPXVIR2 3-8	TP1											=	302	SPPV/GTPV	Do Lamien 2011 to discriminate between SPPV and GTPV	
	CAPXVIR2 3-9	BN1											=	0	NEG	NEG	
	CAPXVIR2 3-10	TP4											=	302	SPPV/GTPV	Do Lamien 2011 to discriminate between SPPV and GTPV	
97617 (2)	CAPXVIR2 3-1	BP1	Lamien et al. 2011	Indical Bioscience		No	Other - Lamien et al. 2011	PCR	N/A	100	0	=		NA (not analysed)			
	CAPXVIR2 3-2	BP1											=		NA (not analysed)		
	CAPXVIR2 3-3	TP3											=		NA (not analysed)		
	CAPXVIR2 3-4	TP5											=		NA (not analysed)		
	CAPXVIR2 3-5	TN1											=		NA (not analysed)		
CAPXVIR2 3-6	TP2	=		NA (not analysed)													
CAPXVIR2 3-7	TP6	=		NA (not analysed)													
CAPXVIR2 3-8	TP1	=	172	GTPV	GTPV												

lab ID	Random ID	Sample ID	Protocol ref / SOP	Producer Extraction protocol / kit	Name Extraction protocol / kit	Internal modifications to the extraction protocol/kit	RT-PCR protocol / kit	Methodology	Cut-off	Positive control	Negative control	Operator	Raw data (Ct cp value)	Interpretation	Comments per sample	General comments
	CAPXVIR2 3-9	BN1										=		NA (not analysed)		
	CAPXVIR2 3-10	TP4										=	151	SPPV	SPPV	
	CAPXVIR2 3-1	TP2										=		LSDV	LSDV Pendik	
	CAPXVIR2 3-2	BP1										=		LSDV	LSDV Pendik	
	CAPXVIR2 3-3	BN1										=		NEG		
	CAPXVIR2 3-4	BP1										=		LSDV	LSDV Pendik	
	CAPXVIR2 3-5	TN1										=		NEG		
97618	CAPXVIR2 3-6	TP3	in house	Invitrogen/ThermoFisher Scientific			Other - Galaye et al. 2015	Gelaye E. et al. 2015				=		LSDV	Lumpy skin disease virus isolate Cro2016 complete genome on gene RPO30-OL1	RT-PCR protocol / kit - Qiagen/Hot Star Taq Polymerase
	CAPXVIR2 3-7	TP4										=		SPPV	Lumpy skin disease virus isolate LSD-148-GP-RSA-1997 complete genome on gene RPO30-OL1	For these results a Sanger sequencing method was used. For these reason there are no figures values mentioned as the sequencing is based on a PCR test.
	CAPXVIR2 3-8	TP5										=		SPPV	SPPV vaccine Saudi Arabia	
	CAPXVIR2 3-9	TP1										=		GTPV	GTPV wild Turkey	
	CAPXVIR2 3-10	TP6										=		LSDV	LSDV Pendik	
	CAPXVIR2 3-1	TP4										=	29	SPPV		
97619	CAPXVIR2 3-2	TP2	Lamien et al 2011	Other		The chemagic Viral DNA/RNA 300 Kit special H96 using chemagic 360 instrument	Other - Lamien et al. 2011	SGreen PCR/ Sanger sequencing	N/A	33	38	=	29	LSDV		
	CAPXVIR2 3-3	TP6										=	31	LSDV		

lab ID	Random ID	Sample ID	Protocol ref / SOP	Producer Extraction protocol / kit	Name Extraction protocol / kit	Internal modifications to the extraction protocol/kit	RT-PCR protocol / kit	Methodology	Cut-off	Positive control	Negative control	Operator	Raw data (Ct cp value)	Interpretation	Comments per sample	General comments
	CAPXVIR2 3-4	TN1											<	38	NEG	
	CAPXVIR2 3-5	TP3											=	29	LSDV	
	CAPXVIR2 3-6	BP1											=	31	LSDV	
	CAPXVIR2 3-7	TP5											=	25	SPPV	
	CAPXVIR2 3-8	BN1											<	38	NEG	
	CAPXVIR2 3-9	TP1											=	29	SPPV/GTPV	
	CAPXVIR2 3-10	BP1											=	31	LSDV	
	CAPXVIR2 3-1	TP3	Lamien CE Le GC	Invitrogen/ThermoFisher Scientific									=	172	LSDV	
	CAPXVIR2 3-2	BP1	Silber R Wallace DB Gulyaz V										=	172	LSDV	
97620	CAPXVIR2 3-3	TP6	Tuppurainen E Madani H Caufour P Adam T El HM Luckins AG Albina E				Qiagen - MO-TO 47	PCR capillary electrophoresis		172	-		=	172	LSDV	Conventional PCR for species differentiation based on the PCR product length. Result is expressed as PCR product length in bp
	CAPXVIR2 3-4	TP4											=	154	SPPV	
	CAPXVIR2 3-5	TP2											=	172	LSDV	

lab ID	Random ID	Sample ID	Protocol ref / SOP	Producer Extraction protocol / kit	Name Extraction protocol / kit	Internal modifications to the extraction protocol/kit	RT-PCR protocol / kit	Methodology	Cut-off	Positive control	Negative control	Operator	Raw data (Ct cp value)	Interpretation	Comments per sample	General comments	
	CAPXVIR2 3-6	TN1	Diallo A. Use of the Capripoxvirus homologues of vaccinia virus 30 kDa RNA polymerase subunit (RPO30) gene as a novel diagnostic and genotyping target: development of a classical PCR method to differentiate goat poxvirus from sheep poxvirus. Vet Microbiol. 2011; 149:30-9.									=	-	NEG			
	CAPXVIR2 3-7	BN1											=	-	NEG		
	CAPXVIR2 3-8	TP1											=	172	GTPV		
	CAPXVIR2 3-9	BP1											=	172	LSDV		
	CAPXVIR2 3-10	TP5											=	154	SPPV		
97630	CAPXVIR2 3-1	TP6	Wolff et al. 2021 (Möller et al. 2019)	IDVET			Other - Wolff et al. 2021 (Both duplexes)		40	28,998	45	=	33,958	LSDV		High difference in the ct value between Capripox and species differentiation (LSDV) 31.94 v 39.86!	
	CAPXVIR2 3-2	BP1							=	30,809	LSDV						
	CAPXVIR2 3-3	BN1							>	45	NEG						

lab ID	Random ID	Sample ID	Protocol ref / SOP	Producer Extraction protocol / kit	Name Extraction protocol / kit	Internal modifications to the extraction protocol/kit	RT-PCR protocol / kit	Methodology	Cut-off	Positive control	Negative control	Operator	Raw data (Ct cp value)	Interpretation	Comments per sample	General comments
	CAPXVIR2 3-4	TP2										=	30,689	LSDV		
	CAPXVIR2 3-5	TP4										=	32,208	SPPV		
	CAPXVIR2 3-6	TP3										>	39,866	LSDV		
	CAPXVIR2 3-7	TP5										=	27,817	SPPV		
	CAPXVIR2 3-8	TP1										=	33,255	GTPV		
	CAPXVIR2 3-9	BP1										=	30,15	LSDV		
	CAPXVIR2 3-10	TN1										>	45	NEG		
	CAPXVIR2 3-1	TP6										=	30,72	LSDV		
	CAPXVIR2 3-2	TP2										=	28,97	LSDV		
	CAPXVIR2 3-3	TP4										=	27,32	SPPV		
9763 1	CAPXVIR2 3-4	TP3	Vidanovic et al. 2016	Roche			Qiagen - MO-TO 47		38	29,52	-	=	27,12	LSDV		
	CAPXVIR2 3-5	TP1	Lamien et al. 2011									=		GTPV	Performed by conventional PCR (Lamien et al 2011)	
	CAPXVIR2 3-6	BP1										=	26,71	LSDV		
	CAPXVIR2 3-7	BN1										=	-	NEG		
	CAPXVIR2 3-8	BP1										=	27,41	LSDV		
	CAPXVIR2 3-9	TP5										=	24,95	SPPV		
	CAPXVIR2 3-10	TN1										=	-	NEG		
9763 2	CAPXVIR2 3-1	BP1	Protocol based on Wolff et al 2021				Other - Wolff et al. 2021 (Both duplexes)		38	30	0	=	29,5	LSDV		As per the diagnostic workflow (Capripox positive followed by negative result using Wolff et al assays for species-specific and LSDV-vaccine specific this sample is further tested with additional assay based on Sprygin et al 2019 publication to check for possibility of recombinant LSDV strains
	CAPXVIR2 3-2	TP4	publication: Probe-Based Real-Time qPCR Assays for a Reliable Differentiation of Capripox	Sacace Biotechnologies		n/a						=	26,2	SPPV		
	CAPXVIR2 3-3	TN1										=	0	NEG		
	CAPXVIR2 3-4	TP3										=	0	NEG		
	CAPXVIR2 3-5	TP2										=	27,6	LSDV		
	CAPXVIR2 3-6	BP1										=	30,7	LSDV		

lab ID	Random ID	Sample ID	Protocol ref / SOP	Producer Extraction protocol / kit	Name Extraction protocol / kit	Internal modifications to the extraction protocol/kit	RT-PCR protocol / kit	Methodology	Cut-off	Positive control	Negative control	Operator	Raw data (Ct cp value)	Interpretation	Comments per sample	General comments
	CAPXVIR2 3-7	TP5	Virus Species									=	23,2	SPPV		
	CAPXVIR2 3-8	BN1										=	0	NEG		
	CAPXVIR2 3-9	TP1										=	29,4	GTPV		
	CAPXVIR2 3-10	TP6										=	32,8	LSDV		
	CAPXVIR2 3-1	TP5										=	29,44	SPPV	Unpublished qPCR assay specific for SPPV was used	
	CAPXVIR2 3-2	TP6										=	34,36	LSDV		
	CAPXVIR2 3-3	BN1										>	40	NEG		
97634	CAPXVIR2 3-4	TP2					Other - Vidanovic et al. 2021	qPCR	40	25,1	45	=	30,85	LSDV		We combined already published assay for LSDV (DIVA) for detection of LSDV with two unpublished qPCR assays for detection of SPPV and GTPV.
	CAPXVIR2 3-5	TP3	Vidanovic	Biosellal								=	30	LSDV	Unpublished qPCR assay specific for SPPV was used	
	CAPXVIR2 3-6	TP4										=	33,94	SPPV	Unpublished qPCR assay specific for GTPV was used	
	CAPXVIR2 3-7	TP1										=	29,75	GTPV		
	CAPXVIR2 3-8	BP1										=	28,49	LSDV		
	CAPXVIR2 3-9	TN1										>	45	NEG		
	CAPXVIR2 3-10	BP1										=	29,19	LSDV		
97642	CAPXVIR2 3-1	TP5					Other - Lamien et al. 2011	fluorescence melting curve analysis(FMCA)	40	19,63	40	=	31,63	SPPV		DNA extraction kit:Maxwell RSC viral Total Nucleic Acid Purification Kit(Promega Cat# AS1330) and Maxwell RSC whole Blood DNA kit(Promega Cat #AS1520)
	CAPXVIR2 3-2	TP3										=	30,29	LSDV		
	CAPXVIR2 3-3	TP1										=	28,57	GTPV		
	CAPXVIR2 3-4	TN1	CE Lamien(2011) virological Methods 171 1 134:140	Other								=	0	NEG		
	CAPXVIR2 3-5	TP2										=	29,3	LSDV		In the above reference paper the melting peak point of SPPV LSDV GTPV is 52 61 69 degrees of celsius respectively By using our viral DNA We modify the temperature of detection to 51(SPPV)
	CAPXVIR2 3-6	TP4										=	31,96	SPPV		
	CAPXVIR2 3-7	BN1										=	0	NEG		
	CAPXVIR2 3-8	TP6										=	32,08	LSDV		
	CAPXVIR2 3-9	BP1										=	28,02	LSDV		

lab ID	Random ID	Sample ID	Protocol ref / SOP	Producer Extraction protocol / kit	Name Extraction protocol / kit	Internal modifications to the extraction protocol/kit	RT-PCR protocol / kit	Methodology	Cut-off	Positive control	Negative control	Operator	Raw data (Ct cp value)	Interpretation	Comments per sample	General comments
	CAPXVIR2 3-10	BP1										=	28,17	LSDV		59(LSDV) 66(GTPV) degrees of celcius
	CAPXVIR2 3-1	TP3										=	39,08	LSDV		
	CAPXVIR2 3-2	TP6										=	32,04	LSDV		
	CAPXVIR2 3-3	TP2										=	28,75	LSDV		
	CAPXVIR2 3-4	TP5										=	28,37	SPPV		
97643	CAPXVIR2 3-5	BP1	NVR-SOP-46	Invitrogen/ThermoFisher Scientific	MagMAX Core Nucleic Acid Purification Kit	/	Lamien et al. 2011	Melt curve analysis	45	45	45	=	28,28	LSDV		
	CAPXVIR2 3-6	BP1										=	30,83	LSDV	LSDV in one replicate only	
	CAPXVIR2 3-7	BN1										>	45	NEG		
	CAPXVIR2 3-8	TP4										=	29,85	SPPV		
	CAPXVIR2 3-9	TP1										=	29,04	GTPV		
	CAPXVIR2 3-10	TN1										>	45	NEG		
	CAPXVIR2 3-1	TP6										=	34,3	LSDV	Positive for LSDV (WT) DNA; Negative for LSDV (Vacc) SPPV and GTPV DNA	
	CAPXVIR2 3-2	BN1										>	45	NEG		
97647	CAPXVIR2 3-3	TP4	TM-039	Thermo Fisher Scientific	MagMAX-96 Viral RNA Isolation Kit	n/a	Wolff et al. 2021 (Both duplexes) AgPath-ID One-Step RT-PCR Reagents (by Thermo Fisher Scientific)	Duplex 1: LSDV ORF008 (Vacc) LSDV ORF126 (WT) Duplex 2: SPPV ORF041 GTPV ORF095		Ct values : <40 POS 40-45 IND >45 NEG	27,4	45	=	33	SPPV	CAPXVIR23-3: based on the qPCR methods used and limited sequence data generated from ORF074 this sample was positive for SPPV DNA (differentiation between WT and RM-65 vaccine was not undertaken).
	CAPXVIR2 3-4	TP2										=	30,4	LSDV		
	CAPXVIR2 3-5	TP1										=	33,1	GTPV		CAPXVIR23-5: based on the qPCR methods used and limited sequence data generated from ORF074 this sample was positive for GTPV DNA (differentiation between WT and vaccine virus was not undertaken).

lab ID	Random ID	Sample ID	Protocol ref / SOP	Producer Extraction protocol / kit	Name Extraction protocol / kit	Internal modifications to the extraction protocol/kit	RT-PCR protocol / kit	Methodology	Cut-off	Positive control	Negative control	Operator	Raw data (Ct cp value)	Interpretation	Comments per sample	General comments
	CAPXVIR2 3-6	BP1											=	31,3	LSDV	
	CAPXVIR2 3-7	BP1											=	30	LSDV	
	CAPXVIR2 3-8	TN1											>	45	NEG	
	CAPXVIR2 3-9	TP5											=	28,8	SPPV	CAPXVIR23-9: based on the qPCR methods used and limited sequence data generated from ORF074 this sample was positive for SPPV DNA (differentiation between WT and RM-65 vaccine was not undertaken).
	CAPXVIR2 3-10	TP3											>	45	NEG	

10.1.6 VIRO: DIVA PCR

lab ID	Random ID	Sample ID	Protocol ref / SOP	Producer Extraction protocol / kit	Internal modifications to the extraction protocol/kit	RT-PCR protocol / kit	Methodology	Cut-off	Positive control	Negative control	Operator	Raw data (Ct cp value)	Interpretation	Comments per sample	General comments
	CAPXVIR23-1	BN1									=	50	NEG		
	CAPXVIR23-2	TP2									=	32,29	LSDV wild	Positive for LSDV wild in Agianniotaki et al. 2017 and Haegeman et al. 2023	
	CAPXVIR23-3	TP4									=	50	SPPV wild	Positive for SPPV wild in Chibssa et al. 2018 which is a gel based PCR	
	CAPXVIR23-4	TN1									=	50	NEG		
	CAPXVIR23-5	TP3									=	30,46	LSDV wild	Positive for LSDV vaccine in Agianniotaki et al. 2017 and for LSDV wild in Haegeman et al. 2023 Thus LSDV recombinant	Extraction Kit: See primary PCR
97506	CAPXVIR23-6	TP1	Chibssa et al. 2018 Agianniotaki et al. 2017 and Haegeman et al. 2023	Other	Addition of EC to buffer B3	Other - Other			23,67	50	=	50	GTPV		Protocol: Chibssa et al. 2018 Agianniotaki et al. 2017 and Haegeman et al. 2023
	CAPXVIR23-7	BP1									=	30,18	LSDV wild	Positive for LSDV wild in Agianniotaki et al. 2017 and Haegeman et al. 2023	
	CAPXVIR23-8	BP1									=	30,73	LSDV wild	Positive for LSDV wild in Agianniotaki et al. 2017 and Haegeman et al. 2023	
	CAPXVIR23-9	TP6									=	34,25	LSDV wild	Positive for LSDV wild in Agianniotaki et al. 2017 and Haegeman et al. 2023	
	CAPXVIR23-10	TP5									=	50	SPPV Vaccin	Positive for SPPV vaccine in Chibssa et al. 2018 which is a gel based PCR	
	CAPXVIR23-1	TP3									=		LSDV wild		RT-PCR protocol / kit : Bio-T kit@ Lumpy Skin Disease DIVA
	CAPXVIR23-2	TP4									=		NEG		Nom Protocole / kit d'extraction : BioExtract Colum
	CAPXVIR23-3	TN1									=		NEG		
97514	CAPXVIR23-5	BN1	Bio-T kit@ Lumpy Skin Disease DIVA	Biosellal		Other - Other			26,38	0	=		LSDV wild		
	CAPXVIR23-6	TP5									=		NEG		
	CAPXVIR23-7	BP1									=		LSDV wild		
	CAPXVIR23-8	BP1									=		LSDV wild		Le Bio-T kit@ Lumpy Skin Disease DIVA
	CAPXVIR23-9	TP1									=		NEG	permet de différencier une souche sauvage de LSDV de la souche vaccinale Neethling.	
	CAPXVIR23-10	TP6									=		LSDV wild		
	CAPXVIR23-1	BP1									=	35,01	LSDV wild		SPPV were also performed with classical PCR published by Haegemann et al. 2014; Haegemann et al. 2023
97600	CAPXVIR23-2	TP6	Haegemann et al. 2023 Viruses 15	Biosellal	no	Other - Haegeman et al. 2023		50	26,81	50	=	38,91	LSDV wild	= GTPV-Isolat	
	CAPXVIR23-3	TP1	870.								=		GTPV	= LSD field (recombinant)	
	CAPXVIR23-4	TP3									=	39,62	LSDV wild		

lab ID	Random ID	Sample ID	Protocol ref / SOP	Producer Extraction protocol / kit	Internal modifications to the extraction protocol/kit	RT-PCR protocol / kit	Methodology	Cut-off	Positive control	Negative control	Operator	Raw data (Ct cp value)	Interpretation	Comments per sample	General comments
	CAPXVIR23-5	TP2									=	38,51	LSDV wild		is only for differentiation of LSDV-isolates
	CAPXVIR23-6	BN1									>	50	NEG		
	CAPXVIR23-7	TP4									=		SPPV wild	after Haegemann et al. 2014 (classical PCR)	
	CAPXVIR23-8	TN1									>	50	NEG		
	CAPXVIR23-9	BP1									=	33,12	LSDV wild		
	CAPXVIR23-10	TP5									=		SPPV Vaccin	after Haegemann et al. 2014 (classical PCR)	
97602	CAPXVIR23-1	TP3									=	33,47	LSDV wild	recombinant (GPCR sequencing)	
	CAPXVIR23-2	TP6									=	35,66	LSDV wild		
	CAPXVIR23-3	TP4									=	0	SPPV wild	Chibssa DIVA gel	
	CAPXVIR23-4	BP1									=	33,37	LSDV wild		
	CAPXVIR23-5	BN1	Haegeman et al. 2023/ Chibssa et al. 2018/ GPCR sequencing	Invitrogen/ThermoFisher Scientific		Qiagen - MO-TO 47			31,83	0	=	0	NA (not analysed)		
	CAPXVIR23-6	BP1									=	32,86	LSDV wild		
	CAPXVIR23-7	TP5									=	0	SPPV Vaccin	Chibssa DIVA gel	
	CAPXVIR23-8	TN1									=	0	NA (not analysed)		
	CAPXVIR23-9	TP2									=	32,27	LSDV wild		
	CAPXVIR23-10	TP1									>	43	NA (not analysed)		
97604	CAPXVIR23-1	TP5									=		SPPV wild	vaccine or field strain not differentiated	
	CAPXVIR23-2	TP2									=		LSDV wild		
	CAPXVIR23-3	BN1									=		NEG		
	CAPXVIR23-4	TP4									=		SPPV wild	vaccine or field strain not differentiated	
	CAPXVIR23-5	TP3	6.3.51	Roche		Qiagen - MO-TO 47					=		LSDV Vaccin		
	CAPXVIR23-6	TN1									=		NEG		
	CAPXVIR23-7	TP1									=		GTPV		
	CAPXVIR23-8	BP1									=		LSDV wild		
	CAPXVIR23-9	TP6									=		LSDV wild		
	CAPXVIR23-10	BP1									=		LSDV wild		
97608 (1)	CAPXVIR23-1	BP1									=	27,4	LSDV wild		
	CAPXVIR23-2	TP6									=	30,7	LSDV wild		
	CAPXVIR23-3	TP1									>	40	NI (not interpretable)		
	CAPXVIR23-4	BN1									>	40	NEG		
	CAPXVIR23-5	BP1									=	27,3	LSDV wild		Extraction: Machery-Nagel - NucleoMagVet kit
	CAPXVIR23-6	TP5	Wolff et al 2021	Other		Other - Wolff et al. 2021	Wolff et al 2021 LSDV field-Vaccine	40	28,7	40	>	40	NI (not interpretable)		
	CAPXVIR23-7	TP4									>	40	NI (not interpretable)		qPCR: Perfecta qPCR kit (Quanta Biosciences)
CAPXVIR23-8	TP2									=	29,7	LSDV wild			
CAPXVIR23-9	TP3									>	40	LSDV wild	LSDV-Recombinant because cattle sample and both LSDV assays (field and vaccine assay) reacted negative		
97608 (2)	CAPXVIR23-10	TN1									>	40	NEG		
	CAPXVIR23-1	BP1	Haegeman et al 2023	Other				38	26,7	38	=	28,3	LSDV wild		Extraction: Machery-Nagel - NucleoMagVet
	CAPXVIR23-2	TP6									=	31,6	LSDV wild		

lab ID	Random ID	Sample ID	Protocol ref / SOP	Producer Extraction protocol / kit	Internal modifications to the extraction protocol/kit	RT-PCR protocol / kit	Methodology	Cut-off	Positive control	Negative control	Operator	Raw data (Ct cp value)	Interpretation	Comments per sample	General comments
	CAPXVIR23-3	TP1										38	NI (not interpretable)		kit
	CAPXVIR23-4	BN1										38	NEG		qPCR: Perfecta qPCR kit (Quanta Biosciences)
	CAPXVIR23-5	BP1				Other - Haegeman et al. 2023	Haegeman et al 2023 Field Assay					27,6	LSDV wild		
	CAPXVIR23-6	TP5										38	NI (not interpretable)		
	CAPXVIR23-7	TP4										38	NI (not interpretable)		
	CAPXVIR23-8	TP2										30,9	LSDV wild		
	CAPXVIR23-9	TP3										31	LSDV wild		
	CAPXVIR23-10	TN1										38	NEG		
	CAPXVIR23-1	BP1											LSDV wild		
	CAPXVIR23-2	TP6											LSDV wild		
	CAPXVIR23-3	TP1											GTPV		Extraction: Machery-Nagel - NucleoMagVet kit
97608 (3)	CAPXVIR23-4	BN1											NEG		
	CAPXVIR23-5	BP1	Chibssa et al. 2018	Other		Other - Chibssa et al. 2018	Chibssa et al. 2018						LSDV wild	Vacc strain SPPV Romanian	
	CAPXVIR23-6	TP5											SPPV Vaccin		qPCR: Perfecta qPCR kit (Quanta Biosciences)
	CAPXVIR23-7	TP4											SPPV wild		
	CAPXVIR23-8	TP2											LSDV wild		
	CAPXVIR23-9	TP3											LSDV Vaccin		
	CAPXVIR23-10	TN1											NEG		
	CAPXVIR23-1	TP5										39	NEG	Upon repeat extraction and testing late Cts recorded for this sample. We exclude laboratory contamination since the negative and positive controls of the assay behaved as expected. We conclude that the sample is negative. Possible explanation could be a non specific reaction of the sample with the assay. Ct refer to WT target.	Extraction was performed manually using Indispin Pathogen kit and automated method Indimag Pathogen.
97609 (1)			Agianniotaki et al. 2016	Indical Bioscience		Other - Agianniotaki et al. 2016		38	32,66	0				Upon repeat extraction and testing late Cts recorded for this sample. We exclude laboratory contamination since the negative and positive controls of the assay behaved as expected. We conclude that the sample is negative. Possible explanation could be a non specific reaction of the sample with the assay. Ct refer to WT target.	
	CAPXVIR23-2	TP4										41	NEG	Upon repeat extraction and testing late Cts recorded for this sample. We exclude laboratory contamination since the negative and positive controls of the assay behaved as expected. We conclude that the sample is negative. Possible explanation could be a non specific reaction of the sample with the assay. Ct refer to WT target.	
	CAPXVIR23-3	TP6										35,61	LSDV wild		
	CAPXVIR23-4	BP1										31,12	LSDV wild		

lab ID	Random ID	Sample ID	Protocol ref / SOP	Producer Extraction protocol / kit	Internal modifications to the extraction protocol/kit	RT-PCR protocol / kit	Methodology	Cut-off	Positive control	Negative control	Operator	Raw data (Ct cp value)	Interpretation	Comments per sample	General comments
	CAPXVIR23-5	TP3									=	32,09	LSDV Vaccin	The assay classifies the sample as vaccine however we are aware that the assay misclassifies recombinant strains as vaccines. For that reason partial GPCR sequencing was performed and showed 100% to similarity to recombinant Lumpy skin disease virus strain LSDV/HongKong/2020-MW732649.1	
	CAPXVIR23-6	TP2									=	32,29	LSDV wild		
	CAPXVIR23-7	TP1									=	39,3	NEG	Upon repeat extraction and testing late Ct's recorded for this sample. We exclude laboratory contamination since the negative and positive controls of the assay behaved as expected. We conclude that the sample is negative. Possible explanation could be a non specific reaction of the sample with the assay. Ct refer to vaccine target. We are aware of non specific reaction of GTPV to the vaccine target of the assay.	
	CAPXVIR23-8	BN1									=	0	NEG		
	CAPXVIR23-9	TN1									=	0	NEG		
	CAPXVIR23-10	BP1									=	29,8	LSDV wild		
97609 (2)	CAPXVIR23-1	TP5	Haegeman et al. 2015-DIVA for SPPV	Indical Bioscience		Other - Haegeman et al. 2015			0	0	=	27,96	SPPV Vaccin		Real-Time PCR was performed using Quantifast Sybr Green. Real-Time PCR amplicons were
	CAPXVIR23-2	TP4									=	30,82	SPPV wild		

lab ID	Random ID	Sample ID	Protocol ref / SOP	Producer Extraction protocol / kit	Internal modifications to the extraction protocol/kit	RT-PCR protocol / kit	Methodology	Cut-off	Positive control	Negative control	Operator	Raw data (Ct cp value)	Interpretation	Comments per sample	General comments
	CAPXVIR23-3	TP6									=	0	NEG		analysed by gel electrophoresis (as described in Haegeman et al. 2015) for sample classification as vaccine or wild type. Maschine: Rororgene Q
	CAPXVIR23-4	BP1									=	0	NEG		
	CAPXVIR23-5	TP3									=	0	NEG		
	CAPXVIR23-6	TP2									=	0	NEG		
	CAPXVIR23-7	TP1									=	0	NEG		
	CAPXVIR23-8	BN1									=	0	NEG		
	CAPXVIR23-9	TN1									=	0	NEG		
	CAPXVIR23-10	BP1									=	0	NEG		
	CAPXVIR23-1	TP5									=	0	NEG		
	CAPXVIR23-2	TP4									=	33,11	SPPV wild		
	CAPXVIR23-3	TP6									=	0	NEG		
97609 (3)	CAPXVIR23-4	BP1	Haegeman et al. 2015 / SPPV WT	Indical Bioscience		Other - Haegeman et al. 2015					=	0	NEG		Real-Time PCR was performed using Quantifast Sybr Green.
	CAPXVIR23-5	TP3	Sybr Green Real Time PCR					35			=	0	NEG		
	CAPXVIR23-6	TP2									=	0	NEG		
	CAPXVIR23-7	TP1									=	0	NEG		Maschine: Rororgene Q
	CAPXVIR23-8	BN1									=	0	NEG		
	CAPXVIR23-9	TN1									=	0	NEG		
	CAPXVIR23-10	BP1									=	0	NEG		
97610	CAPXVIR23-1	TP3									=		LSDV Vaccin		
	CAPXVIR23-2	TN1									=				
	CAPXVIR23-3	BN1									=				
	CAPXVIR23-4	TP2									=		LSDV wild		
	CAPXVIR23-5	TP5	Vidanovic et al	Indical Bioscience		Other - Vidanovic et al. 2021					=				
	CAPXVIR23-6	BP1									=		LSDV wild		
	CAPXVIR23-7	BP1									=		LSDV wild		
	CAPXVIR23-8	TP6									=		LSDV wild		
	CAPXVIR23-9	TP1									=				
	CAPXVIR23-10	TP4									=				
	CAPXVIR23-1	BP1									=	27,43	LSDV wild	overall: LSDV wild	DIVA Reagent: TaqMan
	CAPXVIR23-2	TP5									=		NEG	overall: SPPV (our lab only performs LSD DIVA)	Fast Virus 1-step Master Mix
	CAPXVIR23-3	TP3									=	29,25	LSDV Vaccin	overall: LSDV vaccine	Lot:01271240 Exp: 30-11-23
	CAPXVIR23-4	TN1									=		NEG	overall: Negative	
97611	CAPXVIR23-5	TP4	Agianiotaki et al 2017	QIAGEN		Other - Other	Multiplex real time-LSDV	37	27	37	=		NEG	overall: SPPV (our lab only performs LSD DIVA)	Methodology: Multiplex real time - LSDV
	CAPXVIR23-6	BP1									=	26,57	LSDV wild	overall: LSDV wild	Ref Methodology: Agianiotaki et al 2017
	CAPXVIR23-7	TP1									=		GTPV	overall: GTPV (our lab only performs LSD DIVA)	
	CAPXVIR23-8	TP2									=	28,26	LSDV wild	overall: LSDV wild	DIVA: LSDV Field Pos: Cq 27.07 LSDV Vaccine Pos: Cq

lab ID	Random ID	Sample ID	Protocol ref / SOP	Producer Extraction protocol / kit	Internal modifications to the extraction protocol/kit	RT-PCR protocol / kit	Methodology	Cut-off	Positive control	Negative control	Operator	Raw data (Ct cp value)	Interpretation	Comments per sample	General comments	
	CAPXVIR23-9	BN1									=		NEG	overall: Negative	28.02	Neg:
	CAPXVIR23-10	TP6									=	29,37	LSDV wild	overall: LSDV wild	No Cq or >37	
	CAPXVIR23-1	TP3									=		LSDV wild	the test is unable to discriminate between LSDV field and LSDV vaccine		
	CAPXVIR23-2	TP1									=		GTPV			
	CAPXVIR23-3	TP5									=		SPPV Vaccin			
	CAPXVIR23-4	BN1									=		NEG			
	CAPXVIR23-5	BP1									=		LSDV wild	the test is unable to discriminate between LSDV field and LSDV vaccine		
97612 (1)	CAPXVIR23-6	TP2	Chibssa et al. 2018	Roche		Other - Chibssa et al. 2018	gel-based PCR				=		LSDV wild	the test is unable to discriminate between LSDV field and LSDV vaccine	To discriminate LSDV field from LSDV vaccine we added the Method 2 (ID Gene LSD DIVA Triplex - IdVet)	
	CAPXVIR23-7	TP4									=		SPPV wild			
	CAPXVIR23-8	TN1									=		NEG			
	CAPXVIR23-9	TP6									=		LSDV wild	the test is unable to discriminate between LSDV field and LSDV vaccine		
	CAPXVIR23-10	BP1									=		LSDV wild	the test is unable to discriminate between LSDV field and LSDV vaccine		
	CAPXVIR23-1	TP3									=	27,2	LSDV wild			
	CAPXVIR23-2	TP1									>	38	NEG			
	CAPXVIR23-3	TP5									>	38	NEG			
	CAPXVIR23-4	BN1									>	38	NEG			
97612 (2)	CAPXVIR23-5	BP1	ID GENE LSD DIVA Triplex (ID.VET)			ID.VET - ID GENE@ LSD DIVA TRIPLEX	PCR real time	>38	28,2	38	=	24,3	LSDV wild		This method discriminate between LSDV wild and LSDV Neethling vaccine; the results can be interpreted with method 1	
	CAPXVIR23-6	TP2									=	26	LSDV wild			
	CAPXVIR23-7	TP4									>	38	NEG			
	CAPXVIR23-8	TN1									>	38	NEG			
	CAPXVIR23-9	TP6									=	26,5	LSDV wild			
	CAPXVIR23-10	BP1									=	24	LSDV wild			
	CAPXVIR23-1	TP6									=	30,57	LSDV wild			
	CAPXVIR23-2	TP3									=	28,81	LSDV Vaccin			
	CAPXVIR23-3	BP1									=	27,9	LSDV wild			
	CAPXVIR23-4	TN1									>	40	NEG			
97613 (1)	CAPXVIR23-5	TP4	Agianniotaki et al. 2017			Other - Agianniotaki et al. 2017		40	27,02	40	>	40	NEG			
	CAPXVIR23-6	TP1									>	40	NEG			
	CAPXVIR23-7	BP1									=	27,56	LSDV wild			
	CAPXVIR23-8	TP2									=	29,31	LSDV wild			
	CAPXVIR23-9	TP5									>	40	NEG			
	CAPXVIR23-10	BN1									>	40	NEG			

lab ID	Random ID	Sample ID	Protocol ref / SOP	Producer Extraction protocol / kit	Internal modifications to the extraction protocol/kit	RT-PCR protocol / kit	Methodology	Cut-off	Positive control	Negative control	Operator	Raw data (Ct cp value)	Interpretation	Comments per sample	General comments																										
97613 (2)	CAPXVIR23-1	TP6	Chibssa et al. 2018			Other - Chibssa et al. 2018							=	LSDV wild																											
	CAPXVIR23-2	TP3														=	LSDV Vaccin																								
	CAPXVIR23-3	BP1																=	LSDV wild																						
	CAPXVIR23-4	TN1																		=	NEG																				
	CAPXVIR23-5	TP4																				=	SPPV wild																		
	CAPXVIR23-6	TP1																						=	NEG																
	CAPXVIR23-7	BP1																								=	LSDV wild														
	CAPXVIR23-8	TP2																										=	LSDV wild												
	CAPXVIR23-9	TP5																												=	SPPV Vaccin										
	CAPXVIR23-10	BN1																														=	NEG								
CAPXVIR23-1	BP1	=	LSDV wild																																						
CAPXVIR23-2	TP1			=	GTPV																																				
CAPXVIR23-3	TP2					=	NI (not interpretable)																																		
CAPXVIR23-4	TP4							=	SPPV Vaccin																																
CAPXVIR23-5	TP5									=	NI (not interpretable)																														
CAPXVIR23-6	BP1											=	NI (not interpretable)																												
CAPXVIR23-7	TP6													=	LSDV wild																										
CAPXVIR23-8	TN1															=	NEG																								
CAPXVIR23-9	TP3																	=	LSDV Vaccin																						
CAPXVIR23-10	BN1																			=	NEG																				
CAPXVIR23-1	BP1	=	57																			LSDV wild	LSDV wild																		
CAPXVIR23-2	BP1			=	57																			LSDV wild	LSDV wild																
CAPXVIR23-3	TP3					=	57,65																			LSDV Vaccin	LSDV vaccine														
CAPXVIR23-4	TP5							=	NA (not analysed)																			SPPV vaccine													
CAPXVIR23-5	TN1									=	NA (not analysed)																		NEG												
CAPXVIR23-6	TP2											=	57																	LSDV wild	LSDV wild										
CAPXVIR23-7	TP6													=	57																	LSDV wild	LSDV wild								
CAPXVIR23-8	TP1															=	NA (not analysed)																	GTPV							
CAPXVIR23-9	BN1																	=	NA (not analysed)																NEG						
CAPXVIR23-10	TP4																			=	NA (not analysed)															SPPV wild					
CAPXVIR23-1	TP2	=	34,73																			LSDV wild																			
CAPXVIR23-2	BP1			=	32,74																			LSDV wild																	
CAPXVIR23-3	BN1					=	0																			NEG															
CAPXVIR23-4	BP1							DIVA GREECE Agianniotaki et al 2017	Invitrogen/ThermoFisher Scientific																			Other - Other									Real-time PCR method for bovine samples 1 2 3 4 5 6 and 10	30,83	0	=	32,63
CAPXVIR23-5	TN1									PCR method for ovine samples 7 and 8	0																		NEG												

lab ID	Random ID	Sample ID	Protocol ref / SOP	Producer Extraction protocol / kit	Internal modifications to the extraction protocol/kit	RT-PCR protocol / kit	Methodology	Cut-off	Positive control	Negative control	Operator	Raw data (Ct cp value)	Interpretation	Comments per sample	General comments
	CAPXVIR23-6	TP3									=	33,07	LSDV Vaccin	based PCR method to differentiate sheeppox virus field isolates from vaccine strains (Chibssa et.al. 2018) The interpretation of this sample as SPPV Vaccine is based on the DIVA gel based PCR method to differentiate sheeppox virus field isolates from vaccine strains (Chibssa et.al. 2018).	
	CAPXVIR23-7	TP4									=	0	SPPV wild	The interpretation of this sample as GTPV Wild is based on the sequencing test. It is possible that the DIVA GREECE Agianniotaki et al 2017 protocol to not differentiate the recombinant strains of LSDV Based on the sequencing test this sample seems to be a wild recombinant strain of a LSDV Vaccine.	
	CAPXVIR23-8	TP5									=	0	SPPV Vaccin	The interpretation of this sample as SPPV Wild is based on the DIVA gel based PCR method to differentiate sheeppox virus field isolates from vaccine strains (Chibssa et.al. 2018)	
	CAPXVIR23-9	TP1									=	0	GTPV wild		
	CAPXVIR23-10	TP6									=	40	LSDV wild		
97620 (1)	CAPXVIR23-1	TP3	Chibssa T.R.								=	336	NI (not interpretable)		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.
	CAPXVIR23-2	BP1	Grabherr R. Loitsch A. et al.								=	336	NI (not interpretable)		
	CAPXVIR23-3	TP6	A gel-based PCR method to differentiate sheeppox virus field isolates from vaccine strains.	Invitrogen/ThermoFisher Scientific							=	336	NI (not interpretable)		
	CAPXVIR23-4	TP4									=	302	SPPV wild		
	CAPXVIR23-5	TP2				Qiagen - MO-TO 47	PCR capillary electrophoresis		218		=	336	NI (not interpretable)		
	CAPXVIR23-6	TN1									=	-	NEG		
	CAPXVIR23-7	BN1									=	-	NEG		
	CAPXVIR23-8	TP1	J 15 59 (2018)								=	302	GTPV	sample from goat	
	CAPXVIR23-9	BP1									=	336	NI (not interpretable)		
	CAPXVIR23-10	TP5									=	218	SPPV Vaccin		
97620 (2)	CAPXVIR23-1	TP3	Haegeman A.; De Leeuw I.; Philips W.; De Regge N.	Invitrogen/ThermoFisher Scientific							=	34,73	LSDV wild	positive with probe dye FAM result in Ct value	
	CAPXVIR23-2	BP1				Qiagen - MO-TO 47	real time PCR		26,77		=	33,97	LSDV wild	positive with probe dye FAM result in Ct value	
	CAPXVIR23-3	TP6	Development and								=	36,63	LSDV wild	positive with probe dye FAM result in Ct value	

lab ID	Random ID	Sample ID	Protocol ref / SOP	Producer Extraction protocol / kit	Internal modifications to the extraction protocol/kit	RT-PCR protocol / kit	Methodology	Cut-off	Positive control	Negative control	Operator	Raw data (Ct cp value)	Interpretation	Comments per sample	General comments
	CAPXVIR23-4	TP4	Validation of a New DIVA Real-Time PCR Allowing to Differentiate Wild-Type Lumpy Skin Disease Virus Strains Including the Asian Recombinant Strains from Neethling-Based Vaccine Strains. Viruses 2023 15 870.										NEG		
	CAPXVIR23-5	TP2										35,36	LSDV wild	positive with probe dye FAM result in Ct value	
	CAPXVIR23-6	TN1											NEG		
	CAPXVIR23-7	BN1											NEG		
	CAPXVIR23-8	TP1											NEG		
	CAPXVIR23-9	BP1										34,75	LSDV wild	positive with probe dye FAM result in Ct value	
	CAPXVIR23-10	TP5											NEG		
97621 (1)	CAPXVIR23-1	TP3				Other - Agianniotaki et al. 2017	Duplex RT-PCR (LSDV wild or vaccine)	35	35	40		23,5	LSDV Vaccin		
	CAPXVIR23-2	TP6		QIAGEN								30	LSDV wild		
	CAPXVIR23-3	TP5										40	NEG		
	CAPXVIR23-4	TN1										40	NEG		
	CAPXVIR23-5	BP1										24,2	LSDV wild		
	CAPXVIR23-6	BN1										40	NEG		
	CAPXVIR23-7	BP1										25,2	LSDV wild		
	CAPXVIR23-8	TP1										40	NEG		
	CAPXVIR23-9	TP4										40	NEG		
	CAPXVIR23-10	TP2										25,1	LSDV wild		
	CAPXVIR23-1	TP3											NEG		
	CAPXVIR23-2	TP6											NEG		
	CAPXVIR23-3	TP5											SPPV Vaccin		
97621 (2)	CAPXVIR23-4	TN1				Other - Haegeman et al. 2015	Conventional PCR (differential amplicon by size)						NEG		
	CAPXVIR23-5	BP1		QIAGEN									NEG		
	CAPXVIR23-6	BN1											NEG		
	CAPXVIR23-7	BP1											NEG		
	CAPXVIR23-8	TP1											NEG		
	CAPXVIR23-9	TP4											SPPV wild		
	CAPXVIR23-10	TP2											NEG		
97630	CAPXVIR23-1	TP6				Other - Wolff et al. 2021		40	28,998	45		33,958	LSDV wild		
	CAPXVIR23-2	BP1										30,809	LSDV wild		
	CAPXVIR23-3	BN1										45	NEG		
	CAPXVIR23-4	TP2										30,689	LSDV wild		
	CAPXVIR23-5	TP4											NA (not analysed)		
	CAPXVIR23-6	TP3	Wolf et al. 2021	IDVET								39,866	LSDV wild	High difference in the ct value between Capripox and species differentiation (LSDV) 31.94 v 39.86!!	For samples CAPXVIR23-5 and CAPXVIR23-7 DIVA SPPV was not performed. Therefore we did not determine weather SPPV positivity was due to the vaccination or it is a field strain.
	CAPXVIR23-7	TP5										27,817	NA (not analysed)		
	CAPXVIR23-8	TP1										33,255	GTPV		
	CAPXVIR23-9	BP1										30,15	LSDV wild		

lab ID	Random ID	Sample ID	Protocol ref / SOP	Producer Extraction protocol / kit	Internal modifications to the extraction protocol/kit	RT-PCR protocol / kit	Methodology	Cut-off	Positive control	Negative control	Operator	Raw data (Ct cp value)	Interpretation	Comments per sample	General comments																								
	CAPXVIR23-10	TN1										>	45	NEG																									
97632	CAPXVIR23-1	BP1										=	29,5	LSDV wild	LSDV recombinant strain																								
	CAPXVIR23-2	TP4	Protocol based on Wolff et al 2021 publication: Probe-Based Real-Time qPCR Assays for a Reliable Differentiation of Capripox Virus Species	Sacace Biotechnologies	Other - Wolff et al. 2021	38	30	0	=	=	=	=	26,2	SPPV wild																									
	CAPXVIR23-3	TN1														=	0	NEG																					
	CAPXVIR23-4	TP3																	=	0	LSDV wild																		
	CAPXVIR23-5	TP2																				=	27,6	LSDV wild															
	CAPXVIR23-6	BP1																							=	30,7	LSDV wild												
	CAPXVIR23-7	TP5																										=	23,2	SPPV wild									
	CAPXVIR23-8	BN1																													=	0	NEG						
	CAPXVIR23-9	TP1																																=	29,4	NA (not analysed)			
	CAPXVIR23-10	TP6																																			=	32,8	LSDV wild
CAPXVIR23-1	TP5	>													45																								
CAPXVIR23-2	TP6		=	34,36	LSDV wild																																		
CAPXVIR23-3	BN1					>	45	NEG																															
CAPXVIR23-4	TP2								=	32,09	LSDV wild																												
CAPXVIR23-5	TP3											=	30	LSDV Vaccin																									
CAPXVIR23-6	TP4															>	45	NEG																					
CAPXVIR23-7	TP1																		>	45	NEG																		
CAPXVIR23-8	BP1																					=	28,97	LSDV wild															
CAPXVIR23-9	TN1																								>	45	NEG												
97634																												Vidanovic et al. 2021	Biosellal	Other - Vidanovic et al. 2021	qPCR	40	27,84	45					
	CAPXVIR23-10	BP1																																	=	29,19	LSDV wild		
97637	CAPXVIR23-1	BN1	Vidanovic et al 2016	Roche	Other - Vidanovic et al. 2016										38													26,08	-	=	=	=	=	-	NEG				
	CAPXVIR23-2	TP4				=	-	NI (not interpretable)																															
	CAPXVIR23-3	TN1							=	-	NEG																												
	CAPXVIR23-4	TP1										=	-	NI (not interpretable)																									
	CAPXVIR23-5	BP1														=	30,15	LSDV wild																					
	CAPXVIR23-6	TP3																	=	26,76	LSDV Vaccin																		
	CAPXVIR23-7	TP6																				=	32,2	LSDV wild															
	CAPXVIR23-8	TP5																							=	-	NI (not interpretable)												
	CAPXVIR23-9	BP1																																		=	28,73	LSDV wild	
	CAPXVIR23-10	TP2																																					=
97642 (1)	CAPXVIR23-1	TP5	Eirini I.Agianniotaki (2017)	Other	Other - Agianniotaki et al. 2017										40													27,32	40	=	=	=	=	0	NEG				
	CAPXVIR23-2	TP3				=	29,6	LSDV Vaccin																															
	CAPXVIR23-3	TP1							=	0	NEG																												
	CAPXVIR23-4	TN1										=	0	NEG																									
	CAPXVIR23-5	TP2														=	30,15	LSDV wild																					

lab ID	Random ID	Sample ID	Protocol ref / SOP	Producer Extraction protocol / kit	Internal modifications to the extraction protocol/kit	RT-PCR protocol / kit	Methodology	Cut-off	Positive control	Negative control	Operator	Raw data (Ct cp value)	Interpretation	Comments per sample	General comments
	CAPXVIR23-6	TP4									=	0	NEG		AS1330) and Maxwell
	CAPXVIR23-7	BN1									=	0	NEG		RSC whole Blood DNA
	CAPXVIR23-8	TP6									=	31,25	LSDV wild		kit(Promega Cat
	CAPXVIR23-9	BP1									=	29,04	LSDV wild		#AS1520)
	CAPXVIR23-10	BP1									=	28,75	LSDV wild		
	CAPXVIR23-1	TP5						-			=	0	SPPV Vaccin	218bp	
	CAPXVIR23-2	TP3						-			=	0	LSDV Vaccin	336bp	
	CAPXVIR23-3	TP1						-			=	0	GTPV	302bp	
	CAPXVIR23-4	TN1						-			=	0	NEG	-	
97642 (2)	CAPXVIR23-5	TP2	Chibssa et al. 2018	Other		Other - Chibssa et al. 2018	cPCR	-	0	0	=	0	LSDV wild	336bp	
	CAPXVIR23-6	TP4						-			=	0	SPPV wild	302bp	
	CAPXVIR23-7	BN1						-			=	0	NEG	-	
	CAPXVIR23-8	TP6						-			=	0	LSDV wild	336bp	
	CAPXVIR23-9	BP1						-			=	0	LSDV wild	336bp	
	CAPXVIR23-10	BP1						-			=	0	LSDV wild	336bp	
	CAPXVIR23-1	TP3									=	39,08	LSDV Vaccin	Very late CT suggest recombinant LSDV	
	CAPXVIR23-2	TP6									=	32,038	LSDV wild		
	CAPXVIR23-3	TP2									=	28,7515	LSDV wild		
	CAPXVIR23-4	TP5									=		NA (not analysed)		
97643	CAPXVIR23-5	BP1	Sprygin et al. 2018/ Unpublished assay	Invitrogen/ThermoFisher Scientific - KingFisher Flex	none	Other - Other	real time PCR for differentiation of field and vaccine LSDV	45	45	45	=	28,275	LSDV wild		
	CAPXVIR23-6	BP1									=	30,825	LSDV wild		
	CAPXVIR23-7	BN1									=		NA (not analysed)		
	CAPXVIR23-8	TP4									=		NA (not analysed)		
	CAPXVIR23-9	TP1									=		NA (not analysed)		
	CAPXVIR23-10	TN1									=		NA (not analysed)		
	CAPXVIR23-1	TP6									=	34,3	LSDV wild		VIR23-2 & VIR23-8: based on the qPCR methods used these samples tested
	CAPXVIR23-2	BN1									>	45	NEG		negative for capripoxvirus DNA. VIR23-1, VIR23-4, VIR23-6 & VIR23-7: based on the qPCR methods used these samples were
	CAPXVIR23-3	TP4									>	45	NEG		positive for LSDV (WT) DNA and negative for LSDV (Vacc) DNA consistent with infection with LSDV.
	CAPXVIR23-4	TP2									=	30,4	LSDV wild		VIR23-10: although this sample tested negative for SPPV GTPV and LSDV DNA using the Wolff et al. 2021 duplex assays it tested positive for capripoxvirus DNA using the Bowden et al. 2008 pan-capripoxvirus ORF074 assay. It also tested positive for LSDV using the Alexander Sprygin et al. 2019 pan-LSDV ORF044 assay. Although testing has not established whether this was due to an actual infection or the results of a
	CAPXVIR23-5	TP1									>	45	NEG		
	CAPXVIR23-6	BP1									=	31,3	LSDV wild		
	CAPXVIR23-7	BP1									=	30	LSDV wild		
	CAPXVIR23-8	TN1									>	45	NEG		
	CAPXVIR23-9	TP5		Ambion MagMAX-96 Viral RNA Isolation Kit (by Thermo Fisher Scientific)	NA	Other - Wolff et al. 2021 AgPath-ID One-Step RT-PCR Reagents (by Thermo Fisher Scientific)	LSDV ORF008 (Vacc) LSDV ORF126 (WT)	<40 POS 40-45 IND >45 NEG	27,4	45	>	45	NEG		
97647	CAPXVIR23-10	TP3	TM-039								>	45	NEG		

lab ID	Random ID	Sample ID	Protocol ref / SOP	Producer Extraction protocol / kit	Internal modifications to the extraction protocol/kit	RT-PCR protocol / kit	Methodology	Cut-off	Positive control	Negative control	Operator	Raw data (Ct cp value)	Interpretation	Comments per sample	General comments
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recent vaccination the pattern of the results obtained may be due to infection of this animal with a recombinant variant LSDV strain.

10.2 Annex 2: 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 standardize their different diagnostic tests. The boxplots, shown down below, were created by using the following software programme: shiny.chemgrid.org/boxplotr/.

10.2.1 SERO: ELISA

For the serology ELISA part, box plots of the optical density (OD) per reference sample and per participating laboratory are shown in Figures I and II. Since there was one repetition, only the sample PT2023CAPXSER_P6 was included in the figures down below.

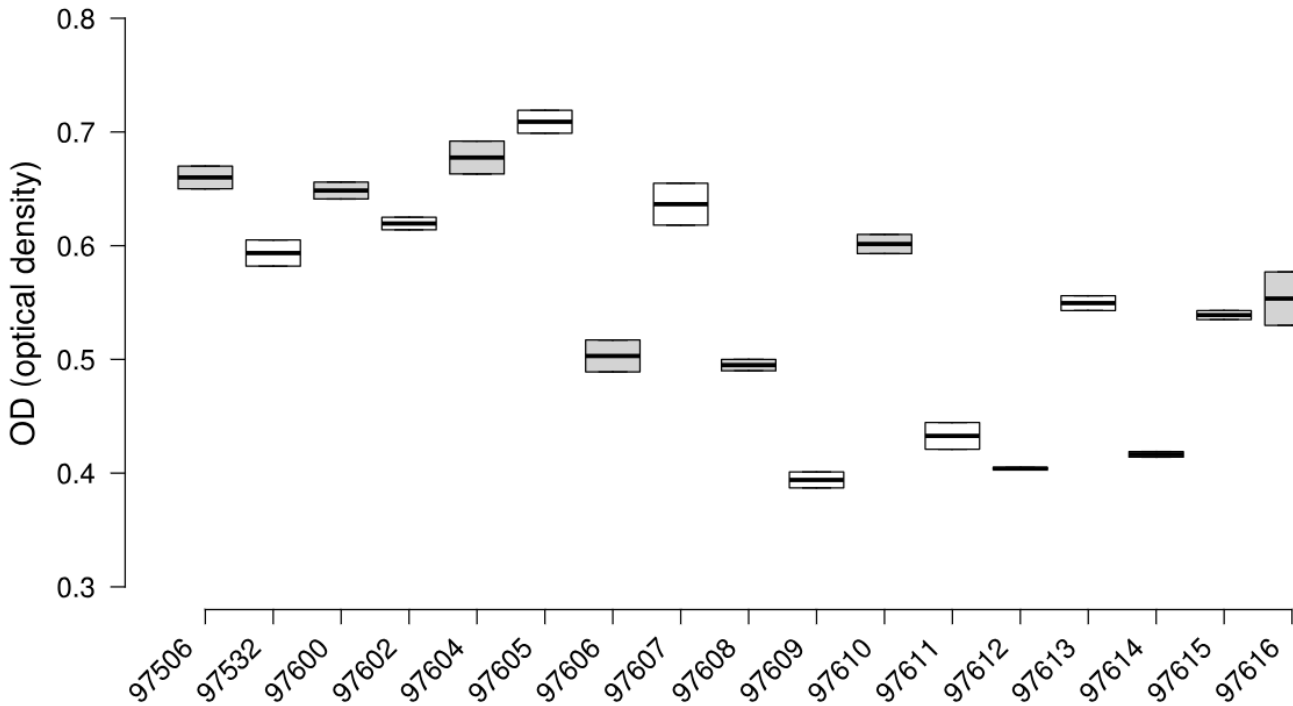


Figure I: Distribution of the optical density (OD) (boxplots) for the PT2023CAPXVIR_P6 samples (LAB97506-LAB97616).

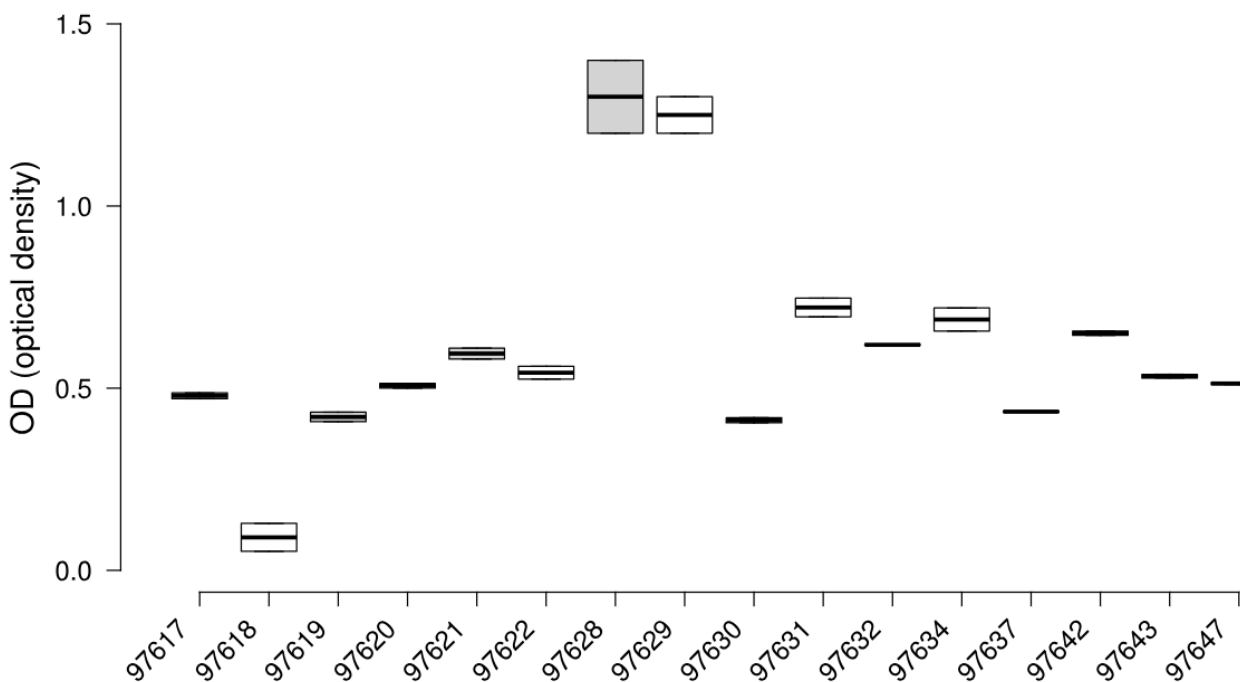


Figure II: Distribution of the optical density (OD) (boxplots) for the PT2023CAPXVIR_P6 samples (LAB97617-LAB97647).

10.2.2 VIRO: RT-QPCR

For the virology RT-qPCR part, box plots of the Ct-values per reference sample and per participating laboratory are shown in Figures III and IV. This panel included one repetition, therefore only the sample PT2023CAPXVIR_BP1 was included in the figures down below. Laboratories that used two different methods are represented twice in the boxplot. Note that lab 97604 is not shown as they only entered an interpretation as a result.

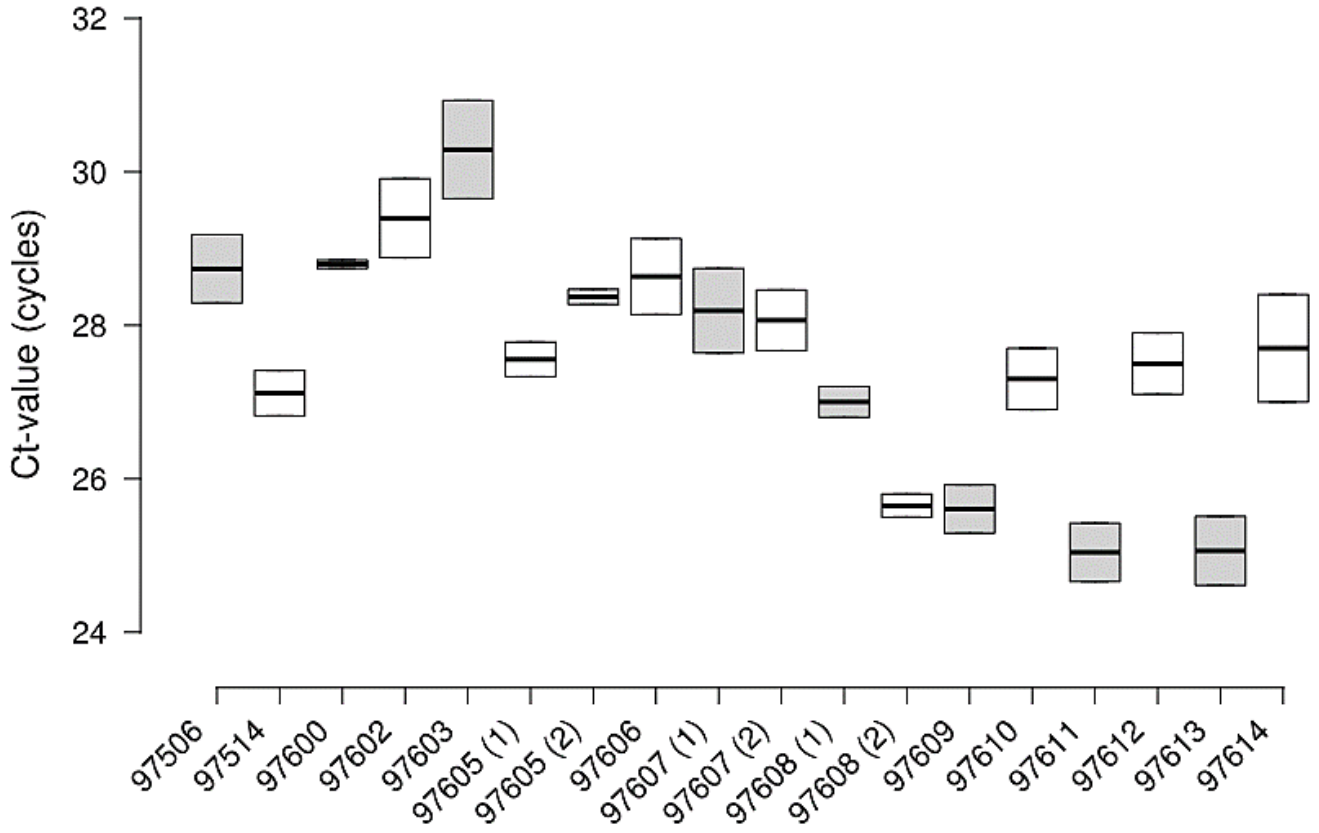


Figure III: Distribution of the Ct-values data (boxplots) for the PT2023CAPXVIR_BP1 samples (LAB97506-LAB97614).

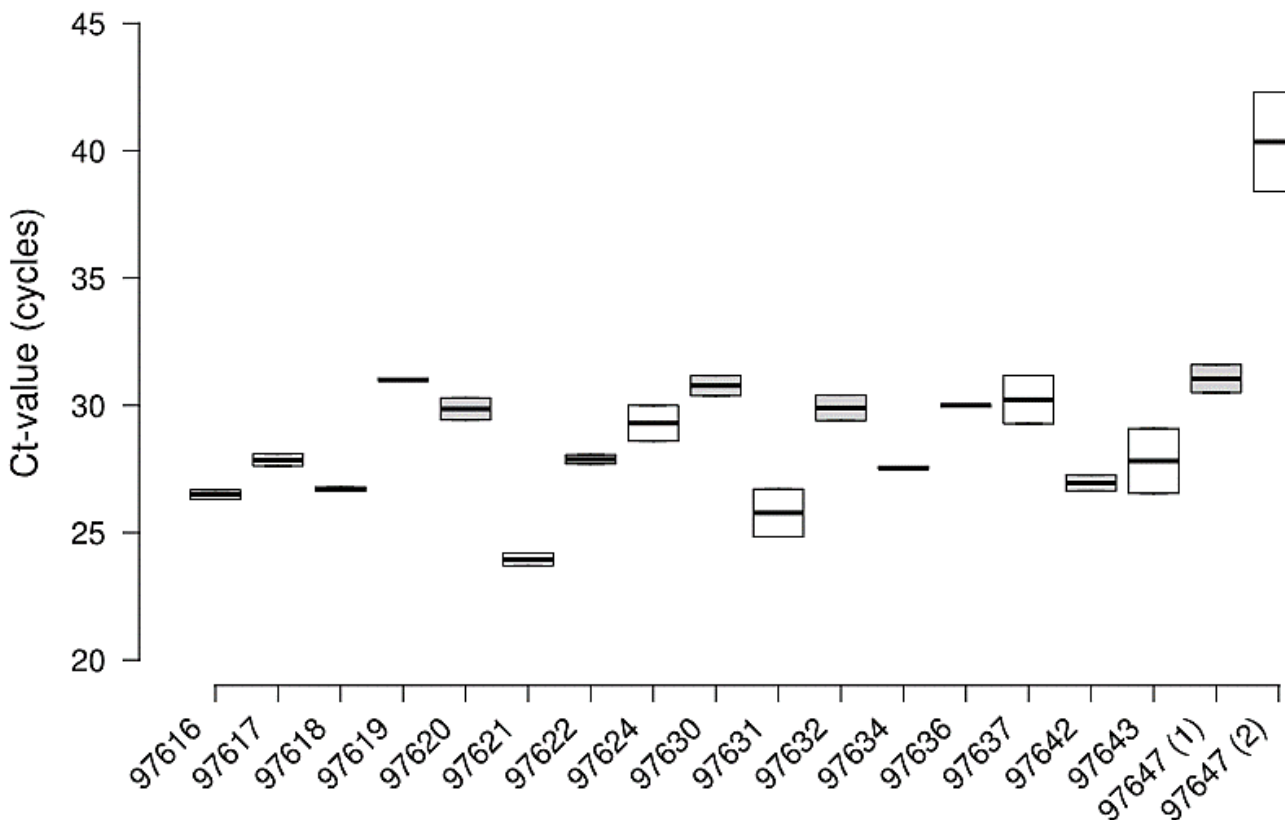


Figure IV: Distribution of the Ct-values data (boxplots) for the PT2023CAPXVIR_BP1 samples (LAB97616-LAB97647).

10.3 Annex 3: Additional information

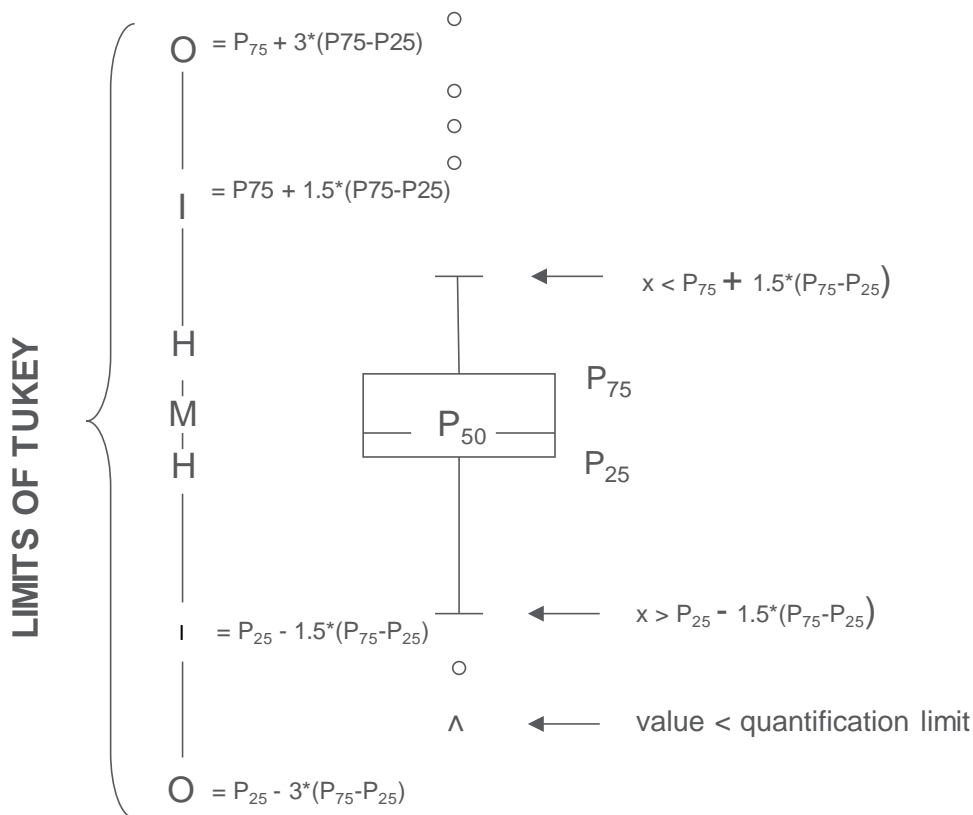
The **calendar** for Proficiency Testing in Veterinary diagnosis is available on our website:

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

Graphical representation

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

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



Corresponding limits in case of normal distribution

END

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