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EXTERNAL QUALITY ASSESSMENT IN VETERINARY DIAGNOSIS

DEFINITIVE GLOBAL REPORT

Proficiency Testing in Veterinary Diagnosis Capripox viruses

SURVEY 2020/3

Corrected version

Virology & Serology

Sciensano/PT VET CPX/1-E-cv

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This corrected version precises on page 4 that this EQA was performed under ISO1743 Standard.

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All the reports are also available on our webpage:

https://www.wiv-isp.be/QML/activities/PT%20VET/fr/originaux/rapports_annee.htm

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I. Introduction

Details relevant to the proficiency test (PT) are available in the procedure SOP 25/01 'Management of the proficiency tests organized by the scientific directorate infectious diseases in animals', which is summarized in the 'Manual for the participant'. The PT was organized according to the ISO17043 'Conformity assessment - General requirements for proficiency testing' norm.

<u>II. Aim</u>

The aim of this PT was to evaluate the ability of the participating laboratories to identify the absence or presence of antibodies to capripox viruses in serum of bovidae origin (Serology component of the PT) and/or to assess the diagnostic capability of the participating laboratories to detect capripox (CAPX) virus nucleic acid in samples containing material for CAPX virus molecular diagnostic (Virology component of the PT).

III. Materials and methods

III.1. Conduct 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 cell culture supernatant, and tissue homogenate samples using their primary diagnostic assay(s) for molecular diagnosis. 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.

III.2. Reference samples

Thirty-one laboratories received the PT2020CAPXSER panel containing 10 aliquots of serum and the PT2020CAPXVIR panel containing 10 aliquots of cell culture supernatant or tissue homogenate samples. One NRL received the PT2020CAPXSER panel and 6 NRLs received the PT2020CAPXVIR panel, only. The PT panels were prepared separately and within each panel samples were numbered from 1 to 10. The samples were prepared by the European Union Reference Laboratory for diseases caused by capripox viruses, Infectious diseases in Animals Directorate, Sciensano.

III.2.1. PT2020CAPXVIR panel: reference cell culture supernatant and tissue homogenate samples

Replicates of 4 reference cell culture supernatants, either free from detectable capripox virus nucleic acid (n = 1; coded PT2020CAPXVIR_VN1) or containing detectable capripox virus nucleic acid (n = 3; coded PT2020CAPXVIR_VP1, PT20CAPXVIR_VP2 and PT2020CAPXVIR_VP3) were used as well as replicates of 6 reference tissue homogenate samples, either free from detectable capripox nucleic acid (n = 1; coded PT2020CAPXVIR_TN1) or containing detectable CAPX nucleic acid (n = 5; coded PT2020CAPXVIR_TP1,

PT2020CAPXVIR_TP2, PT2020CAPXVIR_TP3, PT2020CAPXVIR_TP4 and PT2020CAPXVIR_TP5). PT20CAPXVIR_VP2 was a ten-fold dilution of PT2020CAPXVIR_VP1 and PT2020CAPXVIR_TP2 was a ten-fold dilution of PT2020CAPXVIR_TP1.

In total, 370 aliquots were distributed to 37 participating laboratories. These participants received 10 aliquots: 1 aliquot of each sample. The positions of the reference samples were randomized for each participant.

For each sample, its 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 (Agianniotaki et al. 2016; Haegeman et al. 2016; Chibssa et al. 2018).

Reference sample	origin	background	strain(s)	status
PT2020CAPXVIR_VN1	Cell culture	Culture medium	NA	Capx negative
PT2020CAPXVIR_TN1	Bovine tissue	Uninfected/unvaccinated	NA	Capx negative
PT2020CAPXVIR_VP1	Cell culture	GTPV culture	Gorgon field strain1/1000	Capx positive/GTPV/Wildtype
PT2020CAPXVIR_VP2	Cell culture	GTPV culture	Gorgon field strain1/10 000	Capx positive/GTPV/Wildtype
PT2020CAPXVIR_VP3	Cell culture	LSDV culture	Bulgarian field strain	Capx positive/LSDV/field
PT2020CAPXVIR_TP1	Bovine tissue	Infected	Israeli field strain 1/3	Capx positive/LSDV/field
PT2020CAPXVIR_TP2	Bovine tissue	Infected	Israeli field strain 1/30	Capx positive/LSDV/field
PT2020CAPXVIR_TP3	Bovine tissue	Infected	Neethling strain	Capx positive/LSDV/Vaccine
PT2020CAPXVIR_TP4	Bovine tissue	Infected	Israeli field strain	Capx positive/LSDV/field
PT2020CAPXVIR_TP5	Ovine Tissue	Infected	Moroccan field strain	Capx positive/SPPV/field

Table 1. The samples of the PT2020CAPXVIR panel

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). 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 nucleic acid. In addition, 3 aliquots of each reference sample were tested after the PT using the real-time PCR for capripox D5R (Haegeman et al., 2013) in order to confirm the stability and status of the samples (post-verification).

In conclusion, for the **detection of capripox virus nucleic acids**, the samples PT2020CAPXVIR_VN1 and PT2020CAPXVIR_TN1 were considered as capripox virus negative samples and the samples PT2020CAPXVIR_VP1, PT2020CAPXVIR_VP2, PT2020CAPXVIR_TP1, PT2020CAPXVIR_TP2, PT2020CAPXVIR_TP3, PT2020CAPXVIR_VP3, PT2020CAPXVIR_TP4, PT2020CAPXVIR_TP5 positive samples. For sample PT2020CAPXVIR_VN1 no value could obtained for the internal control in the real-time PCR due to its origin (cell culture medium). Therefore in addition to a negative result, a non-interpretable (doubtful) result was also accepted.

For the **capripox virus species differentiation**, the samples PT2020CAPXVIR_VN1 and PT2020CAPXVIR_TN1 were considered as negative samples, the sample PT2020CAPXVIR_TP5 as SPPV positive samples (where SPPV or SPPV/GTPV results were considered acceptable), and the samples PT2020CAPXVIR_TP1, PT2020CAPXVIR_TP2 and PT2020CAPXVIR_TP3, PT2020CAPXVIR_TP4 and PT2020CAPXVIR_VP3 as LSDV positive samples (where LSDV or GTPV/LSDV results were considered acceptable) and samples PT2020CAPXVIR_VP1 and PT2020CAPXVIR_VP2 as GTPV positive samples (SPPV/GTPV or GTPV/LSDV were considered acceptable).

Finally, for the **field or vaccine strain differentiation**, the samples PT2020CAPXVIR_VN1 and PT2020CAPXVIR_TN1 were considered as negative samples, the samples PT2020CAPXVIR_VP3, PT2020CAPXVIR_TP1 PT2020CAPXVIR_TP2, PT2020CAPXVIR_TP4 and PT2020CAPXVIR_TP5 as field strain and the sample PT2020CAPXVIR_TP3 as vaccine strain. For PT2020CAPXVIR_VP1, PT2020CAPXVIR_VP2, no field or vaccine differentiation was required, hence all answers were considered in agreement with the assigned status.

Final Sample Status

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

sample ID	final status RT-PCR	final status Species Differentiation PCR	final status DIVA PCR	final diagnostic
PT2020CAPXVIR_VN1	Negative/ Doubtful	Negative	Negative	CAPX Negative
PT2020CAPXVIR_TN1	Negative	Negative	Negative	CAPX Negative
PT2020CAPXVIR_VP1	Positive	GTPV	GTPV field	CAPX positive/GTPV/field
PT2020CAPXVIR_VP2	Positive	GTPV	GTPV field	CAPX positive/GTPV/field
PT2020CAPXVIR_TP1	Positive	LSDV	LSDV field	CAPX positive/LSDV/field
PT2020CAPXVIR_TP2	Positive	sitive LSDV LSDV		CAPX positive/LSDV/field
PT2020CAPXVIR_TP3	Positive	LSDV	LSDV vaccine	CAPX positive/LSDV/vaccine
PT2020CAPXVIR_VP3	Positive	LSDV	LSDV field	CAPX positive/LSDV/field
PT2020CAPXVIR_TP4	Positive	LSDV	LSDV field	CAPX positive/LSDV/field
PT2020CAPXVIR_TP5	Positive	SPPV	SPPV field	CAPX positive/SPPV/field

Table 2. The final status of each sample

Randomisation and panel composition

Since a specific number has been assigned to each laboratory, the randomisation was performed as follows:

Sample Order	Odd Laboratories	Even Laboratories
V.CPX 2001	PT2020CAPXVIR_VP3	PT2020CAPXVIR_VP1
V. CPX 2002	PT2020CAPXVIR_VP1	PT2020CAPXVIR_VP2
V.CPX 2003	PT2020CAPXVIR_TP2	PT2020CAPXVIR_TP1
V.CPX 2004	PT2020CAPXVIR_VP2	PT2020CAPXVIR_TP2
V.CPX 2005	PT2020CAPXVIR_TP4	PT2020CAPXVIR_TP3
V.CPX 2006	PT2020CAPXVIR_TP3	PT2020CAPXVIR_VP3
V.CPX 2007	PT2020CAPXVIR_TN1	PT2020CAPXVIR_TP4
V.CPX 2008	PT2020CAPXVIR_TP5	PT2020CAPXVIR_TP5
V.CPX 2009	PT2020CAPXVIR_VN1	PT2020CAPXVIR_VN1
V.CPX 2010	PT2020CAPXVIR_TP1	PT2020CAPXVIR_TN1

Table 3. PT2020CAPXVIR Panel composition for odd and even laboratories

The PT2020CAPXVIR panel was constituted of 10 samples of 600 µl.

III.2.2. PT2020CAPXSER panel: reference serum samples

Replicates of 9 reference serum samples, either free from detectable antibodies to capripox viruses (n=2; coded PT2020CAPXSER_SERN1 and PT2020CAPXSER_SERN2) or containing detectable antibodies to capripox viruses (n=7; coded PT2020CAPXSER_SERP1, PT2020CAPXSER_SERP2,

PT2020CAPXSER_SERP3, PT2020CAPXSER_SERP4, PT2020CAPXSER_SERP5,

PT2020CAPXSER_SERP6 and PT2020CAPXSER_SERP7) were used. In total, 320 aliquots were distributed to 32 participating laboratories.

These participants received 10 aliquots: 2 aliquots of the sample PT2020CAPXSER_SERP4 and 1 aliquot of the samples PT2020CAPXSER_SERN1, PT2020CAPXSER_SERN2,

PT2020CAPXSER_SERP1, PT2020CAPXSER_SERP2, PT2020CAPXSER_SERP3,

PT2020CAPXSER_SERP5, PT2020CAPXSER_SERP6 and PT2020CAPXSER_SERP7. The serum samples were randomized for each participant.

For each serum sample, its 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), the virus neutralisation test with the serum titrated against a constant titre of capripox virus (VN1) and the virus neutralisation test with a capripox virus titrated against a constant dilution of serum (neutralisation index) (VN2).

Table 4. Samples of the PT2020CAPXSER panel

Reference serum sample	origin	background	status
PT2020CAPXSER_SERN1	ovine	Commercial serum	negative
PT2020CAPXSER_SERN2	bovine	Uninfected/unvaccinated	negative
PT2020CAPXSER_SERP1	ovine	Vaccinated + infected	positive
PT2020CAPXSER_SERP2	bovine	Vaccinated only	positive
PT2020CAPXSER_SERP3	bovine	Vaccinated + infected	positive
PT2020CAPXSER_SERP4	bovine	Infected	positive
PT2020CAPXSER_SERP5	bovine	Vaccinated + Infected	positive
PT2020CAPXSER_SERP6	bovine	Vaccinated + Infected	positive
PT2020CAPXSER_SERP7	ovine	Infected	positive

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, VN1 and VN2. For each sample, the same qualitative result was obtained for all 10 aliquots of the same reference serum sample for each test. However when IPMA, VN1 and VN2 were performed using heterologous virus (LSDV for SPPV-positive samples or vice versa), for sample PT2020CAPXSER_SERP1 and PT2020CAPXSER_SERP7 not all aliquots yielded the same results. Since IPMA, VN1 and VN2 are usually only performed using only one virus species, IPMA, VN1, VN2 are considered doubtful for this sample. The results of PT2020CAPXSER_SERP2 in VN was doubtful, yet in ELISA the same qualitative result was obtained for all 10 aliquots, therefore VN1 and VN2 were considered doubtful for this sample. All serum samples were considered as reliable samples in order to evaluate the ability of laboratories to identify the absence or presence of antibodies to capripox viruses in serum. In addition, 3 aliquots of each serum sample were tested once after the PT in order to confirm their stability and status (post-verification) using the ELISA ID Screen® Capripox Double Antigen Multi-species (ID.Vet), IPMA and VN1.

Taken together, the reference serum samples PT2020CAPXSER_SERN1 and PT2020CAPXSER_SERN2 were considered as negative samples, and the reference serum samples, PT2020CAPXSER_SERP3, PT2020CAPXSER_SERP4, PT2020CAPXSER_SERP5 and PT2020CAPXSER_SERP6 as positive samples. The reference serum PT2020CAPXSER_SERP1 and and PT2020CAPXSER_SERP7 were considered as positive samples in the ELISA ID Screen® Capripox Double Antigen Multi-species (ID.Vet), while they were considered as doubtful samples in the IPMA, VN1 and VN2. Reference sample PT2020CAPXSER_SERP2 was considered as doubtful in VN1 and VN2. For these samples, positive, non-interpretable (doubtful) or negative results will be considered acceptable.

Final Sample Status

The final sample status was determined by the EURL for diseases caused by capripoxvirusses using the pre-PT results.

Table 5. Final sample status of each sample

sample ID	Status
PT2020CAPXSER_SERP1	POS
PT2020CAPXSER_SERP2	POS
PT2020CAPXSER_SERP3	POS
PT2020CAPXSER_SERP4	POS
PT2020CAPXSER_SERP5	POS
PT2020CAPXSER_SERP6	POS
PT2020CAPXSER_SERP7	POS
PT2020CAPXSER_SERN1	NEG
PT2020CAPXSER_SERN2	NEG

Randomisation and panel composition

Since a specific number has been assigned to each laboratory, the randomisation has been performed as follow:

Sample Order	Odd laboratories	Even laboratories
S.CPX 2001	PT2020CAPXSER_SERN2	PT2020CAPXSER_SERP1
S.CPX 2002	PT2020CAPXSER_SERN1	PT2020CAPXSER_SERP2
S.CPX 2003	PT2020CAPXSER_SERP4	PT2020CAPXSER_SERP3
S.CPX 2004	PT2020CAPXSER_SERP7	PT2020CAPXSER_SERP4
S.CPX 2005	PT2020CAPXSER_SERP5	PT2020CAPXSER_SERP5
S.CPX 2006	PT2020CAPXSER_SERP3	PT2020CAPXSER_SERP6
S.CPX 2007	PT2020CAPXSER_SERP6	PT2020CAPXSER_SERP7
S.CPX 2008	PT2020CAPXSER_SERP4	PT2020CAPXSER_SERP4
S.CPX 2009	PT2020CAPXSER_SERP2	PT2020CAPXSER_SERN1
S.CPX 2010	PT2020CAPXSER_SERP1	PT2020CAPXSER_SERN2

Table 6. PT2020CAPXSER panel composition for odd and even laboratories

The PT2020CAPXSER panel consisted of 10 serum samples of 500 $\mu l.$

III.3. Classification of results, level of agreement and threshold for qualification

III.3.1. Classification of results

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

III.3.2. Level of agreement

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

III.3.3. Threshold for qualification

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

IV. Results

IV.1. The participants

Twenty-four NRL's of European Union Member States and 14 laboratories from third countries participated to the Capripox Virology survey.

Table 7. The EO Member		Participation in	Participation in
Country	Name	serology survey	virology survey
Austria	Austrian Agency for Health and Food Safety Inst. for veterinary Disease Control Mödling, NRL for CaPV	1	1
Belgium and Luxembourg	Sciensano, NRL for CaPV	1	1
Bulgaria	National Diagnostic and Research Veterinary Medical Institute; Department "Exotic diseases"	0	1
Croatia	Croatian Veterinary Institute	1	1
Cyprus	Laboratory for animal health, virology section	0	1
Czech Republic	State Veterinary Institute Prague	1	1
Denmark	DTU National Veterinary Institute	1	1
Finland	Finnish Food Authority, Virology Unit	0	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 Office, Veterinary Diagnostic Directorate, Laboratory for Molecular Biology	1	1
Ireland	Central Veterinary Research Laboratory	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 molecula Biology and Genetically Modified organisms, Department of serology	1	1
Malta	Veterinary and Phytosanitary Regulation Department, National Veterinary Laboratory	1	0
Poland	National Veterinary Research Institute;	1	1
Portugal	Instituto Nacional de Investigação 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,	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 7.	The	ΕU	Member	State	NRL's
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Table 8. Non EU Member State participants

Country	Name	Participation in serology survey	Participation in virology survey
United Kingdom	The Pirbright Institute	1	1
Albania	Food Safety and Veterinary Insitute, Dep of Animal Health, Molecular Biology	0	1
Belarus	Belarusian State Veterinary Centre	0	1
Bosnia Herzegovina	Veterinary Faculty of the University of Sarajevo Avian and lagomorphic virology laboratory	1	1
Georgia	Laboratory of the Ministry of Agriculture (LMA) of Georgia	0	1
Kazakhstan	National Veterinary Reference Centre Astana	1	1
Kazakhstan	National Veterinary Reference Centre Almaty	1	1
Kosovo	Kosovo Food And Veterinary Laboratory, Kosovo Food And Veterinary Agency	1	1
Montenegro	Diagnostic Veterinary Laboratory	1	1
Republic of North Macedonia	Faculty of Veterinary Medicine Skopje, Laboratory for serology and molecular diagnostics	1	1
Russian Federation	Federal Center for Animal Health "FGBI ARRIAH" Reference laboratory for bovine diseases	1	1
Serbia	Veterinary Specialized Institute Kraljevo	1	1
Turkey	Istanbul Pendik Veterinary Control Institute, Capripoxvirus National Laboratory	1	1
Ukraine	The State Scientific and Research Institute of Laboratory Diagnostics and Veterinary and Sanitary Expertise	1	1

IV.2. Survey Timeline

Transfer of the samples from NRL to QL: 24/04/2020

Randomization of the samples by QL: from 24/04 to 30/04

Sending samples to participants: from 05/05/2020 to 05/06/2020. The samples were sent on dry ice.

NB: due to COVID problem, some borders were closed and the shipment of the samples was not allowed.

Therefore, the shipments were performed at different dates for the different countries.

Deadline for the results encoding: 30/06/2020

Preliminary report: 24/07/2020

The preliminary report is available at:

https://www.wiv-isp.be/QML/activities/PT%20VET/fr/originaux/rapports annee.htm

Final report: 24/09/2020

Amended final report:30/11/2020

IV.3. Compliance with the procedure

All participating laboratories have provided a duly dated and signed copy of the results, except laboratory 97626, who did not report results for the virology component of the PT2020.

IV.4. Qualitative data analysis

IV.4.1. Virology

IV.4.1.1. pan-capripox real time PCR

IV.4.1.1.1. Results per sample

36 laboratories encoded results. 32 laboratories encoded 1 dataset and 4 laboratories encoded 2 datasets. In total, 40 datasets were encoded in total.

Sample ID	Expected result	Positive	Negative	NI	Comment	Status*
PT2020CAPXVIR_VP1	Positive	38	2	0	2 false negative results	Frequently detected
PT2020CAPXVIR_VP2	Positive	33	7	0	7 false negative results	detected
PT2020CAPXVIR_TP1	Positive	39	1	0	1 false negative result	Frequently detected
PT2020CAPXVIR_TP2	Positive	40	0	0	ОК	Frequently detected
PT2020CAPXVIR_TP3	Positive	39	1	0	1 false negative result	Frequently detected
PT2020CAPXVIR_VP3	Positive	37	2	1	2 false negative result and 1 doubtful result	Frequently detected
PT2020CAPXVIR_TP4	Positive	40	0	0	Ok	Frequently detected
PT2020CAPXVIR_TP5	Positive	39	1	0	1 false negative result	Frequently detected
PT2020CAPXVIR_VN1	Negative	0	38	2	2 doubtful results	Negative/ Doubtful
PT2020CAPXVIR_TN1	Negative	0	40	0	ОК	Negative

Table 9 Results per sample

*: 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 (<u>www.qcmd.org</u>).

Summary of the results

Table 10 Summary of the results

Parameter	N	%		
Number of results	400	100		
Number of correct results	385	96,25		
Wrong results	17	4,25		
False negative	14	82,35		
NI=Doubtful	1	5,88		

IV.4.1.1.2 Results per method

Table	11.	Proficiency	per	method
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Method	Target gene	Ν	NR	NCR	%	FP	FN	NI
Real-Time PCR detection of CAPXV, SPPV, GTPV, LSDV	ORF074	1	10	10	100	0	0	0
Haegeman et al., 2013	D5R/E3L	3	30	29	96,7	0	1	0
Bowden et al., 2008	P32	16	160	158	98,75	0	2	0
Babiuk et al., 2008	P32	2	20	20	100	0	0	0
Dietze et al. 2018	P32	1	10	10	100	0	0	0
Vidanović et al. 2016.	EEV	1	10	6	60	0	4	0
IDVET-ID gene Capripox virus triplex		3	30	30	100	0	0	0
Lumpy skin disease DNA detection Kit (Fractabio)		2	20	15	75	0	5	0
RT-PCR (Pan-Capripox)	RPO30	1	10	10	100	0	0	0
6.3.51.1 Capripoxviruses qPCR	P32	1	10	10	100	0	0	0
Balinsky et al., 2008	ORF 068	1	10	10	100	0	0	0
In-house Path-ID qPCR	P32	1	10	9	90	0	0	1
00-14-0959 (In-house)	P32	1	10	10	100	0	0	0
Stubbs pirbright	P32	1	10	10	100	0	0	0
Lamien et al., 2011	RPO30	1	10	8	80	0	2	0
NVR-SOP-20 (Bowden et al., 2013)	ORF074	1	10	10	100	0	0	0
In-house rt-qPCR	ORF074	1	10	10	90	0	0	0
FGBI ARRIAH		1	10	10	100	0	0	0
In-house	P32	1	10	10	100	0	0	0
Total		40	400	383	96,3	0	14	3

N: number of laboratories; NR: number of results; NCR: Number of correct results; %: % success; FP: false positive; FN: false negative; NI: not interpretable (doubtfull)

IV.4.1.1.3 Results per laboratory

For the detection of capripox virus nucleic acid by **real-time PCR (RT-PCR) in the PT panel** : 31 out of 36 participating laboratories provided qualitative results that were in full agreement with the assigned status of the 10 reference samples (100% of agreement), whereas LAB97602, LAB97605 and LAB97616 misclassified 1 aliquot (90% of agreement), LAB97629 misclassified 2 aliquots (80% of agreement). LAB97628 misclassified 3 aliquots (70% agreement).

Among the 4 laboratories that performed a second optional RT-PCR, 1 NRL (LAB97607) was in full agreement (100% agreement) with the assigned status of the 10 reference samples, 1 laboratory (LAB97605) misclassified 1 aliquot (90% of agreement). In their primary PCR they misclassified this aliquot as well. Two laboratories misclassified 4 and 2 aliquots (LAB97620 and LAB97621, respectively) in their secondary PCR, while they classified all aliquots correct in their primary PCR.

Table 12. Results per laboratory

Laboratory	VP1	VP2	TP1	TP2	TP3	VP3	TP4	TP5	VN1	TN1	%
	Pos	Neg/ NI	Neg								
97506	Pos	Neg	Neg	100							
97600	Pos	Neg	Neg	100							
97602	Pos	Neg	Pos	Pos	Pos	Pos	Pos	Pos	Neg	Neg	90
97604	Pos	Neg	Neg	100							
97606	Pos	Neg	Neg	100							
97608	Pos	Neg	Neg	100							
97610	Pos	Neg	Neg	100							
97612	Pos	Neg	Neg	100							
97614	Pos	Neg	Neg	100							
97616	Pos	Pos	Pos	Pos	Pos	NI	Pos	Pos	Neg	Neg	90
97618	Pos	Neg	Neg	100							
97620	Pos	Neg	Neg	100							
97620	Neg	Neg	Pos	Pos	Neg	Pos	Pos	Neg	Neg	Neg	60
97622	Pos	Neg	Neg	100							
97624	Pos	Neg	Neg	100							
97628	Neg	Neg	Pos	Pos	Pos	Neg	Pos	Pos	Neg	Neg	70
97630	Pos	Neg	Neg	100							
97632	Pos	Neg	Neg	100							
97634	Pos	Neg	Neg	100							
97636	Pos	Neg	Neg	100							
97601	Pos	Neg	Neg	100							
97603	Pos	Neg	Neg	100							
97605	Pos	Neg	Pos	Pos	Pos	Pos	Pos	Pos	Neg	Neg	90
97605	Pos	Neg	Pos	Pos	Pos	Pos	Pos	Pos	Neg	Neg	90
97607	Pos	NI	Neg	100							
97607	Pos	NI	Neg	100							
97609	Pos	Neg	Neg	100							
97611	Pos	Neg	Neg	100							
97613	Pos	Neg	Neg	100							

97617	Pos	Neg	Neg	100							
97619	Pos	Neg	Neg	100							
97621	Pos	Neg	Neg	100							
97621	Pos	Neg	Pos	Pos	Pos	Neg	Pos	Pos	Neg	Neg	80
97623	Pos	Neg	Neg	100							
97625	Pos	Neg	Neg	100							
97627	Pos	Neg	Neg	100							
97629	Pos	Neg	Neg	Pos	Pos	Pos	Pos	Pos	Neg	Neg	80
97631	Pos	Neg	Neg	100							
97633	Pos	Neg	Neg	100							
97635	Pos	Neg	Neg	100							

Pos: positive; Neg: negative; NI: doubtful; %: % success; laboratories that scored less than 90% agreement are indicated in bold

IV.4.1.1.4 Results per thermocyler

For 40 PCR results the used thermocycler was indicated.

Table 13. Proficiency per thermocycler

Thermocycler	N	NR	NCR	%
ABI 7500	4	40	40	100
ABI 7500 Fast	2	20	20	100
ABI 7900 HT Fast Real-Time PCR System	1	10	10	100
ABI Verity	1	10	8	80
Agilent AriaMx	2	20	20	100
Biorad CFX Connect	1	10	10	100
Bio-Rad CFX96	4	40	40	100
Bio-Rad CFX96 Touch	2	20	20	100
Mx3005p	2	20	19	95
QuantStudio5	1	10	10	100
ROCHE LC 96	4	40	40	100
ROCHE LC 480	2	20	20	100
Roche Light Cycler 2.0	1	10	10	100
Rotogene	3	30	30	100
Rotor geneQ	3	30	29	96,7
Rotorgene 3000	1	10	10	100
rotorgene 6000	2	20	15	75

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Smartcycler II Cepheid	1	10	10	100
Stratagene Mx3005P	3	30	26	86.7

N: number of laboratories; NR: number of results; NCR: Number of correct results; %: % success

IV.4.1.2. Species differentiation

IV.4.1.2.1 Results per sample

Twenty-seven laboratories encoded results for species differentiation.

Out of 246 encoded results, 230 were considered as successful by the EURLfor disease caused by capripox viruses (93,5%).

Sample ID	Expected result	GTPV	GTPV/ LSDV	LSDV	SPPV	Neg	ND	NR	NCR	%
PT2020CAPXVIR_VP1	GTPV	19	2	0	2	2	2	25	21	84
PT2020CAPXVIR_VP2	GTPV	18	2	0	1	4	2	25	20	80
PT2020CAPXVIR_TP1	LSDV	0	3	23	0	1	0	27	23	85
PT2020CAPXVIR_TP2	LSDV	0	3	22	0	1	1	26	22	85
PT2020CAPXVIR_TP3	LSDV	1	2	24	0	0	0	27	24	89
PT2020CAPXVIR_VP3	LSDV	0	2	21	1	1	2	25	21	84
PT2020CAPXVIR_TP4	LSDV	0	2	23	0	1	1	26	23	88
PT2020CAPXVIR_TP5	SPPV	0	0	0	24	1	2	25	24	96
PT2020CAPXVIR_VN1	Negative	0	0	0	0	21	6	21	21	100
PT2020CAPXVIR_TN1	Negative	0	0	0	0	22	5	22	22	100

Table 14. Results and proficiency per sample for species differentiation

ND: Not determined; NR: number of results; NCR: Number of correct results; %: % success

IV.4.1.2.2 Results per method

Protocol	Method	Target	N	NR	NCR	%
Agianniotaki et al., 2016 in-house Taqman assay	RT-qPCR	GPCR	1	10	10	100
Lamien et al. 2011a	PCR	GPCR	6	54	50	93,2
Lamien et al. 2011b	RT-qPCR	RPO 030	2	20	20	100
Capripoxviruses GPCR seq	PCR+seq	GPCR	1	10	10	100
Adedeji et al. , 2019 Möller et al., 2019	RT-qPCR	UD	1	10	10	100
Gelaye et al., 2013	RT-qPCR	RPO 030	1	10	10	100
Biosellal Bio-T kit Lumpy Skin disease Dual hybridization probe Assay	RT-qPCR	UD	1	7	7	100
in house	PCR+Seq	RPO 030	1	5	5	100
ID Gene LSD DIVA Triplex	RT-qPCR	UD	2	17	13	76,4
Lamien et al., 2011a Gelaye et al. 2015	PCR + Seq	RPO 30	1	8	8	100
Vidanovic et al., 2016	RT-qPCR	EEV	1	10	10	100
RT-PCR GPCR (Specific LSDV)	RT-qPCR	GPCR	1	9	6	66
Lamien et al., 2011a Haegeman, 2015	PCR + Seq	UD	1	8	8	100
Galaye et al., 2017	RT-qPCR	RPO147	1	8	8	100
Gelaye et al. 2015	RT-qPCR	RPO30	1	10	10	100
Tuppurainen et al., 2014	RT-qPCR	G-protein- coupled chemokine receptor	1	10	10	100
Menasherow et al., 2014	PCR	EEV	1	10	8	80
Home made	RT-qPCR	UD	3	30	27	90
Total			27	246	230	93,5

Table 15. Proficiency per method for species differentiation

UD: undisclosed; N: number of laboratories; NR: number of results; NCR: Number of correct results; %: % success

IV.4.1.2.3 Results per laboratory

For the **differentiation of capripox virus species**: Fourteen out of twenty-seven participating laboratories provided qualitative results that were in full agreement with the assigned status of the 10 reference samples (100% of agreement), whereas LAB97602 misclassified 1 aliquot (90% of agreement), LAB97621 and LAB97625 misclassified 2 aliquots (80% of agreement) and LAB97628 and LAB97629 misclassified 4 aliquots (60% of agreement)

LAB97612, LAB97623 and LAB97636 did the analysis only on 7 out of 10 samples, LAB97609, LAB97614 and LAB97617 did the analysis on 8 samples and LAB97618 only on 5 out of 10 samples. These laboratories provided qualitative results that were in full agreement with the assigned status of the samples. LAB97607 did the analysis of 9 aliquots and misclassified 3 aliquots (67% agreement)

Table 16. Result of the species differentiation	per participating laboratory
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Table To	. Result	or the spe	ecies un	erentiatio	in per pai	ucipating	j laborato	лу			
	VP1	VP2	TP1	TP2	TP3	VP3	TP4	TP5	VN1	TN1	%
Lab ID	GTPV	GTPV	LSDV	LSDV	LSDV	LSDV	LSDV	SPPV	Neg	Neg	(NCR/ NDR)
97506	GTPV	GTPV	LSDV	LSDV	LSDV	LSVD	LSDV	SPPV	Neg	Neg	100 (10/10)
97600	GTPV	GTPV	LSDV	LSDV	LSDV	LSVD	LSDV	SPPV	Neg	Neg	100 (10/10)
97602	GTPV	Neg	LSDV	LSDV	LSDV	LSVD	LSDV	SPPV	Neg	Neg	90 (9/10)
97604	GTPV	GTPV	LSDV	LSDV	LSDV	LSVD	LSDV	SPPV	Neg	Neg	100 (10/10)
97608	GTPV	GTPV	LSDV	LSDV	LSDV	LSDV	LSDV	SPPV	Neg	Neg	100 (10/10)
97610	GTPV	GTPV	LSDV	LSDV	LSDV	LSDV	LSDV	SPPV	Neg	Neg	100 (10/10)
97612	ND	ND	LSDV	LSDV	LSDV	LSDV	LSDV	ND	Neg	Neg	100 (7/7)
97614	GTPV	GTPV	LSDV	LSDV	LSDV	LSDV	LSDV	SPPV	ND	ND	100 (8/8)
97618	GTPV	GTPV	LSDV	ND	LSDV	ND	ND	SPPV	ND	ND	100 (5/5)
97620	GTPV/ LSDV	SPPV	Neg	Neg	100 (10/10)						
97628	Neg	Neg	GTPV/ LSDV	GTPV/ LSDV	GTPV	Neg	LSDV	SPPV	Neg	Neg	60 (6/10)
97632	GTPV	GTPV	LSDV	LSDV	LSDV	LSDV	LSDV	SPPV	Neg	Neg	100 (10/10)
97634	GTPV	GTPV	LSDV	LSDV	LSDV	LSDV	LSDV	SPPV	Neg	Neg	100 (10/10)
97636	ND	ND	LSDV	LSDV	LSDV	LSDV	LSDV	ND	Neg	Neg	100 (7/7)
97603	GTPV/ LSDV	SPPV	Neg	Neg	100 (10/10)						
97607	Neg	Neg	LSDV	LSDV	LSDV	LSDV	LSDV	Neg	ND	Neg	67 (6/9)
97609	GTPV	GTPV	LSDV	LSDV	LSDV	LSDV	LSDV	SPPV	ND	ND	100 (8/8)
97611	GTPV	GTPV	LSDV	LSDV	LSDV	LSDV	LSDV	SPPV	Neg	Neg	100 (10/10)
97613	GTPV	GTPV	LSDV	LSDV	LSDV	LSDV	LSDV	SPPV	Neg	Neg	100 (10/10)
97617	GTPV	GTPV	LSDV	LSDV	LSDV	LSDV	LSDV	SPPV	ND	ND	100 (8/8)
97619	GTPV	GTPV	LSDV	LSDV	LSDV	LSDV	LSDV	SPPV	Neg	Neg	100 (10/10)
97621	GTPV	GTPV	LSDV	Neg	LSDV	LSDV	Neg	SPPV	Neg	Neg	80 (8/10)
97623	GTPV	GTPV	LSDV	LSDV	LSDV	ND	LSDV	SPPV	ND	ND	100 (7/7)
97625	SPPV	SPPV	LSDV	LSDV	LSDV	LSDV	LSDV	SPPV	Neg	Neg	80 (8/10)
97629	SPPV	Neg	Neg	LSDV	LSDV	SPPV	LSDV	SPPV	Neg	Neg	60 (6/10)
97631	GTPV	GTPV	LSDV	LSDV	LSDV	LSDV	LSDV	SPPV	Neg	Neg	100 (10/10)
97633	GTPV	GTPV	LSDV	LSDV	LSDV	LSDV	LSDV	SPPV	Neg	Neg	100 (10/10)

NR: number of results; NCR: Number of correct results; %: % success; (number of correct results/number of determined result)

IV.4.1.3 DIVA PCR

In this section, laboratories could report whether the samples contained field or vaccine strains. Twentyfive laboratories encoded results. For samples PT2020CAPXVIR_VP1 and PT2020CAPXVIR_VP2, DIVA results were not required as there is currently no published DIVA method to differentiate GTPV field from vaccine strains. Therefore, all results were considered correct.

IV.4.1.3.1 Results per sample

	Table 17.	Results	and	proficiency	per	sample	for	DIVA
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Sample ID	Expected	Encoded result	N	NR	NCR	%
PT2020CAPXVIR_VP1	GTPV field	1 SPPV field 17 ND 2 NI 5 Neg	8	8	8	100
PT2020CAPXVIR_VP2	GTPV field	1 SPPV field 18 ND 2 NI 4 Neg	7	7	7	100
PT2020CAPXVIR_TP1	LSDV field	22 LSDV field 1 NI 1 ND 1 Neg	24	24	22	92
PT2020CAPXVIR_TP2	LSDV field	24 LSDV field 1 ND	24	24	24	100
PT2020CAPXVIR_TP3	LSDV vaccine	21 LSDV vaccine 2 LSDV field 1 ND 1 Neg	24	24	21	87,5
PT2020CAPXVIR_VP3	LSDV field	22 LSDV Field 2 ND 1 Neg	23	23	22	96
PT2020CAPXVIR_TP4	LSDV field	21 LSDV field 3 ND 1 Neg	22	22	21	95
PT2020CAPXVIR_TP5	SPPV field	7 SPPV field 15 ND 3 Neg	10	10	7	70
PT2020CAPXVIR_VN1	Negative	10 ND 15 Neg	15	15	15	100
PT2020CAPXVIR_TN1	Negative	11 ND 14 Neg	14	14	14	100

NI: not interpretable; ND: Not determined; N: number of laboratories; NR: number of results, NCR: Number of correct results; %: % success

IV.4.1.3.2 Results per method

Table 18. Results per method for DIVA

Method	Ν	NR	NCR	%
ID gene LSD DIVA triplex	5	37	31	83,7
Agianniotaki et al 2016 In house taqman assay	1	6	6	100
Agianniotaki et al. 2017	3	21	20	95,2
Vidanovic et I. 2016	2	14	14	100
Vidanović et al, 2016 Menasherow et al,2014	1	5	5	100
Gelaye et al.2015	1	8	5	62,5
Chibssa et al. 2018 (SPPV)	1	3	3	100
Agiannotaki et al. DIVA LSDV RT-qPCR Haegeman et al. 2015 SPPV DIVA Conv PCR Haegeman 2015 SPPV DIVA RT-qPCR	1	6	6	100
Home made	5	32	38	84,2
Menasherow et al., 2014; Agianniotaki et al., 2017; Haegeman et al., 2015	1	10	10	10
Möller et al., 2019	2	13	13	100
Real-time PCR for the genome characterisation of LSDV-Field strain and LSDV-vaccine strain	1	10	8	80
Sprygin et al., 2018	1	5	3	60
Total	25	170	162	95,2
				-

N: number of laboratories; NR: number of results; NCR: Number of correct results; %: % success

IV.4.1.3.3 Results per laboratory

For the **differentiation between capripox virus field and vaccine strain**: Eighteen out of twenty-five participating laboratories provided qualitative results that were in full agreement with the assigned status of the samples they analyzed (100% of agreement). Only 1 laboratory (LAB97621) analysed all 10 samples. One other laboratory did not analyse the GTPV samples (LAB97600) and analysed a total of 8 aliquots. Six laboratories analysed 7 aliquots (LAB97610, LAB97612, LAB97632, LAB97634, LAB97636 and LAB97631) and did not analyse the GTPV and SPPV samples. Four laboratories analysed 6 aliquots (LAB97506, LAB97609, LAB97611 and LAB97633). These laboratories classified all 5 LSDV samples and one different additional sample each.

Five laboratories (LAB97602, LAB97608, LAB97618, LAB97620 and LAB97630) analysed 5 LSDV samples and classified all correctly. One laboratory (LAB97617) analysed 3 aliquots; all 3 results were in full agreement with the assigned status of the reference samples. LAB97613 misclassified 1 out of 10 aliquots analysed (90% agreement). LAB97623 and LAB97625 misclassified 2 out of 5 aliquots (60% agreement) and out of 10 aliquots (80% agreement). Three laboratories misclassified 3 aliquots; 2 laboratories had analysed all 10 aliquots (LAB97628 and LAB97629) and LAB97604 had analysed 8 aliquots, resulting in an agreement of respectively 70% and 62,5%. LAB97614 misclassified 4 out of 10 aliquots (agreement 60%)

Table 19. Results per participating laboratory

	VP1	VP2	TP1	TP2	TP3	VP3	TP4	TP5	VN1	TN1	%
Lab	GTPV field /Neg	GTPV field /Neg	LSDV field	LSDV field	LSDV vaccine	LSDV field	LSDV field	SPPV field	Neg	Neg	(NCR/ NDR)
97506	ND	ND	LSDV field	LSDV field	LSDV vaccine	LSDV field	LSDV field	SPPV field	ND	ND	100 (6/6)
97600	ND	ND	LSDV field	LSDV field	LSDV vaccine	LSDV field	LSDV field	SPPV field	Neg	Neg	100 (8/8)
97602	ND	ND	LSDV field	LSDV field	LSDV vaccine	LSDV field	LSDV field	ND	ND	ND	100 (5/5)
97604	NI	NI	LSDV field	LSDV field	LSDV vaccine	LSDV field	LSDV field	NI	ND	ND	62.5 (5/8)
97608	ND	ND	LSDV field	LSDV field	LSDV vaccine	LSDV field	LSDV field	ND	ND	ND	100 (5/5)
97610	ND	ND	LSDV field	LSDV field	LSDV vaccine	LSDV field	LSDV field	ND	Neg	Neg	100 (7/7)
97612	ND	ND	LSDV field	LSDV field	LSDV vaccine	LSDV field	LSDV field	ND	Neg	Neg	100 (7/7)
97614	GTPV field	GTPV field	NI	LSDV field	LSDV field	NI	NI	SPPV field	Neg	Neg	60 (6/10)
97618	ND	ND	LSDV field	LSDV field	LSDV vaccine	LSDV field	LSDV field	ND	ND	ND	100 (5/5)
97620	ND	ND	LSDV field	LSDV field	LSDV vaccine	LSDV field	LSDV field	ND	ND	ND	100 (5/5)
97628	Neg	Neg	LSDV field	LSDV field	Neg	Neg	LSDV field	Neg	Neg	Neg	70 (7/10)
97630	ND	ND	LSDV field	LSDV field	LSDV vaccine	LSDV field	LSDV field	ND	ND	ND	100 (5/5)
97632	ND	ND	LSDV field	LSDV field	LSDV vaccine	LSDV field	LSDV field	ND	Neg	Neg	100 (7/7)
97634	ND	ND	LSDV field	LSDV field	LSDV vaccine	LSDV field	LSDV field	ND	Neg	Neg	100 (7/7)
97636	ND	ND	LSDV field	LSDV field	LSDV vaccine	LSDV field	LSDV field	ND	Neg	Neg	100 (7/7)
97609	ND	ND	LSDV field	LSDV field	LSDV vaccine	LSDV field	LSDV field	SPPV field	ND	ND	100 (6/6)
97611	ND	ND	LSDV field	LSDV field	LSDV vaccine	LSDV field	LSDV field	ND	Neg	ND	100 (6/6)
97613	Neg	Neg	LSDV field	LSDV field	LSDV vaccine	LSDV field	LSDV field	Neg	Neg	Neg	90 (9/10)
97617	ND	ND	ND	ND	ND	ND	ND	SPPV field	Neg	Neg	100 (3/3)
97621	Neg	Neg	LSDV field	LSDV field	LSDV vaccine	LSDV field	LSDV field	SPPV field	Neg	Neg	100 (10/10)
97623	ND	ND	LSDV field	LSDV field	LSDV field	LSDV field	NI	ND	ND	ND	60 (3/5)
97625	SPPV field	SPPV field	LSDV field	LSDV field	LSDV vaccine	LSDV field	LSDV field	SPPV field	Neg	Neg	80 (8/10)
97629	Neg	Neg	Neg	LSDV field	LSDV vaccine	LSDV field	Neg	Neg	Neg	Neg	70 (7/10)
97631	ND	ND	LSDV field	LSDV field	LSDV vaccine	LSDV field	LSDV field	ND	Neg	Neg	100 (7/7)
97633	Neg	ND	LSDV field	LSDV field	LSDV vaccine	LSDV field	LSDV field	ND	ND	ND	100 (6/6)

NR: number of results, NCR: Number of correct results; %: % success; (number of correct results/number of determined result)

IV.4.1.4. Final diagnostic

Thirty-six laboratories encoded results. The encoded results depend on the procedure used by each participating laboratory. Not all the laboratories performed species differentiation or DIVA PCR.

IV.4.1.4.1 Results per sample

Table 20. Results per sample

Sample ID	Expected result	Encoded results
PT2020CAPXVIR_VP1	CAPX pos – GTPV field	21(58,3%) CAPX pos-GTPV (field)
		9 (25%) CAPX pos
		3 (8,3 %) CAPX pos - SPPV/GTPV
		1 (2,8%) CAPX pos- SPPV 1 (2,8%) CAPX pos-
	CADY non-CTDV/field	1 (2,0%) CAPA ney
PI2020CAPAVIR_VP2	CAPA pos - GTPV lield	21(30,3%) CAPA pos-GTPV (ileid)
		3(0,3%) CAPX pos - SPPV/GTPV 1(2.8%) CAPX pos - SPPV/field
		P(2, 0, 0) CAEX pos - SEEV lielu
		3(8.3%) CAPX Negative
	CAPX pos_LSDV field	21 (58.3%) CAPY Pos-I SDV/ field
	CALX pos-LODV field	5 (13 9%)CAPX Pos-I SDV
		8 (22 2%) CAPX pos
		1 (2.8%) CAPX Pos-LSDV/GTPV
		1 (2.8%) CAPX negative
PT2020CAPXVIR TP2	CAPX pos-LSDV field	24 (66.7%) CAPX pos-LSDV Field
_		3 (8.3%) CAPX pos-LSDV
		8 (22,2,%) CAPX-Pos
		1 (2,8%) CAPX Pos-LSDV/GTPV
PT2020CAPXVIR_TP3	CAPX pos-LSDV vaccine	22 (61,1%) CAPX pos-LSDV vaccine
		3 (8,3%) CAPX pos-LSDV
		8 (22,2%) CAPX Pos
		2 (5,6%) CAPX pos-LSDV field
		1 (2,8%)CAPX pos-GTPV
PT2020CAPXVIR_VP3	CAPX pos-LSDV field	23 (63,9%) CAPX pos-LSDV Field
		3 (8,3%) CAPX pos-LSDV
		8 (22,2%) CAPX pos
		1 (2,8%) CAPX pos-SPPV
		1 (2,8%) CAPX Neg
PI2020CAPXVIR_IP4	CAPX pos-LSDV field	24 (66,7%) CAPX pos-LSDV Field
		4 (11,1%) CAPX pos-LSDV
	CADY rea CDDV/field	8 (22,2,%) CAPX-POS
FIZUZUCAPAVIK_IP5	CAPA pos-SPPV liela	11 (30,0%) CAPA pos-SPPV Field 12 (22.2%) CAPA pos SPPV
		12 (00,0 %) OAFA PUS-OFFV 10 (27.8%) CAPY Pag
		3 (8 3 %) CAPX Pos-SPP\/ /GTP\/
PT2020CAPXVIR VN1	CAPX negative/ CAPX	35 (97 2%) CAPX Negative
	doubtful	1 (2 8%) NI
		26 (100%) CAPY pogetive
FIZUZUCAFAVIR_INI		

IV.4.1.4.2. Results per laboratory

For the **final diagnostic interpretation of the detection of capripox virus nucleic acid in cell culture supernatant and tissue homogenate**: Thirty out of thirty-six participating laboratories provided qualitative results that were in full agreement with the assigned status of the 10 reference samples and hence reached 100% of agreement. LAB97614, LAB97605 and LAB97623 misclassified 1 aliquot (90% agreement), LAB97625 misclassified 2 aliquots (80% agreement), LAB97628 and LAB97629 misclassified 4 aliquots (60% of agreement).

Tabla	21	Deculto	nor	oomolo
I able	۷١.	Results	per	sample

Sample	VP1	VP2	TP1	TP2	ТРЗ	VP3	TP4	TP5	VN1	TN1	%
status	CAPX pos -	CAPX pos -	CAPX pos-	CAPX pos-	CAPX pos-	CAPX pos-	CAPX pos-	CAPX pos-	CAPX	CAPX	(NCR/NDR)
Laboratory	GTPV field	GTPV field	LSDV field	LSD field	LSDV vaccine	LSDV field	LSDV field	SPPV field	negative/NI	negative	
97506	CAPX pos -	CAPX pos -	CAPX pos-	CAPX pos-	CAPX pos-	CAPX pos-	CAPX pos-	CAPX pos-	CAPX	CAPX	100
	GTPV	GTPV	LSDV field	LSD field	LSDV vaccine	LSDV field	LSDV field	SPPV field	negative	negative	(10/10)
97600	CAPX pos -	CAPX pos -	CAPX pos-	CAPX pos-	CAPX pos-	CAPX pos-	CAPX pos-	CAPX pos-	CAPX	CAPX	100
	GTPV	GTPV	LSDV field	LSD field	LSDV vaccine	LSDV field	LSDV field	SPPV field	negative	negative	(10/10)
97602	CAPX pos -	CAPX pos -	CAPX pos-	CAPX pos-	CAPX pos-	CAPX pos-	CAPX pos-	CAPX pos-	CAPX	CAPX	100
	GTPV	GTPV	LSDV field	LSD field	LSDV vaccine	LSDV field	LSDV field	SPPV field	negative	negative	(10/10)
97604	CAPX pos -	CAPX pos -	CAPX pos-	CAPX pos-	CAPX pos-L	CAPX pos-	CAPX pos-	CAPX pos-	CAPX	CAPX	100
	GTPV	GTPV	LSDV field	LSD field	SDV vaccine	LSDV field	LSDV field	SPPV field	negative	negative	(10/10)
97606	CAPX pos	CAPX pos	CAPX pos	CAPX pos	CAPX pos	CAPX pos	CAPX pos	CAPX pos	CAPX negative	CAPX negative	100 (10/10)
97608	CAPX pos -	CAPX pos -	CAPX pos-	CAPX pos-	CAPX pos-	CAPX pos-	CAPX pos-	CAPX pos-	CAPX	CAPX	100
	GTPV	GTPV	LSDV field	LSD field	LSDV vaccine	LSDV field	LSDV field	SPPV field	negative	negative	(10/10)
97610	CAPX pos -	CAPX pos -	CAPX pos-	CAPX pos-	CAPX pos	CAPX pos-	CAPX pos-	CAPX pos-	CAPX	CAPX	100
	GTPV	GTPV	LSDV field	LSD field	-LSDV vaccine	LSDV field	LSDV field	SPPV field	negative	negative	(10/10)
97612	CAPX pos-	CAPX pos-	CAPX pos-	CAPX pos-	CAPX pos-	CAPX pos-	CAPX pos-	CAPX pos -	CAPX	CAPX	100
	SPPV/GTPV	SPPV/GTPV	LSDV field	LSD field	LSDV vaccine	LSDV field	LSDV field	SPPV/GTPV	negative	negative	(10/10)
97614	CAPX pos -	CAPX pos -	CAPX pos -	CAPX pos –	CAPX pos –	CAPX pos -	CAPX pos -	CAPX pos-	CAPX	CAPX	90
	GTPV	GTPV	LSDV	LSDV - field	LSDV - field	LSDV	LSDV	SPPV field	negative	negative	(9/10)
97616	CAPX pos	CAPX pos	CAPX pos	CAPX pos	CAPX pos	CAPX pos	CAPX pos	CAPX pos	CAPX negative	CAPX negative	100 (10/10)
97618	CAPX pos -	CAPX pos -	CAPX pos-	CAPX pos-	CAPX pos-	CAPX pos-	CAPX pos-	CAPX pos -	CAPX	CAPX	100
	GTPV	GTPV	LSDV field	LSD field	LSDV vaccine	LSDV field	LSDV field	SPPV	negative	negative	(10/10)
97620	CAPX pos -	CAPX pos -	CAPX pos-	CAPX pos-	CAPX pos-	CAPX pos-	CAPX pos-	CAPX pos-	CAPX	CAPX	100
	GTPV	GTPV	LSDV field	LSD field	LSDV vaccine	LSDV field	LSDV field	SPPV field	negative	negative	(10/10)
97622	CAPX pos	CAPX pos	CAPX pos	CAPX pos	CAPX pos	CAPX pos	CAPX pos	CAPX pos	CAPX negative	CAPX negative	100 (10/10)
97624	CAPX pos	CAPX pos	CAPX pos	CAPX pos	CAPX pos	CAPX pos	CAPX pos	CAPX pos	CAPX negative	CAPX negative	100 (10/10)
97628	CAPX neg	CAPX neg	CAPX pos- LSDV/GTPV	CAPX pos- LSDV/GTPV	CAPX pos- GTPV	CAPX Neg	CAPX pos- LSDV field	CAPX pos- SPPV	CAPX negative	CAPX negative	60 (6/10)
97630	CAPX pos -	CAPX pos -	CAPX pos-	CAPX pos	CAPX pos-	CAPX pos-	CAPX pos-	CAPX pos-	CAPX	CAPX	100
	SPPV/GTPV	SPPV/GTPV	LSDV field	-LSDV field	LSDV vaccine	LSDV field	LSDV field	SPPV /GTPV	negative	negative	(10/10)
97632	CAPX pos - GTPV	CAPX pos - GTPV	CAPX pos- LSDV field	CAPX pos- LSDV field	CAPX pos- LSDV vaccine	CAPX pos- LSDV field	CAPX pos- LSDV field	CAPX pos	CAPX negative	CAPX negative	100 (10/10)

97634	CAPX pos -	CAPX pos -	CAPX pos-	CAPX pos-	CAPX pos	CAPX pos-	CAPX pos-	CAPX pos-	CAPX	CAPX	100
	GTPV	GTPV	LSDV field	LSDV field	-LSDV vaccine	LSDV field	LSDV field	SPPV	negative	negative	(10/10)
97636	CAPX pos	CAPX pos	CAPX pos- LSDV field	CAPX pos- LSDV field	CAPX pos- LSDV vaccine	CAPX pos- LSDV field	CAPX pos- LSDV field	CAPX pos	CAPX negative	CAPX negative	100 (10/10)
97601	CAPX pos	CAPX pos	CAPX pos	CAPX pos	CAPX pos	CAPX POS	CAPX pos	CAPX pos	CAPX negative	CAPX negative	100 (10/10)
97603	CAPX pos -	CAPX pos -	CAPX pos-	CAPX pos-	CAPX pos-	CAPX pos-	CAPX pos-	CAPX pos-	CAPX	CAPX	100
	GTPV	GTPV	LSDV	LSDV	LSDV	LSDV	LSDV	SPPV	negative	negative	(10/10)
97605	CAPX pos	CAPX Neg	CAPX pos	CAPX pos	CAPX pos	CAPX POS	CAPX pos	CAPX pos	CAPX negative	CAPX negative	90 (9/10)
97607	CAPX pos – SPVV/GTPV	CAPX pos - SPPV/ GTPV	CAPX pos- LSDV	CAPX pos- LSDV	CAPX pos- LSDV	CAPX pos- LSDV	CAPX pos- LSDV	CAPX pos- SPPV/GTPV	NI	CAPX negative	100 (10/10)
97609	CAPX pos -	CAPX pos -	CAPX pos-	CAPX pos-	CAPX pos-	CAPX pos-	CAPX pos-	CAPX pos-	CAPX	CAPX	100
	GTPV	GTPV	LSDV field	LSDV field	LSDV Vaccine	LSDV-field	LSDV field	SPPV field	negative	negative	(10/10)
97611	CAPX pos -	CAPX pos -	CAPX pos-	CAPX pos-	CAPX pos-	CAPX pos-	CAPX pos-	CAPX pos-	CAPX	CAPX	100
	GTPV	GTPV	LSDV field	LSDV field	LSDV Vaccine	LSDV field	LSDV field	SPPV	negative	negative	(10/10)
97613	CAPX pos - GTPV	CAPX pos - GTPV	CAPX pos- LSDV field	CAPX pos- LSDV field	CAPX pos- LSDV Vaccine	CAPX pos – LSDV - fields strain	CAPX pos – LSDV - fields strain	CAPX pos- SPPV	CAPX negative	CAPX negative	100 (10/10)
97617	CAPX pos -	CAPX pos -	CAPX pos-	CAPX pos-	CAPX pos-	CAPX pos-	CAPX pos-	CAPX pos-	CAPX	CAPX	100
	GTPV field	GTPV	LSDV	LSDV	LSDV	LSDV field	LSDV	SPPV field	negative	negative	(10/10)
97619	CAPX pos -	CAPX pos -	CAPX pos-	CAPX pos-	CAPX pos-	CAPX pos-	CAPX pos-	CAPX pos-	CAPX	CAPX	100
	GTPV	GTPV	LSDV field	LSDV field	LSDV Vaccine	LSDV field	LSDV field	SPPV	negative	negative	(10/10)
97621	CAPX pos -	CAPX pos -	CAPX pos	CAPX pos-	CAPX pos-	CAPX pos-	CAPX pos-	CAPX pos-	CAPX	CAPX	100
	GTPV	GTPV	-LSDV field	LSDV field	LSDV Vaccine	LSDV field	LSDV field	SPPV	negative	negative	(10/10)
97623	CAPX pos -	CAPX pos -	CAPX pos-	CAPX pos-	CAPX pos	CAPX pos-	CAPX pos-	CAPX pos-	CAPX	CAPX	90
	GTPV	GTPV	LSDV	LSDV field	-LSDV field	LSDV field	LSDV field	SPPV	negative	negative	(9/10)
97625	CAPX pos -	CAPX pos -	CAPX pos-	CAPX pos-	CAPX pos-	CAPX pos-	CAPX pos-	CAPX pos-	CAPX	CAPX	80
	SPPV field	SPPV field	LSDV field	LSDV field	LSDV Vaccine	LSDV field	LSDV field	SPPV field	negative	negative	(8/10)
97627	CAPX pos	CAPX pos	CAPX pos	CAPX pos	CAPX positive	CAPX pos	CAPX pos	CAPX pos	CAPX negative	CAPX negative	100 (10/10)
97629	CAPX pos -	CAPX	CAPX	CAPX pos-	CAPX pos-	CAPX pos-	CAPX pos-	CAPX pos-	CAPX	CAPX	60
	SPPV	negative	negative	LSDV field	LSDV Vaccine	SPPV	LSDV field	SPPV	negative	negative	(6/10)
97631	CAPX pos -	CAPX pos -	CAPX pos	CAPX pos-	CAPX pos-	CAPX pos-	CAPX pos-	CAPX pos-	CAPX	CAPX	100
	GTPV	GTPV	-LSDV field	LSDV field	LSDV Vaccine	LSDV field	LSDV field	SPPV	negative	negative	(10/10)
97633	CAPX pos -	CAPX pos -	CAPX pos-	CAPX pos-	CAPX pos-	CAPX pos-	CAPX pos-	CAPX pos-	CAPX	CAPX	100
	GTPV	GTPV	LSDV field	LSDV field	LSDV Vaccine	LSDV field	LSDV field	SPPV	negative	negative	(10/10)
97635	CAPX pos	CAPX pos	CAPX pos	CAPX pos	CAPX pos	CAPX pos	CAPX pos	CAPX pos	CAPX negative	CAPX negative	100 (10/10)

NR: number of results, NCR: Number of correct results; %: % success; (number of correct results/number of determined result)

IV.4.2. Serology

The PT2020CAPXSER panel was composed of 8 positive samples and 2 negative samples. The sample PT2020CAPXSER-P4 was present in duplo.

IV.4.2.1. ELISA

Thirty laboratories encoded results. All the laboratories used the same ELISA kit (ID-Vet ID Screen Capripox Double Antigen Multispecies). All 30 participating laboratories provided qualitative results that were in full agreement with the assigned status of the reference samples and hence reached 100% agreement.

Lab ID	SERP1	SERP2	SERP3	SERP4 (repet.1)	SERP5	SERP6	SERP7	SERP4 (repet.2)	SERN1	SERN2	% (NCR/ NDR)
97506	Pos	Pos	Pos	Pos	Pos	Pos	Pos	Pos	Neg	Neg	100 (10/10)
97600	Pos	Pos	Pos	Pos	Pos	Pos	Pos	Pos	Neg	Neg	100 (10/10)
97602	Pos	Pos	Pos	Pos	Pos	Pos	Pos	Pos	Neg	Neg	100 (10/10)
97604	Pos	Pos	Pos	Pos	Pos	Pos	Pos	Pos	Neg	Neg	100 (10/10)
97608	Pos	Pos	Pos	Pos	Pos	Pos	Pos	Pos	Neg	Neg	100 (10/10)
97610	Pos	Pos	Pos	Pos	Pos	Pos	Pos	Pos	Neg	Neg	100 (10/10)
97612	Pos	Pos	Pos	Pos	Pos	Pos	Pos	Pos	Neg	Neg	100 (10/10)
97614	Pos	Pos	Pos	Pos	Pos	Pos	Pos	Pos	Neg	Neg	100 (10/10)
97616	Pos	Pos	Pos	Pos	Pos	Pos	Pos	Pos	Neg	Neg	100 (10/10)
97618	Pos	Pos	Pos	Pos	Pos	Pos	Pos	Pos	Neg	Neg	100 (10/10)
97620	Pos	Pos	Pos	Pos	Pos	Pos	Pos	Pos	Neg	Neg	100 (10/10)
97622	Pos	Pos	Pos	Pos	Pos	Pos	Pos	Pos	Neg	Neg	100 (10/10)
97628	Pos	Pos	Pos	Pos	Pos	Pos	Pos	Pos	Neg	Neg	100 (10/10)
97630	Pos	Pos	Pos	Pos	Pos	Pos	Pos	Pos	Neg	Neg	100 (10/10)
97632	Pos	Pos	Pos	Pos	Pos	Pos	Pos	Pos	Neg	Neg	100 (10/10)
97636	Pos	Pos	Pos	Pos	Pos	Pos	Pos	Pos	Neg	Neg	100 (10/10)
97634	Pos	Pos	Pos	Pos	Pos	Pos	Pos	Pos	Neg	Neg	100 (10/10)
97605	Pos	Pos	Pos	Pos	Pos	Pos	Pos	Pos	Neg	Neg	100 (10/10)
97607	Pos	Pos	Pos	Pos	Pos	Pos	Pos	Pos	Neg	Neg	100 (10/10)
97609	Pos	Pos	Pos	Pos	Pos	Pos	Pos	Pos	Neg	Neg	100 (10/10)
97611	Pos	Pos	Pos	Pos	Pos	Pos	Pos	Pos	Neg	Neg	100 (10/10)
97613	Pos	Pos	Pos	Pos	Pos	Pos	Pos	Pos	Neg	Neg	100 (10/10)

Table 22. Results for ELISA

| 97615 | Pos | Neg | Neg | 100
(10/10) |
|-------|-----|-----|-----|-----|-----|-----|-----|-----|-----|-----|----------------|
| 97617 | Pos | Neg | Neg | 100
(10/10) |
| 97619 | Pos | Neg | Neg | 100
(10/10) |
| 97621 | Pos | Neg | Neg | 100
(10/10) |
| 97623 | Pos | Neg | Neg | 100
(10/10) |
| 97629 | Pos | Neg | Neg | 100
(10/10) |
| 97633 | Pos | Neg | Neg | 100
(10/10) |
| 97635 | Pos | Neg | Neg | 100
(10/10) |

NR: number of results, NCR: Number of correct results; %: % success; (number of correct results/number of determined result)

IV.4.2.2. Virus neutralization

Five laboratories encoded results for the VN test. Four out of five laboratories (LAB97600, LAB97612, LAB97618 and LAB97633) provided qualitative results that were in full agreement with the assigned status of the reference serum samples and hence reached 100% agreement. LAB97623 misclassified 3 aliquots (70% of agreements). This laboratory considered the duplicate positive sample once as negative and once as positive.

Lab ID	SERP1	SERP2	SERP3	SERP4 (repet.1)	SERP5	SERP6	SERP7	SERP4 (repet.2)	SERN1	SERN2	% (NCR/ NDR)
97600	Pos	Pos	Pos	Pos	Pos	Pos	Pos	Pos	Neg	Neg	100 (10/10)
97612	Pos	Pos	Pos	Pos	Pos	Pos	Pos	Pos	Neg	Neg	100 (10/10)
97618	Pos	Pos	Pos	Pos	Pos	Pos	Pos	Pos	Neg	Neg	100 (10/10)
97623	Neg	Pos	Neg	Pos	Pos	Neg	Neg	Neg	Neg	Neg	70 (7/10)
97633	Pos	Pos	Pos	Pos	Pos	Pos	Pos	Pos	Neg	Neg	100 (10/10)

Table 23. Results for virus neutralization test

NR: number of results, NCR: Number of correct results; %: % success; (number of correct results/number of determined result)

IV.4.2.3. Immunoperoxydase Monolayer Assay (IPMA)

Only 2 laboratories encoded results for IPMA. These 2 laboratories (LAB97506 and LAB97618) provided qualitative results that were in full agreement with the assigned status of the reference serum samples and hence reached 100% agreement.

Lab ID	SERP1	SERP2	SERP3	SERP4 (repet.1)	SERP5	SERP6	SERP7	SERP4 (repet.2)	SERN1	SERN2	% (NCR/ NDR)
97506	Pos	Pos	Pos	Pos	Pos	Pos	Pos	Pos	Neg	Neg	100 (10/10)
976018	Pos	Pos	Pos	Pos	Pos	Pos	Pos	Pos	Neg	Neg	100 (10/10)

Table 24. Results for IPMA

NR: number of results, NCR: Number of correct results; %: % success; (number of correct results/number of determined result)

IV.4.2.4. Final Diagnostics For the final diagnostic interpretation of the detection of specific antibodies to capripox virus in serum: all participating laboratories provided qualitative results that were in full agreement with the assigned status of the reference serum samples and hence reached 100% of agreement Table 25. Final evaluation

Lab ID	SERP1	SERP2	SERP3	SERP4 (repet.1)	SERP5	SERP6	SERP7	SERP4 (repet.2)	SERN1	SERN2	% (NCR/ NDR)
97506	Pos	Pos	Pos	Pos	Pos	Pos	Pos	Pos	Neg	Neg	100 (10/10)
97600	Pos	Pos	Pos	Pos	Pos	Pos	Pos	Pos	Neg	Neg	100 (10/10)
97602	Pos	Pos	Pos	Pos	Pos	Pos	Pos	Pos	Neg	Neg	100 (10/10)
97604	Pos	Pos	Pos	Pos	Pos	Pos	Pos	Pos	Neg	Neg	100 (10/10)
97605	Pos	Pos	Pos	Pos	Pos	Pos	Pos	Pos	Neg	Neg	100 (10/10)
97607	Pos	Pos	Pos	Pos	Pos	Pos	Pos	Pos	Neg	Neg	100 (10/10)
97608	Pos	Pos	Pos	Pos	Pos	Pos	Pos	Pos	Neg	Neg	100 (10/10)
97609	Pos	Pos	Pos	Pos	Pos	Pos	Pos	Pos	Neg	Neg	100 (10/10)
97610	Pos	Pos	Pos	Pos	Pos	Pos	Pos	Pos	Neg	Neg	100 (10/10)
97611	Pos	Pos	Pos	Pos	Pos	Pos	Pos	Pos	Neg	Neg	100 (10/10)
97612	Pos	Pos	Pos	Pos	Pos	Pos	Pos	Pos	Neg	Neg	100 (10/10)
97613	Pos	Pos	Pos	Pos	Pos	Pos	Pos	Pos	Neg	Neg	100 (10/10)
97614	Pos	Pos	Pos	Pos	Pos	Pos	Pos	Pos	Neg	Neg	100 (10/10)
97615	Pos	Pos	Pos	Pos	Pos	Pos	Pos	Pos	Neg	Neg	100 (10/10)
97616	Pos	Pos	Pos	Pos	Pos	Pos	Pos	Pos	Neg	Neg	100 (10/10)
97617	Pos	Pos	Pos	Pos	Pos	Pos	Pos	Pos	Neg	Neg	100 (10/10)
97618	Pos	Pos	Pos	Pos	Pos	Pos	Pos	Pos	Neg	Neg	100 (10/10)
97619	Pos	Pos	Pos	Pos	Pos	Pos	Pos	Pos	Neg	Neg	100 (10/10)
97620	Pos	Pos	Pos	Pos	Pos	Pos	Pos	Pos	Neg	Neg	100 (10/10)
97621	Pos	Pos	Pos	Pos	Pos	Pos	Pos	Pos	Neg	Neg	100 (10/10)
97622	Pos	Pos	Pos	Pos	Pos	Pos	Pos	Pos	Neg	Neg	100 (10/10)
97623	Pos	Pos	Pos	Pos	Pos	Pos	Pos	Pos	Neg	Neg	100 (10/10)
97628	Pos	Pos	Pos	Pos	Pos	Pos	Pos	Pos	Neg	Neg	100 (10/10)
97629	Pos	Pos	Pos	Pos	Pos	Pos	Pos	Pos	Neg	Neg	100 (10/10)
97630	Pos	Pos	Pos	Pos	Pos	Pos	Pos	Pos	Neg	Neg	100 (10/10)
97632	Pos	Pos	Pos	Pos	Pos	Pos	Pos	Pos	Neg	Neg	100 (10/10)
97633	Pos	Pos	Pos	Pos	Pos	Pos	Pos	Pos	Neg	Neg	100 (10/10)

| 97634 | Pos | Neg | Neg | 100
(10/10) |
|-------|-----|-----|-----|-----|-----|-----|-----|-----|-----|-----|----------------|
| 97635 | Pos | Neg | Neg | 100
(10/10) |
| 97636 | Pos | Neg | Neg | 100
(10/10) |

NR: number of results, NCR: Number of correct results; %: % success; (number of correct results/number of determined result)

V. Discussion

The purpose of this PT was to assess the performances of the participating laboratories when analyzing reference serum samples of bovidae origin for the detection of antibodies to capripox viruses and/or analyzing reference cell culture supernatant and tissue homogenate samples for the detection of capripox virus nucleic acid.

V.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 VN1 with Antibody Titer or IPMA, 29 out of 30 laboratories provided qualitative results that were in full agreement with the assigned status of the reference serum samples. LAB97623 misclassified 3 aliquots in their VN results. However, their results of the ELISA were in full agreement with the assigned status of the reference serum samples.

In accordance, the final diagnostic interpretation was 100% successful for 30 out of 30 laboratories.

V.2 Virology component of the PT

For the detection of capripox virus nucleic acid by **real-time PCR (RT-PCR) in the PT panel** : 31 out of 36 participating laboratories provided qualitative results that were in full agreement with the assigned status of the 10 reference samples (100% of agreement), whereas LAB97602 and LAB97605 and LAB97616 misclassified 1 aliquot (90% of agreement), LAB97629 misclassified 2 aliquots (80% of agreement). LAB97628 misclassified 3 aliquots (70% agreement).

Using the method described by Bowden et al. (2008) LAB97602 misclassified PT2020CAPXVIR_VP2 (Neg instead of Pos). LAB97628 misclassified PT2020CAPXVIR_VP1, PT2020CAPXVIR_VP2 and PT2020CAPXVIR_VP3 (all Neg instead of Pos) and LAB97629 misclassified PT2020CAPXVIR_VP2 and PT2020CAPXVIR_TP1 (Neg instead of Pos) using the Lumpy skin disease detection kit (Fractal Bio). LAB97616 misclassified PT2020CAPXVIR_VP3 (doubtful instead of positive) using the Path-ID QPCR (Applied Biosystems). LAB97605 misclassified PT2020CAPXVIR_VP2 using the method described by Haegeman et al. (2013).

Four participating laboratories performed a secondary pan-capripox PCR. LAB97605 provided qualitative results that were identical in both tests. Nine out of ten aliquots were in agreement with the assigned status of the 10 reference samples (90% of agreement) using the method described by Haegeman et al. 2013 as primary test and the method described by Bowden et al. 2008 as secondary PCR. PT2020CAPXVIR_VP2 was misclassified as negative in both PCR.

LAB97607 was in full agreement with the assigned status of the 10 reference samples in both their primary (Bowden et al. 2008) and secondary (RT PCR RPO30 (PAN-Capripox) PCRs. LAB97620 (Vidanovic et al. 2016) and LAB97621 (Lamien et al. 2011) misclassified respectively 4 aliquots (PT2020CAPXVIR_VP1, PT2020CAPXVIR_VP2, PT2020CAPXVIR_TP3 and PT2020CAPXVIR_TP5) and 2 aliquots

(PT2020CAPXVIR_VP2 and PT2020CAPXVIR_VP3). This was in contrast with the results of their primary PCR, where there results were in full agreement with the assigned status of the 10 reference samples.

For the **differentiation of capripox virus species**: sixteen out of twenty-seven participating laboratories provided qualitative results that were in full agreement with the assigned status of the 10 reference samples (100% of agreement), whereas LAB97602 misclassified 1 aliquot (90% of agreement), LAB97621 and LAB97625 misclassified 2 aliquots (80% of agreement) and LAB97628 and LAB97629 misclassified 4 aliquots (60% of agreement)

LAB97612, LAB97618 and LAB97636 did the analysis only on 7 out of 10 samples and LAB97609 and LAB97623 only on 9 out of 10 samples. These participants provided qualitative results that were in full agreement with the assigned status of the samples.

LAB97607 did the analysis of 9 aliquots and misclassified 3 aliquots (66% agreement).

LAB97602 and LAB97625 reported the use of the RT-PCR protocol described in Lamien et al. (2011a), which allows for the discrimination between all the capripox virus species and was successfully used by other participating laboratories. LAB97602 misclassified PT2020CAPXVIR_VP2 (Neg instead of GTPV), LAB97625 misclassified PT2020CAPXVIR_VP1 and PT2020CAPXVIR_VP2 (SPPV instead of GTPV). LAB97602 misclassified PT2020CAPXVIR_VP2 already as neg in the primary PCR. LAB97625 classified PT2020CAPXVIR_VP2 correctly in the primary PCR.

LAB97607 used an in-house RT-PCR GPCR specific to LSDV to correctly identify all LSDV samples and reported the GTPV and SPPV positive samples as negative instead of ND.

LAB97628 used an ID gene LSD DIVA triplex (IDVET) and misclassified 3 aliquots as negative (PT2020CAPXVIR_VP1, PT2020CAPXVIR_VP2 and PT2020CAPXVIR_VP3). These aliquots were already misclassified as capripox negative in the primary PCR. Additionally, PT2020CAPXVIR_VP3 was misclassified as GTPV instead of LSDV.

Using the method described by Menasherow et al. 2014 LAB97621 misclassified PT2020CAPXVIR_TP2 and PT2020CAPXVIR_TP4 as neg instead of LSDV.

LAB97629 misclassified 4 aliquots (PT2020CAPXVIR_VP1 and PT2020CAPXVIR_VP3 as SPPV instead of GTPV and LSDV, respectively and PT2020CAPXVIR_VP2 and PT2020CAPXVIR_TP1 as negative instead of GTPV and LSDV, respectively). VP2 and TP1 were already misclassified as capripox negative in the primary PCR. PT2020CAPXVIR_VP1 and PT2020CAPXVIR_VP3 were correctly classified as capripox positive in the primary PCR.

For the **differentiation between capripox virus field and vaccine strains**: 18 out of 25 participating laboratories provided qualitative results that were in full agreement with the assigned status of the samples they analyzed (100% of agreement). Only 1 participant (LAB97621) analysed all 10 samples. One participant (LAB97600) did not analyse the GTPV samples and thus reported a total of 8 aliquots. Six participants analysed 7 aliquots (LAB97610, LAB97612, LAB97632, LAB97634, LAB97636 and LAB97631) and did not analyse the GTPV samples. Four laboratories analysed 6 aliquots (LAB97506, LAB97609, LAB97611 and LAB97633). These laboratories reported all 5 LSDV samples and one different additional sample. Five participants only reported 5 LSDV samples and classified all of them correctly

(LAB97602, LAB97608, LAB97618, LAB97620 and LAB97630). One participant (LAB97617) analysed 3 aliquots. All these results were in full agreement with the assigned status of the reference samples. LAB97613 misclassified 1 out of 10 reported aliquots (90% agreement). LAB97623 and LAB97625 misclassified 2 aliquots out of respectively 5 (60% agreement) and 10 aliquots (80% agreement). Three laboratories misclassified 3 aliquots; 2 of them analysed all aliquots (LAB97628 and LAB97629) and LAB97604 analysed 8 aliquots, resulting in an agreement of 70% and 62,5%, respectively. LAB97614 misclassified 6 out of 10 analysed aliquots (agreement 40%).

LAB97604 misclassified PT2020CAPXVIR_VP1, PT2020CAPXVIR_VP2 and PT2020CAPXVIR_TP5 as doubtful using the method described by Gelaye et al. 2015. Since all LSDV samples were correctly classified, it is assumed that a misunderstanding between NI and ND occurred.

LAB97614 used an in-house method and misclassified PT2020CAPXVIR_TP1, PT2020CAPXVIR_VP3 and PT2020CAPXVIR_TP4 as doubtful (NI). PT2020CAPXVIR_TP3 was misclassified as LSDV field instead of LSDV vaccine.

LAB97628 and LAB97629 used the ID Gene LSD DIVA Triplex (IDVET) and misclassified PT2020CAPXVIR_TP3, PT2020CAPXVIR_VP3 and PT2020CAPXVIR_TP5 and PT2020CAPXVIR_TP1, PT2020CAPXVIR_TP4 and PT2020CAPXVIR_TP5, respectively, as negative. This method was used by other laboratories as well and their results were in full agreement with the assigned status of the reference samples.

LAB97613 used the method described by Agianniotaki et al. 2017 and misclassified PT2020CAPXVIR_TP5 as negative. This method was used by other laboratories as well and their results were in full agreement with the assigned status of the reference samples.

LAB97623 used the method by Sprygin et al. 2018 and misclassified PT2020CAPXVIR_TP4 as doubtful instead of LSDV field and PT2020CAPXVIR_TP3 LSDV field instead of LSDV vaccine.

LAB97625 used an undisclosed Real-time PCR and missclassified PT2020CAPXVIR_VP1 and PT2020CAPXVIR_VP2 as SPPV field instead of negative.

For the **final diagnostic interpretation of the detection of capripox virus nucleic acid in cell culture supernatant and tissue homogenate**: Thirty out of thirty-six participating laboratories provided qualitative results that were in full agreement with the assigned status of the 10 reference samples and hence reached 100% of agreement. LAB97614, LAB97605 and LAB97623 misclassified 1 aliquot (90% agreement), LAB97625 misclassified 2 aliquots (80% agreement) and LAB97628 and LAB97629 misclassified 4 aliquots (60% of agreement).

LAB97614 misclassified PT2020CAPXVIR_TP3 as LSD field strain instead of the LSD vaccine.

LAB97628 misclassified 4 aliquots in the final diagnostic. They misclassified the three (PT2020CAPXVIR_VP1, PT2020CAPXVIR_VP2 and PT2020CAPXVIR_VP3) cell culture samples that were present in the panel. The fourth misclassified aliquot (PT2020CAPXVIR_TP3) was was misclassified as GTPV instead of LSDV vaccine.

LAB97605 misclassified PT2020CAPXVIR_VP2 as capripox negative instead of positive. This sample was a ten-fold dilution of PT2020CAPXVIR_VP1. Primary and secondary PCR were reported and both classified PT2020CAPXVIR_VP2 as negative.

LAB97623 misclassified PT2020CAPXVIR_TP3 as LSDV field instead of LSDV vaccine.

LAB97625 misclassified PT2020CAPXVIR_VP1 and PT2020CAPXVIR_VP2 as SPPV instead of GTPV.

LAB97629 misclassified 4 aliquots. PT2020CAPXVIR_VP1 and PT2020CAPXVIR_VP3 as SPPV instead of GTPV and LSDV, respectively. PT2020CAPXVIR_VP2 and PT2020CAPXVIR_TP1 were misclassified as negative instead of GTPV and LSDV, respectively. Neighter 1 of the GTPV aliquots was detected.

VI. Conclusions

According to the procedure currently in force, the performance of a participating laboratory is satisfactory if at least 90% of the results provided by this laboratory is in agreement with the status of the reference samples assigned by the reference laboratory for capripox viruses of the Scientific Directorate Infectious Diseases in Animals of Sciensano (see III.3.3.).

For the detection of specific antibodies to capripox virus in bovine and ovine sera, all participating laboratories achieved a satisfactory performance.

For the detection of capripox virus nucleic acid in the cell culture supernatant ortissue homogenate samples, all participating laboratories except LAB97629 (60% agreement), LAB97628 (60% agreement) and LAB97625 (80% agreement) achieved a satisfactory performance based on the final diagnostic.

ANNEXES (Not under accreditation)

Annex 1a: Raw Primary RT-PCR data

ualu	a samola	Labnr 97506	97600	97601	97602	97603	97604	97605
	PT2020CAPXVIB VP1	35.25	30.67	311	33.01	27.62	311	33.39
	PT2020CAPXVIB VP2	39.71	33.67	35.62	no Ct	31.08	34.6	No Ct
	PT2020CAPXVIR TP1	28,15	28,35	29,52	25,6	25,29	28,2	27,53
œ	PT2020CAPXVIR_TP2	33,24	30,55	32,21	29,74	28,95	33,2	29,55
뵹	PT2020CAPXVIR_TP3	31,26	29,32	30,35	27,18	27,29	30,3	28,55
2	PT2020CAPXVIR_VP3	37,06	32,78	35,54	34,4	31,78	34,1	33,01
2	PT2020CAPXVIR_TP4	34,52	33,76	34,85	31,26	30,42	33,6	30,63
	PT2020CAPXVIR_TP5	26,53	23,875	25,58	19,79	22,29	25,5	22,01
	PT2020CAPXVIR_VN1	no et	no ct	no Ct	no Ct	No Ct	Noct	No Ct
	PT2020CAPXVIR_TN1	noct	no ct	no Ct	no Ct	No Ct	Noct	No Ct
	PT2020CAPXVIR_VP1	POS	POS	POS	POS	POS	POS	POS
	PT2020CAPXVIR_VP2	POS	POS	POS	NEG	POS	POS	NEG
	PT2020CAPXVIR_TP1	POS	POS	POS	POS	POS	POS	POS
lts	PT2020CAPXVIR_TP2	POS	POS	POS	POS	POS	POS	POS
BB	PT2020CAPXVIR_TP3	POS	POS	POS	POS	POS	POS	POS
Ē	PT2020CAPXVIR_VP3	POS	POS	POS	POS	POS	POS	POS
Ē	PT2020CAPXVIR TP4	POS	POS	POS	POS	POS	POS	POS
		POS	POS	POS	POS	POS	POS	POS
	PT2020CAPXVIB VNI	NEG	NEG	NEG	NEG	NEG	NEG	NEG
	PT2020CAPXVIB TN1	NEG	NEG	NEG	NEG	NEG	NEG	NEG
	Protocol / SOP used :	RT-PCR (Haegeman et al. 2013)	Bowden et al 2008, as modified by FLI	STUBBS Pirbright		Bowden et al., 2008; Babiuk et al., 2008; SOP from Pirbright	6.3.51.1 Capripoxviruses qPCR	Bowden et al. 2008
	Producer Extraction protocol / kit:	Machery Nagel	Biosellal	Indical Bioscience	ThermoFisher Scientific	Roche Diagnostics	Roche	Roche MP96 / Viral NA SV Kit
	Name Extraction protocol / kit:	Blood	Biosellal Superball	IndiSpin Pathogen Kit	MagMAX Core Nucleic Acid Purification Kit	MagNA Pure Compact Nucleic Acid Isolation Kit I	MagNA Pure 96 DNA and Viral NA Small Volume Kit	External lysis protocol
	In-house modifications to	Addition of EC to buffer BR	no	N/A	N/A	No		No
	PCR Instrument used:	Lightcycler 480	ABI 7500Fast	BioRad CFX 96 Real- Time system	RotorGene Q	LightCycler 96 (Roche)	Bio-Rad CFX96	Mx3005
ation	Cut-off for positive:	Ct<45: Positive; 45≤Ct<50: Doubtful; Ct≥50: Negative		39		<35	40	No cut-off
nform	Producer RT-PCR protocol / kit:	Roche	Qiagen	Indical Bioscience	Qiagen	Qiagen	Qiagen	Thermofisher 4388644
-	Name RT-PCR protocol / kit:	Fast Start DNA polymerase/ Light cycler	QuaniTect Multiplex PCR Kit NoRox	virotype Mix+IC - DNA	Bowden et al, 2008; QuantiFast Pathogen +IC	QuantiFast Probe PCR kit	Quatitect Probe PCR Kit	Path-ID qPCR Master Mix
	The target of the RT primer:	D5R	P32 gene	P 32 gene (89 bp)	CaPV074F1, CaPV074R1, CaPV074P1	p32	P32 gene	ORF074
	Remark(s):		Ct value					Results given as Ct values from qPCR targeting ORF074 (Bowden et al. 2008) Positive (kit) control is given as the ot value from the positive extraction control

		Labor									
valu	e sample	97606	97607	97608	97609	97610	97611	97612			
	PT2020CAPXVIR_VP1	33,13	30,50	32,31	30,5	29,26	27,7	29			
		36,61	33,07	34,81	35,71	34,74	31,3	32			
_		23,20	20,04	27,31	20	27,04	20,4	24			
at a	PT2020CAPAVIA_TP2	30.09	28.90	29.67	28.63	29.72	20,4	23			
3	PT2020CAPXVIB VP3	35.22	34.01	33.47	32.57	35.3	312	32			
ē	PT2020CAPXVIB TP4	34.02	32.24	32.2	32,29	31.43	31.1	30			
	PT2020CAPXVIB TP5	23.87	24.56	22.29	22.02	23.16	21.4	22			
	PT2020CAPXVIR_VN1	No Ct	No Ct	No Ct	noct	No Ct	No Ct	No Ct			
	PT2020CAPXVIB_TN1	No Ct	No Ct	No Ct	no et	No Ct	No Ct	No Ct			
	PT2020CAPXVIR_VP1	POS	POS	POS	POS	POS	POS	POS			
	PT2020CAPXVIR_VP2	POS	POS	POS	POS	POS	POS	POS			
	PT2020CAPXVIB_TP1	POS	POS	POS	POS	POS	POS	POS			
<u>₽</u>	PT2020CAPXVIR TP2	POS	POS	POS	POS	POS	POS	POS			
BSU		POS	POS	POS	POS	POS	POS	POS			
E.	PT2020CAPXVIB VP3	POS	POS	POS	POS	POS	POS	POS			
Ē	PT2020CAPXVIB TP4	POS	POS	POS	POS	POS	POS	POS			
	PT2020CAPXVIB TP5	POS	POS	POS	POS	POS	POS	POS			
	PT2020CAPXVIB VNI	NEG	NI	NEG	NEG	NEG	NEG	NEG			
	PT2020CAPXVIB_TNI	NEG	NEG	NEG	NEG	NEG	NEG	NEG			
		1920	142.0	146.61		192.91	142.61	146.61			
	Protocol / SOP used :	DNA extraction and real time PCR (Bowden et al., 2008)	RT-PCR P32 (Bowden)	K. Dietze et al. / Veterinary Microbiology 221 (2018) 44–48	Bowden et al 2008	IAEA/FAO/Bowden et al.	Haegeman et al, 2013				
	Producer Extraction protocol / kit:	QIAGEN	Qiagen	Qiagen	Indical	Qiagen/Magattract cador Pathogen Kit	Qiagen	Roche			
	Name Extraction protocol / kit:	QIAamp 96 Virus QIAcube HT Kit, lot: 166020122	DNeasy∅ Blood & Tissue	QIAamp Viral BNA Mini Kit	Indimag Pathogen kit	King Fisher Flex/Magattract cador Pathogen Kit	QiaAmp Viral RNA Mini Kit	High Viral Nucleic Acid kit			
	In-house modifications to extraction protocol (if yes, which?):	Extraction with QIAcube HT/QIAxtractor using manual lusis protocol	N/A	N/A	N/A	N/A	nła	N/A			
	PCR Instrument used:	Bio-Rad CFX96 Touch	LightCycler96 (Roche)	Bio-Rad CFX 96	Rotor-Gene	RotorGene Q	Agilent ABIAmx	LightCycler 96 (Roche)			
ation	Cut-off for positive:	< 40	No cut-off (Observation of a characteristic amplification curve)	40	ct 40	Ct 38	CT38				
- Line	Producer RT-PCR protocol / kit:	QIAGEN	Qiagen	Quantabio	Qiagen	Qiagen/Quantitect	Master mix: Thermofisher	IDVET			
	Name RT-PCR protocol / kit:	QuantiNova Probe PCR Kit, lot: 163028903	QuantiFast Probe PCR kit	PerfeCTa qPCR ToughMix	Quantifast Pathogen + IC		Master mix: TaqMan Fast Virus 1-Step Master mix	ID Gene Capripox Virus Triplex			
	The target of the RT primer:	Intracellular mature virion envelope protein P32 (VACV H3L homolog)	P32	ORF074 p32		p32	E3L	Target sequence of Capripox viral genome (including Lumpy Skin disease, Sheepox and Goatpox virus).			
	Remark(s):				All samples tested for CapV genome detection by Bowden et al Real Time PCR protocol		Results as Ct values				

		Labnr						
valu	e sample	97613	97614	97616	97617	97618	97619	97620
	PT2020CAPXVIR_VP1	26,15	32,4	32,32	31,73	30,66	29,6	32,79
	PT2020CAPXVIR_VP2	29,72	35	33,88	35,08	32,94	33,3	34,29
	PT2020CAPXVIR_TP1	23,41	26,3	26,59	29,21	24,16	28	28,56
at a	PT2020CAPAVIB_TP2	23,63	30,2	31,0	32,60	30,31	32,4	31,00
2	PT2020CAPAVID_TP3	20,23	21,0	25,00	30,30	20,10	23,3	23,61
ē	PT2020CAPXVIB TP4	28.64	34.2	32.42	34.07	29.26	33	33.31
	PT2020CAPXVIB TP5	20.81	20.6	24.81	23.59	22.3	24	22.6
	PT2020CAPXVIR VN1	No Ct	No Ct	No Ct	No Ct	No Ct	no Ct	No Ct
	PT2020CAPXVIR_TN1	No Ct	No Ct	No Ct	No Ct	No Ct	no Ct	No Ct
	PT2020CAPXVIR VP1	POS	POS	POS	POS	POS	POS	POS
	PT2020CAPXVIR VP2	POS	POS	POS	POS	POS	POS	POS
	PT2020CAPXVIR TP1	POS	POS	POS	POS	POS	POS	POS
ta ta	PT2020CAPXVIB TP2	POS	POS	POS	POS	POS	POS	POS
SL	PT2020CAPXVIB TP3	POS	POS	POS	POS	POS	POS	POS
1	PT2020CAPXVIB VP3	POS	POS	NI	POS	POS	POS	POS
Ē	PT2020CAPYVIR TP4	POS	POS	POS	POS	POS	POS	POS
		POS	POS	POS	POS	POS	POS	POS
		F03	NEG	NEG	NEG	NEG	NEG	NEG
	PT2020CAPXVIB TNI	NEG	NEG	NEG	NEG	NEG	NEG	NEG
_		TALCA	NEG	142.04	heor	TALCA	NEG	
	Protocol / SOP used :	Babiuk et al, 2008, Transbound Emerg Dis 55(7):299-307	Babiuk et.al., 2008, Transbound Emerging Diseases., 55(7):299-307	Path-ID qPCR	Bowden et al. 2008	Bowden et al., 2008	Balinsky et al., 2008	Bowden TH, Babluk SL, Parkin Left, Copps JS, Boyle DB (2008): Capripoxvirus tissue tropism and shedding: A quantitative study in experimentally infected sheep and goats. Virology 371 (2008) 380–393
	Producer Extraction protocol / kit:	QIAGEN	Qiagen	Qiagen	Qiagen Biosprint 96	Invitrogen by Thermo Fisher Scientific	Roche	QIAGEN
	Name Extraction protocol / kit:	IndiSpin Pathogen MiniKit	Viral BNA mini kit	QIAmp DNA mini kit	MagAttract 96 cador Pathogen Kit	Pure Link Genomic DNA Mini Kit	The High Pure Viral Nucleic Acid Kit	QIAamp Viral RNA Mini Kit (250)
	In-house modifications to extraction protocol (if ues, which?):	No	No	N/A	N/A	N/A	no	NłA
_	PCR Instrument used:	RotorGeneQ	AB 7500	Roche Lightcycler 96	Bio-Rad C1000/CFX96	ABI 7900 HT Fast Real-Time PCR System	CFX 96 Touch	Stratagene Mx3005P
matio	Cut-off for positive:			35	N/A	N/A	45	Ct < 45
life	Producer RT-PCR protocol / kit:	ThermoFisherScientific	ThermoFisherScientific	Applied Biosystems	Nzytech	Invitrogen by life technologies	Qiagen	QIAGEN
	Name RT-PCR protocol / kit:	Path-ID qPCR	Maxima Probe qPCR Master Mix	Path-ID qPCR Master Mix	NZYTaq 2x Colourless Master Mix	Platinum Quantitative PCR SuperMix-UDG with ROX	QuantiTect Probe PCR Kit/ Primers and Probe synthesised by Microsynth based publication	QuantiTect Virus Kit
	The target of the RT primer:	P32		p32	P32	ORF074 Primers CaPV- 074F15'-AAA ACG GTA TAT GGA ATA GAG TTG GAA-3' and CaPV-074R15'- AAA TGA AAC	ORF 068 [poly(A) polymerase (small subunit) gene	ORF074 of LSDV, SPPV, GTPV
	Remark(s):					The positive and negative controls were not included in kit. The values mentioned are Ct values.		

valu	e samnle	97621	97622	97623	97624	97625	97627	97628
	PT2020CAPXVIB VP1	28.2	36 75 / 37 25	32.065	34.3	28.53	2816	no Ct
	PT2020CAPXVIB VP2	32.8	40.00/38.88	35,735	38.5	3186	30.98	no Ct
	PT2020CAPXVIB TP1	25.9	28.01/28.22	30.68	30.5	25.52	26.78	29.92
	PT2020CAPXVIB TP2	29.3	32.30 / 32.32	33.235	34.6	29.31	29.31	35.56
뵹	PT2020CAPXVIR TP3	30,9	32,28 / 32,28	30,125	37,8	29,29	27,99	30,15
8	PT2020CAPXVIR VP3	34,5	36,00736,84	35,795	38,8	33,67	32,1	no Ct
2	PT2020CAPXVIR_TP4	32	33,08733,21	34,65	38,1	31,36	31,59	33,22
	PT2020CAPXVIR_TP5	24,2	27,22726,91	24,72	29,2	24,17	23,19	23,59
	PT2020CAPXVIR_VN1	No Ct	No Ct / No Ct	No Ct	No Ct	noct	No.Ct	no Ct
	PT2020CAPXVIR_TN1	No Ct	No Ct / No Ct	No Ct	No Ct	noct	No.Ct	no Ct
	PT2020CAPXVIR_VP1	POS	POS	POS	Pos	POS	POS	NEG
	PT2020CAPXVIR_VP2	POS	POS	POS	Pos	POS	POS	NEG
	PT2020CAPXVIB_TP1	POS	POS	POS	Pos	POS	POS	POS
ŧ	PT2020CAPXVIR_TP2	POS	POS	POS	Pos	POS	POS	POS
BSI	PT2020CAPXVIR_TP3	POS	POS	POS	Pos	POS	POS	POS
	PT2020CAPXVIR_VP3	POS	POS	POS	Pos	POS	POS	NEG
Ē	PT2020CAPXVIR_TP4	POS	POS	POS	Pos	POS	POS	POS
	PT2020CAPXVIR_TP5	POS	POS	POS	Pos	POS	POS	POS
	PT2020CAPXVIR VN1	NEG	NEG	NEG	Neg	NEG	NEG	NEG
	PT2020CAPXVIR TN1	NEG	NEG	NEG	Neg	NEG	NEG	NEG
	Protocol / SOP used :	Primers and probe described in Bowden TR et al. Virology 371 (2008) 380-393	00-14-0959	NVR-SOP-20 (Bowden et al., 2013)	IAEA Bowden et.al	Real-Time PCR detection of Capripoxvirus-sheeppox, goatpox and lumpy skin disease viruses		
	Producer Extraction protocol / kit:	Qiagen	Roche	ThermoFisher/KingFisher Flex	Invitrogen	QIAGEN	Qiagen Cat#51306	ThermoFisher
	Name Extraction protocol / kit:	MagAttract 96 CADOR Pathogen Kit	MagNa Pure 96 DNA and	LSI Magvet	Pure link genomic DNA mini kit	DNA Mini Kit	Qiamp DNA Mini Kit	PrepFiler Express Forensic DNA Extraction Kit
	In-house modifications to	Proteinase treatment at 72 °C /						
	extraction protocol (if yes, which?):	Addition of ARN carrier	N/A	None	No	no	N/A	N/A
	PCR Instrument used:	Applied Biosystems 7500	LC480	AB7500 Fast	7500 Real-Time PCR Sistem	Rotor-Gene	Light Cycler 2.0	Rotor-Gene 6000
mation	Cut-off for positive:	Ct < 35: + (35 < Ct < 40: unconclusive)		None				
Infe	Producer RT-PCR protocol / kit:	Life Technologies	Roche	LifeTechnologies	Applied biosystems & Euroffins Genomics		Invitrogen Cat# 10996-026	FractalBio, Russian Federation
	Name RT-PCR protocol / kit:	Path ID Q-PCR Master mix	LightCycler Fast Start DNA Master HybProbe	PathID kit	Taq Man Universal Master Mix & LSDV Primer Set	Path-ID qPCR Master Mix Kit	Taq platinum DNA polymerase	Lumpy skin disease DNA detection Kit
	The target of the RT primer:	p32	P32	ORF074	CAPV074	CaPV074F1 AAAACGGTATATGGAATAGAGTT GGAA CaPV074R1 AAATGAAACCAATGGATGGGATA CaPV074P1FAM- TGGCTCATAGATTCCA- MGB/NFQ	Sigma Forward primer- 5'GGCGATGTCCATTCCCTG 3' Reverse primer-5' AGCATTTCATTTCCGTGAGG A-3' Probe MGB 5' CAATGGGTAAAAGATTTCTA- 3'	
	Remark(s):							

		07000	Labnr	07004	07000	07000	07004	07025	07000
value	PT2020CADVVID_VD1	31523	37630	37531	37632	37633	37639	37630	37636
		31,76	32,9	27,31	31,0	27,60	30,78	30,13	27,01
	PT2020CAPXVIB TP1	DO Ct	26.2	24.2	261	25.46	23.95	26.97	21.21
_	PT2020CAPXVIB TP2	32.98	3178	27.46	316	29,25	27,72	30.48	25.17
Ť.	PT2020CAPXVIB TP3	32.59	28.73	28.04	28.1	26,27	26.41	29.17	22.79
8	PT2020CAPXVIR VP3	35,16	34.17	30,44	33.6	32.83	35,49	34,14	30.47
2	PT2020CAPXVIR_TP4	36,09	32,2	30,82	31,1	31,19	29,56	32,13	29,24
	PT2020CAPXVIB_TP5	25,72	24,98	23,3	22,3	21,99	22,75	24,01	18,15
	PT2020CAPXVIR_VN1	no Ct	Noct	No et	No Ct	No Ct	No Cq	No Ct	No Ct
	PT2020CAPXVIR_TN1	no Ct	Noct	No et	No Ct	No Ct	No Cq	No Ct	No Ct
	PT2020CAPXVIR_VP1	POS	POS	POS	POS	POS	POS	POS	POS
	PT2020CAPXVIR_VP2	NEG	POS	POS	POS	POS	POS	POS	POS
	PT2020CAPXVIR TP1	NEG	POS	POS	POS	POS	POS	POS	POS
<u>1</u>		POS	POS	POS	POS	POS	POS	POS	POS
Ins	PT2020CAPXVIB_TP3	POS	POS	POS	POS	POS	POS	POS	POS
2	PT2020CAPXVIB VP3	POS	POS	POS	POS	POS	POS	POS	POS
,Ë		POS	POS	POS	POS	POS	POS	POS	POS
-		POS	POS	POS	POS	POS	POS	FOS	POS
		PUS	POS	PUS	PUS	PUS	PUS	FUS	PUS
	PT2020CAPXVIR_VIVI	NEG	NEG	NEG	NEG	NEG	NEG	NEG	NEG
	PT2020CAPXVIR_TNI	NEG	NEG	NEG	NEG	NEG	NEG	NEG	NEG
	Protocol / SOP used :				Detection of Capripox viral DNA by real time PCR, based on Bowden et al. 2008, Stubbs et al. 2012		Capripox qPCR assay, Bowden		009
	Producer Extraction protocol / kit:	ThermoFisher	Qiamp Cador pathogen Mini kit	Roche, High Pure Nucleid acid Kit	Sacace Biotechnologies	Qiagen , Germany	LSI	Roche	INDICAL Bioscience
	Name Extraction protocol / kit:	PrepFiler Express Forensic DNA Extraction Kit			Viral Nucleic Acid Extraction Kit	Dna mini kit	Magvet universal kit	High Pure Viral Nucleic Acid Kit/33091000	IndiSpin Pathogen Kit
	In-house modifications to extraction protocol (if yes, which?):	N/A	N/A	N/A	N/A	N/A	N/A	N/A	N/A
	PCR Instrument used:	Rotor-Gene 6000	Smartcycler II Cepheid	AB 7500	QuantStudio5	rotor gene	AriaMx	Biorad CFX Connect	Rotor Gene 3000
ormation	Cut-off for positive:			38		up tp 35 cycles	38	≤38	
Ę	Producer RT-PCR protocol / kit:	FractalBio, Russian Federation	ID gene Capripox virus triplex	Qiagen, QuantiTect PCR kit	Applied Biosystems	FGBI ARRIAH	Agilent	Roche	IDvet Genetics
	Name RT-PCR protocol / kit:	Lumpy skin disease DNA detection Kit	IDCPV ver1217_EN	Bowden et al 2008	Path-ID multiplex One-Step RT- PCR Kit	A real time PCR kit for indentification of capripoxvirus DNA	Brilliant III Ultrafast qPCR kit	Roche LightCycler 480 Probes Master/26874521	ID Gene Capripox Virus Triplex
	The target of the RT primer:				ORF074	not disclosed		P32 Gene,89 bp	
	ricinan(s).								

Annex 1b: Raw Optional RT-PCR data

		Labnr	Labnr	Labnr	Labnr
value	sample	97605	97607	97620	97621
	PT2020CAPXVIR_VP1	34,4	31,61	No Ct	POS++
	PT2020CAPXVIR_VP2	No Ct	35,78	No Ct	NEG
	PT2020CAPXVIR_TP1	32,75	36,29	29,77	NEG
_	PT2020CAPXVIR_TP2	29,59	32,73	32,84	POS++
date	PT2020CAPXVIR_TP3	29	30,29	No Ct	POS++
DE L	PT2020CAPXVIR_VP3	31,3	33,4	36,33	POS+
	PT2020CAPXVIR_TP4	27,27	27,72	34,64	POS+++
	PT2020CAPXVIR_TP5	22,35	26,16	No Ct	POS++
	PT2020CAPXVIR_VN1	No Ct	No Ct	No Ct	NEG
	PT2020CAPXVIR_TN1	No Ct	No Ct	No Ct	NEG
	PT2020CAPXVIR_VP1	POS	POS	NEG	POS
	PT2020CAPXVIR_VP2	NEG	POS	NEG	NEG
	PT2020CAPXVIR_TP1	POS	POS	POS	NEG
ţ2	PT2020CAPXVIR_TP2	POS	POS	POS	POS
nsa	PT2020CAPXVIR_TP3	POS	POS	NEG	POS
E .	PT2020CAPXVIR_VP3	POS	POS	POS	POS
Ξ	PT2020CAPXVIR_TP4	POS	POS	POS	POS
	PT2020CAPXVIR_TP5	POS	POS	NEG	POS
	PT2020CAPXVIR_VN1	NEG	NI	NEG	NEG
	PT2020CAPXVIR_TN1	NEG	NEG	NEG	NEG
	Protocol / SOP used :	Haegeman et al. 2013	RT-PCR RPO30 (Pan- capripox - Under development)	Vidanović, et al. 2016.	Lamien et al., 2011
	Producer Extraction protocol / kit:	Roche MP96 / Viral NA SV Kit	Qiagen	QIAGEN	Qiagen
	Name Extraction protocol	External lysis protocol	DNeasy⊘ Blood & Tissue	QIAamp Viral RNA Mini Kit (250)	MagAttract 96 CADOB Pathogen Kit
	In-house modifications to extraction protocol (if yes, which?):	No cut-off	No	(immin((200)	Proteinase treatment at 72 °C / Addition of carrier ABN
ation	PCR Instrument used:	MX3005p	Stratagene MX3005P (Agilent Technologies)	Stratagene Mx3005P	Verity (Applied Biosystems)
Inform	Cut-off for positive:	No cut-off	No cut-off (Observation of a characteristic amplification curve)	Ct < 45	Clear band at the right height and with the expected intensity + blasting in the NCBI database
	Producer RT-PCR protocol / kit:	Thermofisher 4388644	Biorad	QIAGEN	Promega
	Name RT-PCR protocol /	Path-ID qPCR Master Mix	SsoAdvanced Universal Probes Supermix	QuantiTect Virus Kit	GoTaq® Hot Start Green Master Mix
	The target of the RT primer: Remark(c):	E3L	RP030	EEV gene of LSD field strain	
	neman(s).				

mage 3900 3904 3900 3904 3904 3904 3904 3904 PT20024791(1):0 MG parkin 50.0 100 p.p. F00 ML rependent PT20024791(1):0 MG parkin 50.0 100 p.p. F00 ML rependent PT20024791(1):0 MG parkin 50.0 100 p.p. F00 ML rependent F00 53.0 F00 p.p. F00 ML F00 53.0 F00 p.p. F00 F00 F00 F00 53.0 F00 p.p. F00 53.0 F00 p.p. F00						Labar				
Product NVPL VPL Product NVPL VPL VPL Product NVPL VPL Product NVPL	v	alue	sample	97506	97600	97602	97603	97604	97607	97608
Processes/ Processes/			PT2020CAPXVIR_VP1	NEG	positive	35,01	172bp	POS	No Ct	sequencing
No. State The State State The State PTSSSSCAPUNE_TRT State TTV			PT2020CAPXVIR_VP2	NEG	positive	no Ct	1726p	POS	No Ct	sequencing
No. South South No. Trage South No.			PT2020CAPXVIR_TP1	29,6	positive	30.88	172bp	POS	28.52	27.04
Product Activity 100 Product Activity 101 Product			PT2020CAPXVIB TP2	34.5	positive	35.23	172bp	POS	33.86	29.33
Procession Display			PT2020CAPYVID TP3	32.2	positius	32.55	172ko	 pos	31 305	30.05
Process Answer Tria Display Display <th></th> <td></td> <td>PT2020CARXVID_VP2</td> <td>37.9</td> <td>positive</td> <td>Jos de</td> <td>1726-</td> <td>POS</td> <td>26.775</td> <td>30,00</td>			PT2020CARXVID_VP2	37.9	positive	Jos de	1726-	POS	26.775	30,00
Product Armonic International Control Design (C) Display Display Product (C) Display Display <th></th> <td>8</td> <td>PT2020CAPXVIR_VP3</td> <td>01,0</td> <td>positive</td> <td>NO CC</td> <td>1205-</td> <td>POS</td> <td>30,115</td> <td>32,02</td>		8	PT2020CAPXVIR_VP3	01,0	positive	NO CC	1205-	POS	30,115	32,02
PP20000_ABX/RE_TP3 55.50 pontini 65.51 Stap PC No.C respecting PP20000_ABX/RE_TP3 NA segafities 0.6.5 No.E seg No.C No.C<		No.	PT2020CAPXVIR_TP4	33,3	positive	31,36	1r2bp	P02	34,33	32,23
Product AVXINE_TP3 55.50 pools Fib p P01 No.01 exequating Product AVXINE_TV11 M.A exequating in 0.01		-								
Processor/Res. VM NA segain No. Cr. No. K seg No. Cr. No. Cr. Processor/Res. VM GTV GTV <th></th> <th></th> <th>PT2020CAPXVIR_TP5</th> <th>35,26</th> <th>positive</th> <th>26,93</th> <th>151bp</th> <th>POS</th> <th>No Ct</th> <th>sequencing</th>			PT2020CAPXVIR_TP5	35,26	positive	26,93	151bp	POS	No Ct	sequencing
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P12020AX/VIR_VTM SPFV SPFV <th></th> <td>ú.</td> <td>PT2020CAPXVIR_TP4</td> <td>LSDV</td> <td>LSDV</td> <td>LSDV</td> <td>GTPV/LSDV</td> <td>LSDV</td> <td>LSDV</td> <td>LSDV</td>		ú.	PT2020CAPXVIR_TP4	LSDV	LSDV	LSDV	GTPV/LSDV	LSDV	LSDV	LSDV
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Formation Index				assay				GPCR seq	Under development)	Archives of Virology (2019)
Producer Extraction protocol / Mic Mindcary Nigal Biocellal ThermeFielder Sciatation Rocke Disgon Disgon Name Extraction protocol / Mic Blood Biocellal Superball MagMAX Core Neddic Add Purifications KR MagMAX Parce SDNA and Vari NA Small Volume KR Disayo Blood & Tiscse OlAmp Viral RNA Mini KR Incloses modifications to extraction protocol (I yee, which?): Addition of EC to Buffer B3 no Stratagene MX3009 (Agile) Concort for positive: Concort for positive: Concort for positive: Concort for positive: Red Methodology: Rocke Read-time PCR- Deal hybridication probes assay. Chostine PCR- Deal hybridication probes assay. Chostine CRR applification correct Promogene mic Stratagene MX3009 (Agile) Concort for positive: Correct for positive: Correct for positive: Correct for positive: Red Methodology: Rocke Read-time PCR - Deal hybridication probes assay. Chostine CRR applification probes assay. Chostine CRR applification correct Stratagene MX3009 (Agile) Correct for positive: Correct for positive: Correct for positive: Correct for positive: Red Methodology: Rocke Read-time PCR - Deal hybridication probes assay. Chostine Carl positive: Correct for posit										164:2931-2941
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Name Extraction protocol / kit: Blood Bioselial Bapurball MagMAX Core Nucleic Add Purification Kit MagMA Pure Sc DubA and Viral MA Small Volume Kit DMeary® Blood & Tissee OliAnap Viral RNA Mini Kit In-house modifications to extraction protocol (f yes, which?): Addition of EC to Buffer B3 no Consolution (Group Control (Group Con		Pro	outcor Extraction protocol 7 kit:	Machery Nagel	Biosellal	ThermoFisher Scientific	Roche Diagnostics	Roche	Qiagen	Qiagen
Induction protect frame Blood Blood Blood MgMAX Zores Nucleix Add Purifications Kit MgMAA Pure Compact Nuclei Vrai NA Small Volums Kit MgMA Pure Compact Nuclei Volum			no Extraction protocol d bits							
Inclusion Decision Purifications Kit Virial NA Small Volums Kit Drespy Decision Kit [20] Decis Kit [20] <		No.	me Extraction protocol r kit:	Blood	Biocollal Superhall	MagMAX Core Nucleic Acid	MagNA Pure Compact Nucleic	MagNA Pure 96 DNA and	DNoncu® Blood & Ticsur	QLAsmo Viral DNA Misi Kis
In-house modifications to extraction protocol (if yes, which?): PCR instrument weed: Addition of EC to Buffer B3 no No NA PCR instrument weed: Lightcycler 480 BioRad CPX36 RotorGene@ LightCycler 96 (Roche) Eppendorf Mastergryden ProS Strategrae MX3005P (Aglinet Technologies) GeneAmp 2120 Thermal Cycler Cut-off for positive: Crt4dS: Positive: 45c:Ct/50: Doubtful; Crt50: Nogative Crt4dS: Positive: 45c:Ct/50: Doubtful; Crt50: Nogative Roche Roche Roche Roche Prose Biornd Sanger Sequencing Ref Methodology: Taq Platnium GPCR Carse of the RT primer: LISDV GPCR (field strain) GPCR GPCR GPCR Adedaje at , Transboand Emergy (2015) 164:2307-2341 remarks LISDV GPCR (field strain) GPCR GPCR SPPV (C2 24;6) and SPPV (C2 24;6) and				Diood	Diosenal Superball	Purification Kit	Acid Isolation Kit I	Viral NA Small Volume Kit	Dracasyo Diood & Lissue	woomp virai bino ivini KR
Integer Index		le l	ana madifications to other time			1				
protocol (if yes, which?): Light cycler 480 BioRad CFX36 RetorGane® Light cycler 36 (Rocke) Ependorf Mastercycler ProS Stratagene MX3005P (Agilent Technologier) Cancer App 220 Thermal Cycler Out-off for positive: Cr445: Positive; 450:Ct50: Doubtrid; Cr450: Nogative Cr445: Positive; 450:Ct50: Doubtrid; Cr450: Nogative Red-time PCR - Dual hybridisation probes seasy NNA NNA Nota-ctfridis applification of a characteristic applification curve) Sanger Sequencing Methodology: Red Methodology: Taq Platnium Classical PCR applification probes seasy GoTaq Colories: Master mit Sondarracteristic applification curve) Sanger Sequencing The target of the RT primer: LISDV GPCR (field strain) GPCR GPCR RPCR RPO30 GPCR gene GPCR gene Classical PCR applification curve) For the V-CPX2009 sample, ve have displayed ND because the charles NI is not available.		IN-1	iouse modifications to extraction	Addition of EC to Buffer B3	no		No		No	N/A
PCR Instrument used: Lightcycler 480 BioRad CFX36 RotorGane@ LightCycler 36 (Rochc) Eppendorf Mattercycler ProS Stratagane MXX000P (Agilent ProS GeneAmp 2720 Thermal Cycler Out-off for positive: Cxt43: Positive; 45:Ctx50: Doubtful; Cxt50: Negative Cxt43: Positive; 45:Ctx50: Doubtful; Cxt50: Negative Cxt43: Positive; 45:Ctx50: Doubtful; Cxt50: Negative Roche N/A No No No Cut-off (Discarvation of characteristic amplification ourse) No Methodology: Roche Roche Roche Rest-time PCR - Dual hybridisation probes assay Classical PCR amplification ourse) Promega Biorad Sanger Sequencing Tag Platinium GPCR Rest-time PCR - Dual hybridisation probes assay Classical PCR amplification mix Promega Sind-Advaced Universal Probes Supermix The target of the RT primer: LSDV GPCR (field strain) GPCR GPCR RP030 GPCR gene GPCR Addedji et al., Transbound Emerg Dis.2015;1-11.// Mölist et al., Archives of Vicology (2013) (Ct.25.66), GTPV (Ct.22.16) and SPPV (Ct.22.16) and Mensaberor et al. 2014. SPPV (Ct.22.16) and SPPV (Ct.22.16) and SPPV (Ct.22.16) and Mensaberor et al. 2014. SPPV (Ct.22.16) and ISDV ample ased as positive collectof from real the craste of SPBp for SPPV vind TX26 positi		pro	otocol (if yes, which?):							
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Out-off for positive: Cx45: Positive: 45:CX450: Doubtrity: CX250: Negative Cx465: Positive: 45:CX450: Doubtrity: CX250: Negative N/A								ProS	Technologies)	System
Cut-off for positive: Cut-off for positive: Cut-off for positive: N/A characteristic amplification curve) Methodology: Roche Real-time PCR - Dual hybridisation probes assay Classical PCR amplification Promega Biorad Sanger Sequencing Image: Problem Problem Problem Problem PCR - Dual hybridisation probes assay Classical PCR amplification Promega Biorad Sanger Sequencing The target of the RT prime: LSDV GPCR (field strain) GPCR GPCR RPO30 GPCR gene GPCR Dis-2015-11.1 // Molifier et al., Archivee Of UR 2015-11.1 // Molifier et al., Archivee Of UR 2015-11.1 // Molifier et al., Archivee Of UR 2015-11.1 // Molifier et al., Pro sample: 2005 and 2001 specifier we datemarks SBPC (field strain) GPCR SBPC (field strain)				Chr/45: Regiting: 455Chr/50: Deubasid					No cut-off (Observation of a	
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remarks SPPV (Ct 24,43) For samples 2006 and 2007 species was determined using protocols of Vidanovic et al. 2016, and Menasherow et al., 2014. For the V-CPX2003 sample, we have displayed ND because the choice NI is not available.						(Ct 25.66), GTPV (Ct 22,16) and	genotyped as GTPV if collected			
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was determined using protocols of Vidanovic et al. 2016. and Menasherow et al., 2014. known positive SPPV, GTPV and LSDV sample used as positive control with the results of 151bp for SPPV and 12bp for GTPV and LSDV. we nave approyed ND because		107	barks			For complex 2006 and 2007 crastics	collected from cattle DNA from		we have displayed ND because	
Was determined using protocols of Vidanovic et al. 2016. and Menasherow et al., 2014. LSDV sample used as positive control with the results of 151bp for SPPV and 122bp for GTPV and LSDV. the choice will is not available.		. en				use determined using protocols of	known positive SPPV, GTPV and		the shales Million the Second	
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Menasherow et al., 2014. for SPPV and 172bp for GTPV and LSDV.						vidanovic et al. 2016, and	control with the results of 151bp			
LSDV.						rvienasherow et al., 2014.	for SPPV and 172bp for GTPV and			
							LSDV.			

Annex 2: Raw Capripox Species Differentiation data

value	sample	97609	97610	97611	97612	97613	97614	97617
- dide	PT2020CAPXVIB VP1	gel-based (Lamien et al-ampicon sequencing)	01010	33.1	No Ct	30.46	36.56	POS
	PT2020CAPXVIB VP2	gel-based (Lamien et al-ampicon sequencing)		36.5	No Ct	36.63	40	POS
	PT2020CAPXVIB TP1	27 2 (WT)		27.7	26	26 73	33.9	POS
	PT2020CAPXVIB TP2	32.79 (VT)		29.6	30	35.9	34.7	POS
	PT2020CAPXVIB TP3	29.66 (Viac)		281	27	31.28	35.7	POS
2	PT2020CAPXVIB VP3	36.59 (V(T)		21.8	23	36.56	38.2	POS
-B		22 77 (V/T)		215	23	24 90	29.5	POS
8	T TEOEDONI AMILEIT 4	55,11 (#1)		51,5	51	54,55	30,5	185
-		gel-based (Lamien et al-ampicon sequencing plus						
	PT2020CAPXVIR_TP5	Haegeman 2015 conv SPPV DIVA (ampicon		21,6	No Ct	33,95	33,7	POS
		sequencing) and SPPV WT Real Time PCR)						
	PT2020CAPXVIB_VNI	not tested (Neg CanV)		No Ct	No Ct	No Ct	No Ct	NKA
	PT2020CAPXVIB_TNI	not tested (Neg CapY)		No Ct	No Ct	No Ct	No Ct	N/A
	PT2020CAPXVIB VP1	GTPV	GTPV	GTPV	ND	GTPV	GTPV	GTPV
	PT2020CAPXVIB VP2	GTPV	GTPV	GTPV	ND	GTPV	GTPV	GTPV
	PT2020CAPXVIB TP1	ISDV	LSDV	ISDV	ISDV	LSDV	ISDV	LSDV
£	PT2020CAPXVIB TP2	ISDV	LSDV	LSDV	ISDV	I SDV	ISDV	LSDV
SL	PT2020CAPXVIB TP3	ISDV	LSDV	LSDY	LSDY	LSDV	LSDV	LSDV
2	PT2020CAPXVIB VP3	ISDV	LSDV	LSDV	ISDV	LSDV	ISDV	LSDV
Ë,	PT2020CAPXVIB TP4	LSDV	LSDV	LSDV	ISDV	LSDV	ISDV	LSDV
-	PT2020CAPXVIB TP5	SPPV	SPPV	SPEV	ND	SPPV	SPEV	SPPV
	PT2020CAPXVIB VNI	ND	NEG	NEG	NEG	NEG	ND	NVA
	PT2020CAPXVIB TN1	ND	NEG	NEG	NEG	NEG	ND	N/A
		Lamion of allHangeman 2015 SPRV DIVA					Lamion et al. 2011 Journal	
	Protocold SOP used.	convertional JUbgeman 2015 SPPV DIVA	Golano et al. El OS 2012			Lamien et al 2011, J of Virol	of uirological methods	Golano et al. 2015
	riotocorroor used.	Time PCB (Subr)	Gelage et al., 1 200 2010			Methods 171(1):134-140	171(1).124.140	Gelage et al. 2010
	Destance Enterties and a lit	niner Orr(ogbi)					11 (1),104-140	
	kit:	Qiagen		Qiagen	Roche	QIAGEN	Qiagen	Qiagen Biosprint 96
	Name Extraction protocol / kit:	Qiamp Cador Pathogen	Qiagen/Magattract_cador Pathogen Kit	Qiagen QiaAmp Viral RNA Mini Kit	High Viral Nucleic Acid kit	IndiSpin Pathogen MiniKit	Viral BNA mini kit	MagAttract 96 cador Pathogen Kit
	In-house modifications to extraction protocol (if yes, which?):	no	King Fisher Flex/Magattract cador Pathogen Kit	nła		No	No	N/A
	PCR Instrument used:	Veriti Thermal Cycler (Applied Biosystems)/Rotor- Gene Q	Roto-Gene Q	Agilent AriaMx	LightCycler96 (Roche)	RotorGeneQ	LightCycler 480	UNO II - Biometra
	Cut-off for positive:	N/A	ct 38	37				N/A
5	Methodology:	gel-based PCR and sequencing analysis:	Qiagen/Quantitect	Melt Curve using SYBR	PCR real time	Dual Hybridization Probes	Dual Hybridization Probe	Nzytech
Ξ.		Lamion et al/Haegeman 2015 SPRV DIVA	_	Green chemisty		Assay	Assay	
Inform	Ref Methodology:	conventional /Hageman 2015 SPPV WT Real Time PCR (Sybr)		Galaye et al, 2017, adapted in-house for Quantitect SYBR Green Master mix	Bio-T kit Lumpy Skin Disease (BioSellal)	Lamien et al 2011, J of Virol Methods 171(1):134-140	Lamien et.al.,2011 Journal of virological methods 171(1);134-140	NZYTaq 2x Colourless Master Mix
	The target of the RT primer:		RPO30	RP0147	Target sequence of Lumpy Skin Disease viral genome	chemokine gene		RP030
		No need for species differentiation regarding cattle samples. Only Field strain/Vaccine differentiation done by DIVA LSDV Real Time PCR						
	remarks	G I PV confirmation on goat samples done by Lamien et al conventional PCR and amplicon sequencing SPPV confirmation on sheep samples done by Lamien et al conventional PCR and amplicon sequencing		Hesults as I'm products and Ct values				

		nr			Labni			
value	sample	97618	97619	97620	97621	97623	97625	97628
		OTDUT	01010	4701	5000	01020	0000	0.020
	PT2020CAPXVIR_VP1	GIPVTurkey	pos	172bp	PUS+++		PUS	no Lt
	PT2020CAPXVIR_VP2	GTPV Koppal	pos	172bp	POS+		POS	no Ct
	PT2020CAPXVIB TP1	LSDV Isolate Pendik	DOS	172bp	POS++		POS	25.33
	PT2020CAPXVIB TP2	ND	DOS	172bp	NEG		POS	31.09
		LODVILING CD DCA 1007	pos	1705-	DOC		000	01,00 00.1E
	PT2020CAPXVIR_TP3	LSDV ISOI 148-GP-RSA-1997	pos	1720p	PUS++		PUS	30,15
18	PT2020CAPXVIR_VP3	ND ND	pos	172bp	POS+		POS	no Ct
-	PT2020CAPXVIB TP4	ND	pos	172bp	NEG		POS	31.72
8	_							
-								
	PT2020CAPXVIB_TP5	SPPVaccine Turken	DOS	151bn	POS+		POS	23.74
			P	ionsp	1 001			2011
	PT2020CAPXVIB VNI		Deg	peg	NEG		NEG	no Ct
		ND	neg	neg	NEC		NEC	Ct
_	F12020CAFAVIE_INI	ND	neg	neg	NEG		NEG	no Lt
	PT2020CAPXVIR_VP1	GTPV	GTPV	GTPV/LSDV	GTPV	GTPV	SPPV	NEG
	PT2020CAPXVIR VP2	GTPV	GTPV	GTPV/LSDV	GTPV	GTPV	SPPV	NEG
	PT2020CAPYVID TP1	LSDV	LSDV	GTEV/LEDV	LSDV	LEDV	LEDV	GTEWNEDV
00	PT2020CALAVIT_TET	1 100	LODV	OTDUILODU	2304	LODY	LODV	OTPUILODU
- E	PT2020CAPXVIR_TP2		LSDV	GIPVALSOV	NEG	LSDV	LSDV	GIPWESDV
8	PT2020CAPXVIR_TP3	LSDV	LSDV	GTPV/LSDV	LSDV	LSDV	LSDV	GTPV
-	PT2020CAPXVIR VP3	ND	LSDV	GTPV/LSDV	LSDV	ND	LSDV	NEG
.≝.	PT2020CAPXVIB TP4	ND	L SDV	GTPWI SDV	NEG	L SDV	L SDV	L SDV
ць.		CDDV	CODY		CDDY	CDDY	CDDY	CDDV
	F12020CAFXVIR_TF0	SPPV	SFFV	SPPV	SFFV	SPPV	SPPV	SFFV
	PT2020CAPXVIR_VN1		NEG	NEG	NEG	ND	NEG	NEG
	PT2020CAPXVIB_TN1	ND	NEG	NEG	NEG	ND	NEG	NEG
							Classical PCB Method for	
				Lamion CE at al. Vat	Menasherow S et al. Jounal of	NVD COD 46 (Lamion at	Differential Disenserie of	ID Cose LSD DIVA
	Protocol/SOP used:	in house	Tapputainen et al., 2014	Lamence, et al., vet	Virological Methods 199:95-101.	NVH-SOF-46 (Lamenet	Dimerential Diagnosis or	ID Gene LSD DIVA
				Microbiol. 2011; 149:30–9.	2014	al., 2011)	Capripox: sheep&goat Pox/	Triplex
					2014		Lumpy skin disease	
	Producer Extraction protocol A	_				ThermoFisher/		
	kits	Promega	Roche	QIAGEN	Qiagen	KingEicher Elev	QIAGEN	ThermoFisher
	No					rangi ibner i ieu		Deer Film Frances
	Name Extraction protocol r Kit:	Genomic DNA Purification	The High Pure Viral Nucleic Acid	QIAamo Viral BNA Mini Kit	MagAttract 96 CADOR			FrepFiler Express
		V9	Vit	(250)	Bathogon Kit	LSI Magvet	DNA Mini Kit	Forensic DNA Extraction
		NR.	NR I	(200)	Factogen Kit			Kit
	In-house modifications to		no modification in ext protocol					
	outraction protocol (if use	No	ophuin DCD UDM _ replaced by		Proteinase treatment at 72 °C ł	None		NU O
	extraction protocol (in ges,	NO	Only ITECH HEIM - Teplaced by		Addition of ARN carrier	None	110	DIR.
	which?j:		Sanger sequencing of					
	DCD is shown as to use of	Cintra Thursday	CEV of Tauah	Diameter TO These seconds	Varias (Applied Discourses a)	AD7500 E>		Data: Case 0000
	PCR instrument used:	SimilAmp Thermal Cycler	CFA 36 TOUCh	Biometra 13 Thermocycler	Venty (Applied Biosystems)	AB7000 Fast		Hotor-Gene 6000
					Class band at the right give and			
			-		Clear band at the right size and			
	Cut-off for positive:		45	,	with the expected intensity +	None		
5					blasting in NCBI database			
1. 1.		Sequencing of BPO 030			_		APHI Seibersdorf(Lamien et 1	
Ē	Methodology:	Sequencing of the O 000	Thermo Fisher Scientific	Invitrogen	Promega	Rotogene	-Loot	DIVA PCR
Ę.		gene					ai 2011	
5					GoTag® Hot Start Green			
	Ref Methodology:	Gelaye E. et al., 2015	SS III ONE-STEP HI FI	Platinum PCR Supermix	A star Mar	-		
		-			Iviaster Iviix			
							SoGoDNApolE 5'	
							TCTATGTCTTGATATGTGGT	
	The target of the DT primer.	DD0020 avea	G-protein-coupled chemokine	RP030 of LSDV, SPPV,		CRCR	GGTAG-3'	
	The target of the RT primer:	RP0030 gene	receptor	GTPV		GFCR	SpGpBNApolB 5'-	
							AGTGATTAGGTGGTGTATT	
							ATTICC-3	
							In the time of the second second second	
							It is important to note that this	
				Conventional PCR for			PCR metod produce a product	
				species differentiation based			with similar size (172 bp) for	
	remark s			on the PCB product length			GTPV and LSDV and the	
	remarks			on the Fort productiength.			on ry and copy and the	
				Result is expresed as PCR			interpretation of the results	
				product length in bp.			needs to take into account the	
							species from which the	

alue	sample	97629	97631	97632	97633	97634	97636
	PT2020CAPXVIR_VP1	32,88	No Ct		28,13	31	
	PT2020CAPXVIR_VP2	noCt	No Ct		31,4	34,5	
	PT2020CAPXVIR_TP1	noCt	27,41		23,69	26,22	19,43
	PT2020CAPXVIR_TP2	29,89	29,01		27,56	29,56	23,4
_	PT2020CAPXVIR_TP3	32,59	27,45		26	26,49	23,13
të	PT2020CAPXVIR_VP3	39,23	33,2		31,55	37,64	29,02
3	PT2020CAPXVIR_TP4	31,93	31,93		28,84	31,72	27,59
2							
	PT2020CAPXVIR_TP5	24,66	26,11		21,86	26	
	PT2020CAPXVIB VN1	no Ct	No et		No Ct	No Ca	
	PT2020CAPXVIR TN1	noCt	Neg.		No Ct	NoCq	
	PT2020CAPXVIR_VP1	SPPV	GTPV	GTPV	GTPV	GTPV	ND
	PT2020CAPXVIR_VP2	NEG	GTPV	GTPV	GTPV	GTPV	ND
	PT2020CAPXVIR_TP1	NEG	LSDV	LSDV	LSDV	LSDV	LSDV
뽁	PT2020CAPXVIB_TP2	LSDV	LSDV	LSDV	LSDV	LSDV	LSDV
88	PT2020CAPXVIR_TP3	LSDV	LSDV	LSDV	LSDV	LSDV	LSDV
Ē	PT2020CAPXVIR_VP3	SPPV	LSDV	LSDV	LSDV	LSDV	LSDV
÷Ē	PT2020CAPXVIR_TP4	LSDV	LSDV	LSDV	LSDV	LSDV	LSDV
	PT2020CAPXVIR_TP5	SPPV	SPPV	SPPV	SPPV	SPPV	ND .
	PT2020CAPXVIR_VN1	NEG	NEG	NEG	NEG	NEG	NEG
	PT2020CAPXVIR_TN1	NEG	NEG	NEG	NEG	NEG	NEG
						Four qPCR test were	
				Differentiation of SPPV from		used, first qPCR for held	
				GTPWLSD, based on Lamien et		LSDV, second qPCR for	
	Protocol/SOP used :	Trialan		al. 2011; Genotyping or		Vac LSDV, third qPCR	
		rnpiex		Caphpoxvirus based on		aPCP (or GTPV	
				based on Galave et al. 2015		Vidapouio 2016	
				based off Gelage et al. 2010		Vidanovic Unpublished	
	Producer Extraction protocol /	ThermoFisher		Sacace Biotechnologies	Qiagen , Germanu	LSI. Thermo	INDICAL Bioscience
	Kit: Name Eutroptics protocold kit.	Bree Filer Funces	Dealte High Dura		~ , , , , , , , , , , , , , , , , , , ,		
	Name Exclaction protocol PRIC	Forensic DNA Extraction	Yiral Nucleic Acid	Viral Nucleic Acid Extraction Kit	Doa mini kit	Maguat universal kit	IndiSpin Pathogen Kit
		Kit	Kit	Than doleto Hold Exclaction Act	Diaminikk	reageet aniversarkit	indiopint actogentat
	In-house modifications to	144					
Ξ	extraction protocol (if yes,	N/A			N/A		
atio	which?):						
To To	PCR Instrument used:	Rotor-Gene 6000	AB7500	SimpliAmp	rotor gene	AriaMx	
-	Cut-off for positive:		38		up tp 35 cycles	40	
	Methodologu:	DIVA PCB		conventional PCR and Sanger	FGBI ABBIAH		PCB-BT
	,			sequencing	A real time DCD kit (or		
	Bef Methodology				indeptification of		
	The Methodology.				cantinosvirus DNA		
					saphponings ender		
							ID
	The barnet of the DT arise of			BBO30	nat disala and		Divet Genetics, ID Genetics DDV 6
	The target of the HT primer:			HF030	not disclosed		Gene LSD DIVA
							rupiex

		Labnr	Labnr	Labor	Labor	Labor	Labor	Labor
value	sample	97506	97600	97602	97604	97608	97609	97610
	PT2020CAPXVIR VP1	NEG	No Ct	ND	NA	ND	0	NA
	PT2020CAPXVIR_VP2	NEG	No Ct	ND	NA	ND	0	NA
	PT2020CAPXVIR TP1	29,6	29,3	28,01	NA	27,04	36,59	NA
_	PT2020CAPXVIR_TP2	34,5	31,85	33,28	NA	29,33	32,79	NA
data	PT2020CAPXVIR_TP3	32,2	33,21	no Ct	NA	30,05	29,66	NA
, B	PT2020CAPXVIR_VP3	37,9	35,94	38,94	NA	32,62	33,77	NA
-	PT2020CAPXVIR_TP4	35,9	34,37	33,8	NA	32,23	27,2	NA
	PT2020CAPXVIR_TP5	35,26	25,7	ND	NA	ND	21.62	NA
	PT2020CAPXVIR_VN1	NA	no ct	ND	NA	ND	0	NA
	PT2020CAPXVIR_TN1	NA	no ct	ND	NA	ND	0	NA
	PT2020CAPXVIR_VP1	ND	ND	ND	NI	ND	ND	ND
	PT2020CAPXVIR_VP2	ND	ND	ND	NI	ND	ND	ND
	PT2020CAPXVIR_TP1	LSDV FIELD	LSDV FIELD	LSDV FIELD	LSDV FIELD	LSDV FIELD	LSDV FIELD	LSDV FIELD
ţ	PT2020CAPXVIR_TP2	LSDV FIELD	LSDV FIELD	LSDV FIELD	LSDV FIELD	LSDV FIELD	LSDV FIELD	LSDV FIELD
lesu	PT2020CAPXVIR_TP3	LSDV VACCINE	LSDV VACCINE	LSDV VACCINE	LSDV VACCINE	LSDV VACCINE	LSDV VACCINE	LSDV VACCINE
E	PT2020CAPXVIR_VP3	LSDV FIELD	LSDV FIELD	LSDV FIELD	LSDV FIELD	LSDV FIELD	LSDV FIELD	LSDV FIELD
Ξ	PT2020CAPXVIR_TP4	LSDV FIELD	LSDV FIELD	LSDV FIELD	LSDV FIELD	LSDV FIELD	LSDV FIELD	LSDV FIELD
	PT2020CAPXVIR_TP5	SPPV FIELD	SPPV FIELD	ND	NI	ND	SPPV FIELD	ND
	PT2020CAPXVIR_VN1	ND	NEG	ND	ND	ND	ND	NEG
	PT2020CAPXVIB_TML	ND	NEG	ND	ND	ND	ND	NEG
		148	1426	148	148	NB	140	142.04
	Protocol / SOP used :	Agianniotaki et al., 2016 and in-house Taqman assay	Confidential	Vidanović et al, 2016 Menasherow et al. 2014	Agianniotaki et al., 2016 and in-house Taqman assay	Agianniotaki et al., 2016 and in-house Taqman assay	Agianniotaki et al DIVA LSDV real-time PCR /Haegeman et al 2015 SPPV DIVA conv PCR/ Haegeman 2015 SPPV WT Real Time PCR (Agianniotaki et al., 2016 and in-house Taqman assay
	Protocol / SOP used : Producer Extraction protocol / kit:	Agianniotaki et al., 2016 and in-house Taqman assay Machery Nagel	Confidential Biosellal	Vidanovi: et al, 2016 Menasherow et al. 2014 ThermoFisher Scientific	Agianniotaki et al., 2016 and in-house Taqman assay Roche	Agianniotaki et al., 2016 and in-house Taqman assay Qiagen	Agiannicaki et al DIVA LSDV real-time PCR /Haegeman et al 2015 SPPV DIVA conv PCR/ Haegeman 2015 SPPV WT Real Time PCR (Qiagen	Agianniotaki et al., 2016 and in-house Taqman assay Qiagen/Magattract cador Pathogen Kit
	Protocol / SDP used : Producer Extraction protocol / kit: Name Extraction protocol / kit:	Agianniotaki et al., 2016 and in-house Taqman assay Machery Nagel Blood	Confidential Biosellal Biosellal Superball	Vidanovi: et al, 2016 Menasherow et al. 2014 ThermoFisher Scientific MagMAX Core Nucleic Acid Purification Kit	Agianniotaki et al., 2016 and in-house Taqman assay Roche MagNA Pure 96 DNA and Viral NA Small Volume Kit	Agianniotaki et al., 2016 and in-house Taqman assay Qiagen QIAamp Viral BNA Mini Kit	Agianniotaki et al DIVA LSDV real-time PCR /Haegeman et al 2015 SPPV DIVA conv PCR/ Haegeman 2015 SPPV WT Real Time PCR (Qiagen Qiamp Cador Pathogen	Agianniotaki et al., 2016 and in-house Taqman assay Qiagen/Magattract cador Pathogen Kit King Fisher Flex/ Magattract cador Pathogen Kit
5	Protocol / SOP used : Producer Extraction protocol / kit: Name Extraction protocol / kit: In-house modifications to extraction protocol (if yes, which?):	Agianniotaki et al., 2016 and in-house Taqman assay Machery Nagel Blood Addition of EC to Buffer B3	Confidential Biosellal Biosellal Superball	Vidanovi: et al, 2016 Menasherow et al. 2014 ThermoFisher Scientific MagMAX Core Nucleic Acid Purification Kit	Agianniotaki et al., 2016 and in-house Taqman assay Roche MagNA Pure 96 DNA and Viral NA Small Volume Kit	Agianniotaki et al., 2016 and in-house Taqman assay Qiagen QlAamp Viral BNA Mini Kit N/A	Agianniotaki et al DIVA LSDV real-time PCR /Haegeman et al 2015 SPPV DIVA conv PCR/ Haegeman 2015 SPPV WT Real Time PCR (Qiagen Qiamp Cador Pathogen NO	Agianniotaki et al., 2016 and in-house Taqman assay Qiagen/Magattract cador Pathogen Kit King Fisher Flew Magattract cador Pathogen Kit
imation	Protocol / SOP used : Producer Extraction protocol / kit: Name Extraction protocol / kit: In-house modifications to extraction protocol (if yes, which?): PCR Instrument used:	Agianniotaki et al., 2016 and in-house Taqman assay Machery Nagel Blood Addition of EC to Buffer B3 Lightcycler 480	Confidential Biosellal Biosellal Superball no ABI 7500Fast	Vidanovi: et al, 2016 Menasherow et al. 2014 ThermoFisher Scientific MagMAX Core Nucleic Acid Purification Kit BotorGeneQ	Agianniotaki et al., 2016 and in-house Taqman assay Roche MagNA Pure 96 DNA and Viral NA Small Volume Kit Eppendorf Mastercycler ProS	Agianniotaki et al., 2016 and in-house Tagman assay Qiagen QiAamp Viral BNA Mini Kit N/A Bio-Rad CFX 96	Agianniotaki et al DIVA LSDV real-time PCR /Haegeman et al 2015 SPPV DIVA conv PCR/ Haegeman 2015 SPPV WT Real Time PCR (Qiagen Qiamp Cador Pathogen NO RotorGene	Agianniotaki et al., 2016 and in-house Taqman assay Qiagen/Magattract cador Pathogen Kit King Fisher Flex/ Magattract cador Pathogen Kit Roto-Gene Q
Information	Protocol / SOP used : Producer Extraction protocol / kit: Name Extraction protocol / kit: In-house modifications to extraction protocol (if yes, which?): PCR Instrument used: Cut-off for positive:	Agianniotaki et al., 2016 and in-house Taqman assay Machery Nagel Blood Addition of EC to Buffer B3 Lightoyoler 480 Ctc 45: Positive; 45sCtc 50: Doubtful; Ct250: Negative	Confidential Biosellal Biosellal Superball no ABI 7500Fast	Vidanovi: et al, 2016 Menasherow et al. 2014 ThermoFisher Scientific MagMAX Core Nucleic Acid Purification Kit BotorGeneQ	Agianniotaki et al., 2016 and in-house Taqman assay Roche MagNA Pure 96 DNA and Viral NA Small Volume Kit Eppendorf Mastercycler ProS Ct<45: Positive; 45≤Ct<50: Doubtful; Ct≥50: Negative	Agianniotaki et al., 2016 and in-house Tagman assay Qiagen QiAamp Viral BNA Mini Kit N/A Bio-Rad CFX 96 40	Agiannicaki et al DIVA LSDV real-time PCR /Haegeman et al 2015 SPPV DIVA conv PCR/ Haegeman 2015 SPPV WT Real Time PCR (Qiagen Qiamp Cador Pathogen NO RotorGene	Agianniotaki et al., 2016 and in-house Taqman assay Qiagen/Magattract cador Pathogen Kit King Fisher Flex/ Magattract cador Pathogen Kit Roto-Gene Q
Information	Protocol / SOP used : Producer Extraction protocol / kit: Name Extraction protocol / kit: In-house modifications to extraction protocol (if yes, which?): PCR Instrument used: Cut-off for positive: Methodology	Agianniotaki et al., 2016 and in-house Taqman assay Machery Nagel Blood Addition of EC to Buffer B3 Lightcycler 480 Ct<45: Positive; 45sCt<50: Doubtful; Ct≥50: Negative Roche	Confidential Biosellal Biosellal Superball no ABI 7500Fast	Vidanovi: et al, 2016 Menasherow et al. 2014 ThermoFisher Scientific MagMAX Core Nucleic Acid Purification Kit RotorGeneQ Real-time PCR	Agianniotaki et al., 2016 and in-house Taqman assay Roche MagNA Pure 96 DNA and Viral NA Small Volume Kit Eppendorf Mastercycler ProS Ctc45: Positive; 45sCtc50: Doubtful; Ct250: Negative GPCR sequencing + BLAST analysis	Agianniotaki et al., 2016 and in-house Taqman assay Qiagen QIAamp Viral RNA Mini Kit N/A Bio-Rad CFX 96 40 sPCR	Agianniotaki et al DIVA LSDV real-time PCR /Haegeman et al 2015 SPPV DIVA conv PCR/ Haegeman 2015 SPPV WT Real Time PCR (Qiagen Qiamp Cador Pathogen NO RotorGene Agianniotaki et al DIVA LSDV real-time PCR /Haegeman et al 2015 SPPV DIVA conv PCR/ Haegeman 2015 SPPV WT Real Time PCR	Agianniotaki et al., 2016 and in-house Taqman assay Qiagen/Magattract cador Pathogen Kit King Fisher Flew Magattract cador Pathogen Kit Roto-Gene Q Qiagen/Quantitect
Information	Protocol / SOP used : Producer Extraction protocol / kit: Name Extraction protocol / kit: In-house modifications to extraction protocol (if yes, which?): PCR Instrument used: Cut-off for positive: Methodology Ref Methodology	Agianniotaki et al., 2016 and in-house Taqman assay Machery Nagel Blood Addition of EC to Buffer B3 Lightoyoler 480 Ctc45: Positive: 45sCtc50: Doubtful; Ct250: Negative Roohe Taq Platinium	Confidential Biosellal Biosellal Superball no ABI 7500Fast	Vidanovi: et al, 2016 Menasherow et al. 2014 ThermoFisher Scientific MagMAX Core Nucleic Acid Purification Kit BotorGeneQ Real-time PCR Vidanovi: et al, 2016	Agianniotaki et al., 2016 and in-house Taqman assay Roche MagNA Pure 36 DNA and Viral NA Small Volume Kit Eppendorf Mastercycler ProS Ct<45: Positive; 45sCt<50: Doubt/ul; Ct>50: Negative GPCR sequencing + BLAST analysis Gelaye E, et al., 2015 + BLAST	Agianniotaki et al., 2016 and in-house Taqman assay Qiagen QIAamp Viral RNA Mini Kit N/A Bio-Rad CFX 96 40 sPCR	Agianniotaki et al DIVA LSDV real-time PCR /Haegeman et al 2015 SPPV DIVA conv PCR/ Haegeman 2015 SPPV WT Real Time PCR (Qiamp Cador Pathogen NO RotorGene Agianniotaki et al DIVA LSDV real-time PCR /Haegeman et al 2015 SPPV DIVA conv PCR/ Haegeman 2015 SPPV WT Real Time PCR	Agianniotaki et al., 2016 and in-house Taqman assay Qiagen/Magattract cador Pathogen Kit King Fisher Flex/ Magattract cador Pathogen Kit Roto-Gene Q Qiagen/Quantitect

Annex 3: Raw Capripox field and vaccine strain differentiation data

		Labnr	Labnr	Labnr	Labnr	Labnr	Labnr	Labnr
value	sample	97611	97612	97613	97614	97617	97618	97620
	PT2020CAPXVIR_VP1	No Ct	-	No Ct	NA	N/A		
	PT2020CAPXVIR_VP2	No Ct	-	No Ct	NA	N/A		
	PT2020CAPXVIR_TP1	31,5	22 FAM / - VIC	33,51	NA	Not done	29,52	24,99 (FAM)
	PT2020CAPXVIR_TP2	29,1	26 FAM / - VIC	33,19	NA	Not done	36,14	29,71(FAM)
ġ	PT2020CAPXVIR_TP3	29,6	FAM - / 26 VIC	29,67	NA	Not done	32,19	32,54 (VIC)
B B	PT2020CAPXVIR_VP3	32,4	29 FAM / - VIC	33,14	NA	Not done	37,75	32,19 (FAM)
	PT2020CAPXVIR_TP4	27,3	28 FAM / - VIC	26,2	NA	Not done	34,84	31,28 (FAM)
	PT2020CAPXVIR_TP5	No Ct	-	No Ct	35,26	POS (302 bp)		
	PT2020CAPXVIR_VN1	No Ct	-	No Ct	NA	NłA		
	PT2020CAPXVIR_TN1	No Ct	-	No Ct	NA	NłA		
	PT2020CAPXVIR_VP1	ND	ND	NEG	NI	N/A	ND	ND
	PT2020CAPXVIR_VP2	ND	ND	NEG	NI	N/A	ND	ND
	PT2020CAPXVIR_TP1	LSDV FIELD	LSDV FIELD	LSDV FIELD	NI	NI Not done		LSDV FIELD
ta ta	PT2020CAPXVIR_TP2	LSDV FIELD	LSDV FIELD	LSDV FIELD	LSDV FIELD	Not done	LSDV FIELD	LSDV FIELD
esul	PT2020CAPXVIR_TP3	LSDV VACCINE	LSDV VACCINE	LSDV VACCINE	LSDV FIELD	Not done	LSDV VACCINE	LSDV VACCINE
L E	PT2020CAPXVIR_VP3	LSDV FIELD	LSDV FIELD	LSDV FIELD	NI	Not done	LSDV FIELD	LSDV FIELD
Ē	PT2020CAPXVIR_TP4	LSDV FIELD	LSDV FIELD	LSDV FIELD	NI	Not done	LSDV FIELD	LSDV FIELD
	PT2020CAPXVIR_TP5	ND	ND	NEG	SPPV FIELD	SPPV FIELD	ND	ND
	PT2020CAPXVIR_VN1	NEG	NEG	NEG	NEG	NEG	ND	ND
	PT2020CAPXVIR_TN1	ND	NEG	NEG	NEG	NEG	ND	ND
	Protocol / SOP used :	Agianniotaki et al., 2017	ID Gene LSD DIVA Triplex (Idvet)	Agianniotaki et al., 2017 J of Virol Methods 249; 48- 57		Agianniotaki et al., 2016 and in-house Taqman assay	DIVA GREECE Agianniotaki et al 2017	ID Gene LSD DIVA Triplex, Real time PCR
	Protocol / SOP used : Producer Extraction protocol / kit:	Agianniotaki et al., 2017 Qiagen QiaAmp Viral RNA Mini Kit	ID Gene LSD DIVA Triplex (Idvet) Roche	Agianniotaki et al., 2017 J of Virol Methods 249; 48- 57 QIAGEN		Agianniotaki et al., 2016 and in-house Taqman assay Qiagen Biosprint 96	DIVA GREECE Agianniotaki et al 2017 Invitrogen by Thermo Fisher Scientific	ID Gene LSD DIVA Triplex, Real time PCR QIAGEN
	Protocol / SOP used : Producer Extraction protocol / kit: Name Extraction protocol / kit:	Agianniotaki et al., 2017 Qiagen QiaAmp Viral RNA Mini Kit	ID Gene LSD DIVA Triplex (Idvet) Roche High Viral Nucleic Acid kit	Agianniotaki et al., 2017 J of Virol Methods 249; 48- 57 QIAGEN IndiSpin Pathogen MiniKit		Agianniotaki et al., 2016 and in-house Taqman assay Qiagen Biosprint 96 MagAttract 96 cador Pathogen Kit	DIVA GREECE Agianniotaki et al 2017 Invitrogen by Thermo Fisher Scientific Pure Link Genomic DNA Mini Kit	ID Gene LSD DIVA Triplex, Real time PCR QIAGEN QIAamp Viral RNA Mini Kit (250)
5	Protocol / SOP used : Producer Extraction protocol / kit: Name Extraction protocol / kit: In-house modifications to extraction protocol (if yes, which?):	Agianniotaki et al., 2017 Qiagen QiaAmp Viral RNA Mini Kit n/a	ID Gene LSD DIVA Triplex (Idvet) Roche High Viral Nucleic Acid kit	Agianniotaki et al., 2017 J of Virol Methods 249; 48- 57 QIAGEN IndiSpin Pathogen MiniKit No		Agianniotaki et al., 2016 and in-house Taqman assay Qiagen Biosprint 96 MagAttract 96 cador Pathogen Kit N/A	DIVA GREECE Agianniotaki et al 2017 Invitrogen by Thermo Fisher Scientific Pure Link Genomic DNA Mini Kit No	ID Gene LSD DIVA Triplex, Real time PCR QIAGEN QIAamp Viral RNA Mini Kit (250)
mation	Protocol / SOP used : Producer Extraction protocol / kit: Name Extraction protocol / kit: In-house modifications to extraction protocol (if yes, which?): PCR Instrument used:	Agianniotaki et al., 2017 Qiagen QiaAmp Viral RNA Mini Kit n/a Agilent ABIA	ID Gene LSD DIVA Triplex (Idvet) Roche High Viral Nucleic Acid kit LightCycler 96 (Roche)	Agianniotaki et al., 2017 J of Virol Methods 249; 48- 57 QIAGEN IndiSpin Pathogen MiniKit No RotorGeneQ		Agianniotaki et al., 2016 and in-house Taqman Assay Qiagen Biosprint 96 MagAttract 96 cador Pathogen Kit N/A Bio-Rad C1000/CFX96	DIVA GREECE Agianniotaki et al 2017 Ihvitrogen by Thermo Fisher Scientific Pure Link Genomic DNA Mini Kit No LC 480 Thermal Cycler	ID Gene LSD DIVA Triplex, Real time PCR QIAGEN QIAamp Viral RNA Mini Kit (250) QuantStudio5
Information	Protocol / SOP used : Producer Extraction protocol / kit: Name Extraction protocol / kit: In-house modifications to extraction protocol (if yes, which?): PCR Instrument used: Cut-off for positive:	Agianniotaki et al., 2017 Qiagen QiaAmp Viral RNA Mini Kit n/a Agilent ABIA CT38	ID Gene LSD DIVA Triplex (Idvet) Roche High Viral Nucleic Acid kit LightCycler 96 (Roche)	Agianniotaki et al., 2017 J of Virol Methods 249; 48- 57 QIAGEN IndiSpin Pathogen MiniKit No RotorGeneQ		Agianniotaki et al., 2016 and in-house Taqman assay Qiagen Biosprint 96 MagAttract 96 cador Pathogen Kit N/A Bio-Rad C1000/CFX96 N/A	DIVA GREECE Agianniotaki et al 2017 Ihvitrogen by Thermo Fisher Scientific Pure Link Genomic DNA Mini Kit No LC 480 Thermal Cycler No	ID Gene LSD DIVA Triplex, Real time PCR QIAGEN QIA amp Viral RNA Mini Kit (250) QuantStudio5 Ct<40
Information	Protocol / SOP used : Producer Extraction protocol / kit: Name Extraction protocol / kit: In-house modifications to extraction protocol (if yes, which?): PCR Instrument used: Cut-off for positive: Methodology	Agianniotaki et al., 2017 Qiagen QiaAmp Viral RNA Mini Kit n/a Agilent ARIA CT38 Multiplex real time	ID Gene LSD DIVA Triplex (Idvet) Roche High Viral Nucleic Acid kit LightCycler 96 (Roche) PCR Real Time	Agianniotaki et al., 2017 J of Virol Methods 249; 48- 57 QIAGEN IndiSpin Pathogen MiniKit No RotorGeneQ Duplex real-time PCR with LNA TaqMan probes		Agianniotaki et al., 2016 and in-house Taqman Qiagen Biosprint 96 Cador Pathogen Kit N/A Bio-Rad C1000/CFX96 N/A	DIVA GREECE Agianniotaki et al 2017 Thermo Fisher Scientific Pure Link Genomic DNA Mini Kit No LC 480 Thermal Cycler No Real-time PCR method	ID Gene LSD DIVA Triplex, Real time PCR QIAGEN QIAAmp Viral RINA Mini Kit (250) QuantStudio5 Ct<40 ID.Vet Genetics
Information	Protocol / SOP used : Producer Extraction protocol / kit: Name Extraction protocol / kit: In-house modifications to extraction protocol (if yes, which?): PCR Instrument used: Cut-off for positive: Methodology Ref Methodology	Agianniotaki et al., 2017 Qiagen QiaAmp Viral RNA Mini Kit Agilent ARIA CT38 Multiplex real time Thermofisher TaqMan Fast Virus 1-Step Master Mix	ID Gene LSD DIVA Triplex (Idvet) Roche High Viral Nucleic Acid kit LightCycler 96 (Roche) PCR Real Time ID Gene LSD DIVA Triplex (Idvet)	Agianniotaki et al., 2017 J of Virol Methods 249; 48- 57 QIAGEN IndiSpin Pathogen MiniKit No RotorGeneQ Duplex real-time PCR with LNA TaqMan probes Agianniotaki et al., 2017 J of Virol Methods 249; 48- 57		Agianniotaki et al., 2016 and in-house Taqman Qiagen Biosprint 96 Cador Pathogen Kit N/A Bio-Rad C1000/CFX96 N/A Nzytech NZYTaq 2x Master Mix;	DIVA GREECE Agianniotaki et al 2017 Thermo Fisher Scientific Pure Link Genomic DNA Mini Kit No LC 480 Thermal Cycler No Real-time PCR method Agianniotaki et al 2017	ID Gene LSD DIVA QIAGEN QIAGEN QIAamp Viral RNA Mini Kit (250) QuantStudio5 Ct<40 ID.Vet Genetios ID Gene LSD DIVA Triplex, Real time PCB
Information	Protocol / SOP used : Producer Extraction protocol / kit: Name Extraction protocol / kit: In-house modifications to extraction protocol (if yes, which?): PCR Instrument used: Out-off for positive: Methodology Ref Methodology Remark(s):	Agianniotaki et al., 2017 Qiagen QiaAmp Viral RNA Mini Kit Agilent ARIA CT38 Multiplex real time Thermofisher TaqMan Fast Virus 1-Step Master Mix	ID Gene LSD DIVA Triplex (Idvet) Roche High Viral Nucleic Acid kit LightCycler 96 (Roche) PCR Real Time ID Gene LSD DIVA Triplex (Idvet)	Agianniotaki et al., 2017 J of Virol Methods 249; 48- 57 QIAGEN IndiSpin Pathogen MiniKit No RotorGeneQ Duplex real-time PCR with LNA TaqMan probes Agianniotaki et al., 2017 J of Virol Methods 249; 48- 57		Agianniotaki et al., 2016 and in-house Taqman assay Qiagen Biosprint 96 Cador Pathogen Kit N/A Bio-Rad C1000/CFX96 N/A Nzytech NZYTaq 2x Master Mix;	DIVA GREECE Agianniotaki et al 2017 Thermo Fisher Scientific Pure Link Genomic DNA Mini Kit No LC 480 Thermal Cycler No Real-time PCR method Agianniotaki et al 2017	ID Gene LSD DIVA Triplex, Real time PCB QIAGEN QIAamp Viral RNA Mini Kit (250) QuantStudio5 Ct<40 ID.Vet Genetics ID Gene LSD DIVA Triplex, Real time PCB

value	sample		Labnr 97621		Labnr 97623	Labnr 97625	Labnr 97628	Labnr 97629
	PT2020CAPXVIB VP1	POS+++	No ct	NEG	NA	32,49	no Ct	no Ct
	PT2020CAPXVIR_VP2	POS+	No et	NEG	NA	34,8	no Ct	no Ct
	PT2020CAPXVIR_TP1	POS+	39,1	NEG	NA	34,84	25,33	29,89
_	PT2020CAPXVIB_TP2	NEG	36,8	NEG	NA	29,77	31,09	no Ct
data	PT2020CAPXVIR_TP3	POS++	33,5	NEG	NA	28,96	no Ct	33,03
R.	PT2020CAPXVIR_VP3	NEG	39,1	NEG	NA	33,02	no Ct	32,59
-	PT2020CAPXVIR_TP4	POS++	30,8	NEG	NA	26,23	31,72	no Ct
	PT2020CAPXVIR_TP5	POS+	Noct	POS++	NA	24,95	no Ct	no Ct
	PT2020CAPXVIR_VN1	NEG	No ct	NEG	NA	no ct	no Ct	no Ct
	PT2020CAPXVIR_TN1	NEG	No ct	NEG	NA	no ct	no Ct	no Ct
	PT2020CAPXVIR_VP1		NEG		ND	SPPV FIELD	NEG	NEG
	PT2020CAPXVIR_VP2		NEG		ND	SPPV FIELD	NEG	NEG
	PT2020CAPXVIR_TP1		LSDV FIELD		LSDV FIELD	LSDV FIELD	LSDV FIELD	NEG
	PT2020CAPXVIR_TP2		LSDV FIELD		LSDV FIELD	LSDV FIELD	LSDV FIELD	LSDV FIELD
les	PT2020CAPXVIR_TP3		LSDV VACCINE		LSDV FIELD	LSDV VACCINE	NEG	LSDV VACCINE
	PT2020CAPXVIR_VP3		LSDV FIELD		LSDV FIELD	LSDV FIELD	NEG	LSDV FIELD
Ē	PT2020CAPXVIR_TP4		LSDV FIELD		NI	LSDV FIELD	LSDV FIELD	NEG
	PT2020CAPXVIR_TP5		SPPV FIELD		ND	SPPV FIELD	NEG	NEG
	PT2020CAPXVIR_VN1		NEG		ND	NEG	NEG	NEG
	PT2020CAPXVIR_TN1		NEG		ND	NEG	NEG	NEG
	Protocol / SOP used :	Menasherow et al., 2014;	Agianniotaki et al., 2017;	Haegeman et al., 2015	Sprygin et al., 2018, unpublished assay	Real-time PCR for the genome characterisation of LSDV-Field strain and LSDV-vaccine strain	ID Gene LSD DIVA Triplex	ID Gene LSD DIVA Triplex
	Producer Extraction protocol / kit:		Qiagen		ThermoFisher/ KingFisher Flex	QIAGEN	ThermoFisher	ThermoFisher
	Name Extraction protocol 7 kit:	Mag.	Attract 96 CADOR Pathoge	en Kit	none	DNA Mini Kit	PrepFiler Express Forensic DNA Extraction Kit	PrepFiler Express Forensic DNA Extraction Kit
5	In-house modifications to extraction protocol (if yes, which?):	Proteinase tre	eatment at 72 °C / Addition (of carrier ARN	AB7500 Fast	no	N/A	N/A
mati	PCR Instrument used:		Verity (Applied Biosystems))	nła		Rotor-Gene 6000	Rotor-Gene 6000
ц Ц	Cut-off for positive:	Clear band at the right h	eight and with the expected NCBI database	intensity + blasting in the		Rotor-Gene		
	Methodology		Promega			Bernd Hoffman/QIAGEN	DIVA PCR	DIVA PCR
	Ref Methodology		Taq Platinium			QuantiTect Multiplex PCR NoROX Kit		
	Remark(s):							

		Labnr	Labnr	Labnr	Labnr	Labnr	Labnr
value	sample	97630	97631	97632	97633	97634	97636
	PT2020CAPXVIR_VP1	No et	0		30,65		
	PT2020CAPXVIR_VP2	No et	0				
	PT2020CAPXVIR_TP1	23,95	33,2	25,1	28,51	19,43	19,43
_	PT2020CAPXVIR_TP2	29,04	29,01	31		23,4	23,4
ğ	PT2020CAPXVIR_TP3	30,18	27,45	28,9	29,13	23,13	23,13
2	PT2020CAPXVIR_VP3	31,26	31,93	32,8	26,36	29,02	29,02
	PT2020CAPXVIR_TP4	29,38	27,41	30,4		27,59	27,59
	PT2020CAPXVIR_TP5	No et	0	21,5			
	PT2020CAPXVIR_VN1	No et	0	No Ct			
	PT2020CAPXVIR_TN1	No et	0	No Ct	23,88		
	PT2020CAPXVIR_VP1	ND	ND	ND	NEG	ND	ND
	PT2020CAPXVIR_VP2	ND	ND	ND	ND	ND	ND
	PT2020CAPXVIR_TP1	LSDV FIELD	LSDV FIELD	LSDV FIELD	LSDV FIELD	LSDV FIELD	LSDV FIELD
ţ2	PT2020CAPXVIR_TP2	LSDV FIELD	LSDV FIELD	LSDV FIELD	LSDV FIELD	LSDV FIELD	LSDV FIELD
lesu	PT2020CAPXVIR_TP3	LSDV VACCINE	LSDV VACCINE	LSDV VACCINE	LSDV VACCINE	LSDV VACCINE	LSDV VACCINE
Ē	PT2020CAPXVIR_VP3	LSDV FIELD	LSDV FIELD	LSDV FIELD	LSDV FIELD	LSDV FIELD	LSDV FIELD
Ē	PT2020CAPXVIR_TP4	LSDV FIELD	LSDV FIELD	LSDV FIELD	LSDV FIELD	LSDV FIELD	LSDV FIELD
	PT2020CAPXVIR_TP5	ND	ND	ND	ND	ND	ND
	PT2020CAPXVIR_VN1	ND	NEG	NEG	ND	NEG	NEG
	PT2020CAPXVIR_TN1	ND	NEG	NEG	ND	NEG	NEG
	Protocol / SOP used :		Vidanovic et al, 2016	Real time PCR for genomic characterisation of LSDV vaccine strain, LSDV field strain and SPPV (Moller et al 2019- Experimental LSD	FGBI ARRIAH	For DIVA LSDV qPCR assay Vidanovic was used	
	Producer Extraction protocol / kit:	Qiamp Cador pathogen Mini kit	High Pure Viral Nucleic Acid Kit	Sacace Biotechnologies	Qiagen , Germany	LSI, Thermo	INDICAL Bioscience
	Name Extraction protocol	panoganiania		Viral Nucleic Acid Extraction Kit	Dna mini kit	Magvet universal kit	IndiSpin Pathogen Kit
5	In-house modifications to extraction protocol (if yes, which?):				N/A		
ati L	PCR Instrument used:	Smartcycler II Cepheid	AS7500		rotor gene	AriaMx	Rotor Gene 3000
linfo	Cut-off for positive:		38		up tp 35 cycles	40	
	Methodology						
		Triplex			FGBIARRIAR		
	Ref Methodology	Triplex			A real time PCR kit for indentification of capripoxvirus DNA		

Annex 4: Raw ELISA data

مىلدىر	cample	97506	97600	97602	97604	97605	97607	97609	97609	97610	97611	97612	97612	97614
value	nositius kit control (mean)	1054	0.896	0.83	0.863	1022	0.807	0.739	0.039	107	0.836	0.849	0.844	1.011
	positive kit control (mean)	0.051	0,035	0,03	0,0057	0.062	0,007	0,755	0,033	0.047	0,050	0,043	0.052	0.05
	DT2020CADVEED CEDD1	0,001	0,045	0,047	0,007	0,003	0,045	0,007	0,000	0,047	0,000	0,002	0,032	0,05
	PT2020CAPASEN_SENFT	0,032	0,010	0,071	0,022	0,007	0,400	0,070	0,001	0,606	0,012	0,001	0,001	0,727
	PT2020CAFASEN_SENF2	0,0	0,710	0,701	0,017	0,003	0,004	0,733	0,002	0,000	0,640	0,63	1,005	0,763
	PT2020CAFASEN_SENF3	1,107	1,035	0,365	0,363	1,105	0,073	0,334	1,031	1,004	0,074	1,033	1,005	1,102
8	PT2020CAPASER_SERP4	1,177	0,366	1,021	0,366	1,029	0,778	0,721	0,380	1,01	0,738	0,357	0,333	1,03
	PT2020CAPASER_SERPS	2,349	1,801	1,678	1,311	1,812	1,063	0,572	1,332	2,067	1,739	1,606	1,888	2,281
	PT2020CAPASER_SERP6	2,196	1,72	1,603	1,327	1,753	1,432	0,77	1,365	2,012	1,705	1,604	1,811	2,199
	PT2020CAPASER_SERP7	0,334	0,762	0,707	0,36	0,801	0,632	0,360	0,324	0,807	0,769	0,704	0,827	0,38
	PT2020CAPASER_SERP4	1,142	0,991	0,365	1,048	1,079	0,734	0,659	0,947	0,974	0,860	0,981	0,931	1,143
	PT2020CAPASER_SERNI	0,054	0,048	0,059	0,071	0,065	0,055	0,045	0,041	0,054	0,062	0,048	0,052	0,049
	PIZUZUCAPXSER_SERNZ	0,054	0,047	0,049	0,062	0,053	0,055	0,044	0,043	0,051	0,061	0,052	0,051	0,053
	positive kit control (mean)		100		100%	100	100	100			100	100%		
	negative kit control <u>[mean]</u>	50.054	0		0%	0	0	70	50.074	54.04	0	0%		70.404
	PT2020CAPASER_SERPT	53,954	67	66,30	07%	63	55	76	03,37%	94,64	58,435	63%	68,02	70,484
ata	PT2020CAPASER_SERP2	/4,638	78	83,50	34%	63	/1	108,9	88,51%	62,66	/0,406	80%	80,48	/4,857
P	PT2020CAPASER_SERP3	111,23	123	119,80	110%	109	109	137,4	122,11%	101,37	104,387	131%	120,33	117,855
zec	PT2020CAPASER_SERP4	112,297	110	214,40	116%	100	36	37,4	109,81%	34,13	35,22	114%	118,85	108,277
ile	PT2020CAPASER_SERPS	223,101	206	208,30	230%	182	133	70,6	226,64%	137,46	210,603	201%	231,36	232,279
E	PT2020CAPASER_SERP6	213,873	197	198,70	233%	1/6	182	104,6	223,51%	192,08	211,36	201%	222,18	223,738
ž	PT2020CAPASER_SERP7	34,046	04	84,20	102%	11	00	133,1	102,73%	74,23	30,76	00%	37,32	36,820
	PT2020CAPASER_SERP4	108,796		117,20	123%	106	38	88,3	0.174	90,62	103,753	10726	111,04	()0.104
	PT2020CAPASER_SERNI	0,263	0	1,46	47.	0	1	-1,0	0,17%	0,66	0,472	-124	-0,06	(-) 0,104
	FIZUZUCAFASEN_SENNZ	0,313	U DOC	0,13	1/4	DOC	PO2	-1,0 BOC	0,472	0,33	0,420	DOC	-0,07	0,312
	positive kit control (mean)		NEG	NEG		NEG	NEG	NEG			NEG	NEG	NEG	
	PT2020CAPYSER SERPI	POS	POS	POS	POS	POS	POS	POS	POS	POS	POS	POS	POS	POS
	PT2020CAPXSEB SEBP2	POS	POS	POS	POS	POS	POS	POS	POS	POS	POS	POS	POS	POS
ts	PT2020CAPXSEB_SEBP3	POS	POS	POS	POS	POS	POS	POS	POS	POS	POS	POS	POS	POS
IS I	PT2020CAPXSEB_SEBP4	POS	POS	POS	POS	POS	POS	POS	POS	POS	POS	POS	POS	POS
2	PT2020CAPXSER SERP5	POS	POS	POS	POS	POS	POS	POS	POS	POS	POS	POS	POS	POS
L a	PT2020CAPXSER_SERP6	POS	POS	POS	POS	POS	POS	POS	POS	POS	POS	POS	POS	POS
Ľ.	PT2020CAPXSER_SERP7	POS	POS	POS	POS	POS	POS	POS	POS	POS	POS	POS	POS	POS
	PT2020CAPXSER_SERP4	POS	POS	POS	POS	POS	POS	POS	POS	POS	POS	POS	POS	POS
	PT2020CAPXSER_SERN1	NEG	NEG	NEG	NEG	NEG	NEG	NEG	NEG	NEG	NEG	NEG	NEG	NEG
	PT2020CAPXSER_SERN2	NEG	NEG	NEG	NEG	NEG	NEG	NEG	NEG	NEG	NEG	NEG	NEG	NEG
	Name ELISA kit producer:	ID-VE1	IDVet	ID Vet	IDvet	ID VE I	IDVE1	ID.vet	ID.vet	IDEXX	IDVet	ID Vet	ID.Vet	ID.vet
		ID screen	Caprinov	ID Screen	ID Screen	Caprinov	Capripor		Capripov	IDScreen	ID Screen,	Capripov	Capripox	Capripox
	Name FLISA kit-	Captinos	Double	Capripox	Capripox	Double Antigen	Double	Caprinos	Double	Capripox	Capripox Double	Double	Double	double
		Double antigen	Antigen	double antigen	Double Antigen	Multi-species	antigen multi-	Double Antigen	Antigen Multi-	Double	Antigen Multi	Antigen Multi-	Antigen Multi-	antigen multi-
		j	Multispecies	multispecies	Multi-species	· ·-···	species		species	Antigen	Species	species	species	species
_	Short or long incubation		short			short		short			short		short	short
tio	prococor (in approable) :	SIP-V-					SIP*/-							SIR V - (00
ma		(OD(Sample)-	S/P%=(ODSa	S/P%=[(Odsa	(ODsample-	S/P%=((ODsa	(ODsample -	S/P% = (OD	S/P%=(ODsa	S/P%=((ODs -	((OD Sample-OD	S/P%=	S/P% = ((OD	sample - OD
for	Formula to calculate the	ODINCIVIODI	mple-	mple-	ODNCHOOP	mple-		Probe - OD	mple-		Negative)/(OD	[(Odsample-	sample - OD	NC)/(OD
<u>-</u>	normalized data:	PC)-	ODNC)/(ODP	Odneg)/(Odpo	C-ODNC1*100	ODnc)/(ODpc-	PC-OD	nK)ł(OD pK -	ODnc)x100/(O	ODn11*100	Positive- 0D	Odneg)/(Odpo	NC)/(OD PC -	PC-OD NC1
		OD(NC))*100	C-ODNC)x100	s-Odneg)]*100	,	ODnc)) *100	NC))*100	OD nK)*100	Dpc-ODnc)	,	Negative))*100	s-Udneg)]*100	UD NC)*100	× 100
			NEG - S/P*/			S/P%<30%		S/P%> 30% -	S/P%<30%					
	Used Cut-off ·	30%	<30% POS =	30%	30%	Negativ,	S/P% < 30%	Pos: S/P%	negative,	S/P%>=30	>=30% positive,	S/P%>=30%	>= 30. NEG #	30%
	See Sur Sur .		S/P%≥30%			S/P%>30%	NEGATIVE	30% = Nea	S/P%>=30%	Positive	<30% negative	POS	S/P% < 30	
	Datah ELICA kit.	E02	E92.01	E02	E02	Positiv Eoo	Gee	Gee	DOSITIVE	E02	Lot E92	E02	E02	E02
	Daton ELISA KIC	E00	E03-01	EOJ	E03	E00	000	(100	EOJ	EOJ	LUCE03	EOJ	EOJ	EOJ

مىبادىر	cample	97615	97616	97617	97619	97619	97620	97621	97622	97623	97628
value	nositius kit control (mean)	0.795	0.856	0.775	1121	0.922	0.767	0.919	0.95010.92910.927	0.926	0.882
	positive kit control (mean)	0,100	0,050	0,113	0.052	0,000	040.0	0,010	0,05210,02010,050	0,000	0,002
	DT2020CADVCCD CEDD1	0,05	0,004	0,032	0,002	0,000	0,040	0,0435	0,00010,00110,000	0.012	0,002
	F12020CAFASEN_SENFI	0,063	0,626	0,036	0,620	0,013	0,047	0,744	0,00110,07110,040	0,612	0,000
	PT2020CAPASER_SERP2	0,733	0,646	0,689	0,824	0,687	0,773	0,344	0,84870,86870,737	0,661	0,778
	PT2020CAPXSER_SERP3	1,024	0,992	0,386	1,118	1,089	1,051	1,288	1,19271,13371,198	1,107	1,080
9	PT2020CAPXSER_SERP4	0,991	1,044	1,31	1,006	0,955	0,989	1,262	1,17471,15071,036	1,083	1,056
	PT2020CAPXSER_SERP5	1,8835	1,83	1,795	1,93	1,891	1,881	2,159	2,04772,10871,765	1,786	2,171
	PT2020CAPXSER_SERP6	1,845	1,717	1,836	1,765	1,693	1,774	2,042	1,94771,98671,705	1,655	2,109
	PT2020CAPXSER_SERP7	0,801	0,716	0,88	0,892	0,791	0,858	1,166	0,91170,91770,704	0,766	0,832
	PT2020CAPXSER_SERP4	0,996	1,023	0,983	1,069	0,918	0,985	1,301	1,114 / 1,101 / 1,011	1,084	1,054
	PT2020CAPXSER_SERN1	0,05	0,053	0,027	0,066	0,054	0,054	0,059	0,05470,05570,056	0,055	0,047
	PT2020CAPXSER_SERN2	0,049	0,054	0,071	0,055	0,071	0,048	0,054	0,05670,05570,056	0,055	0,049
	positive kit control <u>(mean)</u>	N/A		100		100%	100	100	100 / 100 / 100	100	
	negative kit control <u>(mean</u>)	N/A		0		0%	0	0	01010	0	
	PT2020CAPXSER_SERP1	68,792	72	75,9	S/P% = 53,38%	66,10%	69,47	80	68770767	59,58	64,5394
5	PT2020CAPXSER_SERP2	91,611	74	88,4	S/P% = 71,54%	67,55%	100,9	103	89793777	64,92	87,4172
ф.	PT2020CAPXSER_SERP3	130,738	117	128,4	S/P% = 98,79%	110,95%	139,56	142	127 / 124 / 125	113,05	124,383
red	PT2020CAPXSER_SERP4	126,309	123	172	S/P% = 88,41%	96,45%	130,94	139	125 / 126 / 111	110,45	120,891
zile	PT2020CAPXSER_SERP5	246,107	222	237,3	S/P% = 174,05%	197,15%	255	243	22272367193	186,15	255,148
Ē	PT2020CAPXSER_SERP6	240,939	207	242,8	S/P% = 158,75%	175,90%	240,12	229	211 / 222 / 187	172,05	247,682
No	PT2020CAPXSER_SERP7	100,805	83	114,1	S/P% = 77,85%	78,85%	112,72	128	96799774	76,24	93,9193
-	PT2020CAPXSER_SERP4	126,913	121	128	S/P% = 94,25%	92,50%	130,38	14.4	118 / 120 / 108	110,45	120,65
	PT2020CAPXSER_SERN1	< 0.001	0	-0,7	S/P% = 1,29%	-0,40%	0,9	1	07-171	-0,3877	0,769
	PT2020CAPXSER_SERN2	< 0.001	0	5,2	S/P% = 0,28%	1,30%	0,067	1	07-171	-0,34475	0,808
	positive kit control <u>(mean</u>)	POS	POS	POS		POS	POS	POS			
	negative kit control <u>(mean</u>)	NEG	NEG	NEG		NEG	NEG	NEG			
	PT2020CAPXSER_SERP1	POS	POS	POS	POS	POS	POS	POS	POS	POS	POS
in the	PT2020CAPXSER_SERP2	POS	POS	POS	POS	POS	POS	POS	POS	POS	POS
-H	PT2020CAPXSER_SERP3	POS	POS	POS	POS	POS	POS	POS	POS	POS	POS
es	PT2020CAPXSER_SERP4	POS	POS	POS	POS	POS	POS	POS	POS	POS	POS
-	PT2020CAPXSER_SERP5	POS	POS	POS	POS	POS	POS	POS	POS	POS	POS
Ē	PT2020CAPXSER_SERP6	POS	POS	POS	POS	POS	POS	POS	POS	POS	POS
_	PT2020CAPXSER_SERP7	PUS	PUS	PUS	PUS	PUS	PUS	PUS	PUS	PUS	PUS
	PT2020CAPASER_SERP4	PUS NEG	PUS	PUS	PUS	PUS	PUS	FUS NEC	PUS NEG	PUS NEG	PUS NEG
	PT2020CAPASEN_SERNI	NEG	NEG	NEG	NEG	NEG	NEG	NEG	NEG	NEG	NEG
	Name ELISA kit producer	ID Vet	IDV-st	IDVot	IDust	IDust	IDuat	IDVET	ID-Vet	IDVat	Iduat Eranoa
	Name ELISA KIC producer.	ID Screen	IDVet	IDVet	ID Screen	ID Screen	ID.Vet	ID Screen	ID-Yet	IDScreen	lavermance
		Capripox	ID Screen	ID Screen	Capripox	Capripox	ID Screen	Capripox	ID Screen Capripox	Capripox	
	Name ELISA kit:	Double	Capripox Deuble Aexieve	Capripox Devide Actions	Double	Double	Capripox	Double Antigen	double antigen multi	double	ID Screen Capripox
		Antigen Multi-	Double Antigen	Double Antigen	Antigen Multi-	Antigen Multi-	double antigen	MULTI-	species	antigen multi-	Antigen Multi-species
		species	Multi-species	iviula-species	species/CPVD	species	Multi-species	SPECIES		species	
	Short or long incubation	short		N/A							
-	protocol <i>(if applicable)</i> :		0.0		0.00						
lo			S/P ratio = test		S/P%= (UD	CID Mar					
ma		Similar (LOD	sample OD -	S/P% = (OD	sample - OD	ODcomple	S/P%=(ODsa	((OD sample -	SID V = (Odesmole		SID:/_((OD comple
5	Formula to calculate the	Neg Ctrl) / (OD	control	sample-OD	control/OD		mple-	OD NC)/(OD	Odpe)/(Odpe-		OD NC MOD PC.OD
Ē	normalized data:	Pos Ctrl, OD	OD/positiue	NC)/(OD PC-	pozitiu control -	PC.ODNC)	ODnc)/(ODpc-	PC - OD NC))	Odpe)*100		NC1)*100
		Neg Ctrl))x 100	control OD -	OD NC)x100	OD negativ	100	ODnc)*100	*100	Cancy loo		140)) 100
			negative		control) x 100	100					
		Positive >- to			NEGATIVE =		S/P greather		S/P v		
	Used Cut-off ·	30 Menative /	30%	0.254	S/P%k	30.00%	or equal to	POS>= 30%;	<30%=negative-S/P	30%	less than 30% are
	osea our on .	30	0071	0,201	30%.POSITIVE	00,0074	30%	NEG < 30%	%>= 30%=positive	0071	considered negative
	Datab ELICA bit.	502	E02	E02	S/P%≥30%	Let E02	E02	E02	E02	FOO	202
	DAICH ELISA KIC	EõJ	EðJ	EðJ	(166	LOUE83	EõJ	EõJ	EðJ	E03	

		07000	07000	Labnr	07000	07004	07025	07000
value	sample	37623	37630	97632	97633	37634	37633	37636
	positive kit control [mean]	0,882	0,697	0,789	0,981	0,845	0,755	1,13
	negative kit control <u>[mean]</u>	0,052	0,05	0,046	0,063	0,137	0,057	0,062
	PT2020CAPXSER_SERP1	0,536	0,469	0,849	0,645	0,651	0,575	0,826
	PT2020CAPXSER_SERP2	0,745	0,414	0,828	0,076	0,773	0,618	0,797
	PT2020CAPXSER_SERP3	1,026	0,778	1,269	1,215	1,198	0,995	1,467
	PT2020CAPXSER_SERP4	0,989	0,722	1,188	1,165	1,031	0,906	1,316
0	PT2020CAPXSER_SERP5	2,025	1,271	1,88	1,84	1,704	1,782	2,125
	PT2020CAPXSER_SERP6	1,964	1,256	1,808	1,73	1,61	1,615	1,97
	PT2020CAPXSER_SERP7	0,808	0,497	0,99	0,85	1,01	0,77	1,059
	PT2020CAPXSER SERP4	0,977	0,745	1,168	1,16	1,071	0,954	1,367
	PT2020CAPXSER SERNI	0.048	0.073	0.054	0.055	0,092	0.06	0.051
	PT2020CAPXSEB SEBN2	0.048	0.049	0.054	0.057	0.108	0.063	0.054
	nositive kit control (mean)			100		100		
	pegative kit control (mean)			0		0		
	DT2020CADVEED CEDD1	E0 2701	64.01	100.1	E9 20#/	72.610	02.2702225	72
	DT2020CALASEL_SELLET	00,2101	50,01 EC 0	105,1	70.50%	00,000	02,0102200	60
ata	PT2020CAPASER_SERPZ	03,9937	06,2	109,2	117.40%	03,308	00,00000130	63
D	PT2020CAPASER_SERP3	117,279	112,93	164,5	117,90%	193,829	142,0001433	132
zec	PT2020CAPXSER_SERP4	112,824	103,99	153,7	112,30%	126,253	129,7994269	11/
	PT2020CAPXSER_SERP5	237,568	188,95	246,7	182,70%	221,313	255,3008596	193
E	PT2020CAPXSER_SERP6	230,223	186,62	237	170,00%	208,045	231,3753582	179
No	PT2020CAPXSER_SERP7	91,0295	69,1	127	80,0%	123,359	110,3151862	93
	PT2020CAPXSER_SERP4	111,379	107,5	150,9	111,80%	131,898	136,6762178	122
	PT2020CAPXSER_SERN1	0,481	3,47	1,1	0,00%	-6,21	0,429799427	0
	PT2020CAPXSER_SERN2	0,481	-0,28	1,1	0,00%	-4,023	0,859598854	0
	positive kit control (mean)			POS		POS		
	negative kit control (mean)			NEG		NEG		
	PT2020CAPXSER SERP1	POS	POS	POS	POS	POS	POS	POS
	PT2020CAPXSEB SERP2	POS	POS	POS	POS	POS	POS	POS
lts	PT2020CAPXSEB SERP3	POS	POS	POS	POS	POS	POS	POS
DS II	PT2020CAPXSEB_SEBP4	POS	POS	POS	POS	POS	POS	POS
2	PT2020CAPXSEB SEBP5	POS	POS	POS	POS	POS	POS	POS
Ľ	PT2020CAPXSEB SEBP6	POS	POS	POS	POS	POS	POS	POS
iΞ.	PT2020CAPXSEB SEBP7	POS	POS	POS	POS	POS	POS	POS
	PT2020CAPXSEB SERP4	POS	POS	POS	POS	POS	POS	POS
	PT2020CAPXSER SERNI	NEG	NEG	NEG	NEG	NEG	NEG	NEG
	PT2020CAPXSER SERN2	NEG	NEG	NEG	NEG	NEG	NEG	NEG
	Name ELISA kit producer:	Idvet France	ID.VET	ID Vet	IDVET	ID-VET	IDVet .	ID vet
		ID Course	ID screen	ID Screen	ID Screen	ID Screen	ID Course	ID Screen
		D Screen	Capripox	Capripox	Capripox	Capripox	Double Double	Capripox
	Name ELISA kit:	Capripox Apticop Multi	Double Antigen	Double	Double	double	Antigon Multi	Double
		Anagen Maia-	Multi-species	Antigen Multi-	Antigen Multi-	antigen	Anagen Maia Coopies	Antigen Multi-
		species	ELISA	species	species	Multispecies	opecies	species
	Short or long incubation	short		short				
_	protocol <i>(if applicable)</i> :	Short		511011				
6								S/P%= OD
nat		S/P%=((OD				S/P% =	S/P %=	sample - OD
E	Formula to calculate the	sample - OD		As per kit	S/P%= (ODS -	(ODSample -	ODSample-	neg
nfo	normalized data:	NC1/IOD PC		instructions	OD (K-)/ OD	ODNo/ODPo	ODNo /ODPo	control/OD
_		OD NC 11*100		(kit manual)	(K+))×100%	ODNc1 x 100	ODNc x 100	pos control -
								ODneg
		0/01/2			01044-0044			control*100
		SfP%: -less	Sp% < 30%		S/P%(30% -			
	Used Cut-off :	than 30% are	NEG; Sp% >	30	negative,	40	S/P %≥ 30%	no
		considered	30% POS		S/P%>30% -			
	Batch FLISA kit-	F83	F83	D15	E83	F83	F83	F 83
	DAVOI LEION KIL	200	200	010	200	200	200	200

Annex 5: Raw VN1 with Antibody Titer data

				Labnr		
value	sample	97600	97612	97618	97623	97633
	positive kit control (mean)	120	1:20	1:20		1/128
	negative kit control (mean)	<10	<1:5	< 1:5		1/4
	PT2020CAPXSER_SERP1	15	1:20	1:160	1/3	1/8
	PT2020CAPXSER_SERP2	60	1:20	1:20	1/8	1/8
ter	PT2020CAPXSER_SERP3	60	1:160	1:40	1/4	1/64
y ti	PT2020CAPXSER_SERP4	120	1:320	1:160	1/4	1/32
bod	PT2020CAPXSER_SERP5	120	1:320	1:160	1/3	1/64
ntil	PT2020CAPXSER_SERP6	60	1:160	1:160	1/6	1/32
A	PT2020CAPXSER SERP7	20	1:10	1:5	<1/2	1/8
	PT2020CAPXSER_SERP4	240	1:320	1:160	1/48	1/64
	PT2020CAPXSER SERN1	<10	<1:5	< 1:5	<1/2	1/4
	PT2020CAPXSER SERN2	<10	1:5	< 1:5	<1/2	1/4
	positive kit control (mean)	POS	POS	POS		POS
	negative kit control (mean)	NEG	NEG	NEG		NEG
	PT2020CAPXSEB SEBP1	POS	POS	POS	NEG	POS
	PT2020CAPXSEB SEBP2	POS	POS	POS	POS	POS
2	PT2020CAPXSEB SEBP3	POS	POS	POS	NEG	POS
Ins	PT2020CAPXSEB SEBP4	POS	POS	POS	NEG	POS
2	PT2020CAPXSEB_SEBP5	POS	POS	POS	NEG	POS
ina	PT2020CAPXSEB SEBP6	POS	POS	POS	POS	POS
<u> </u>	PT2020CAPXSEB_SEBP7	POS	POS	POS	NEG	POS
	PT2020CAPXSEB SEBP4	POS	POS	POS	POS	POS
	PT2020CAPXSEB_SEBN1	NEG	NEG	NEG	NEG	NEG
	PT2020CAPXSEB_SEBN2	NEG	NEG	NEG	NEG	NEG
	Protocol/SOP used	yes after OIE manual	In house protocol	Pirbright Protocol/IDAH SOP 39"Serum neutralization test for the detection of	NVR-SOP-42	Home method
	Name (+Reference) cell type used	ESH	OA3.Ts (ATCC CRL- 6546)	Capripoxvirus OA3Ts	МОВК	LSDV "Dagestan/2 015"
nation	Starting dilution of PT serum samples tested:	5	01:05	01:05	43862	0,5
Inforr	Yirus dose used in test (TCID50): e.g. 100 TCID50	TCID50 103	100 TCID50	100 TCID50	100 TCID 50	100 TCID50
	Positive control serum used:	home made	in house	Antiserum Pirbright	VIN83	0,0078125
	Cut-off for positive:	> 10	>1:5	≥ 1:5	1/5	0,125
	Name (• reference) virus strain used:	LSDV Neethling	SIS Neethling-	Neethling, Pirbright	LSDV Neethling	
	samples tested:	2	1:5-1:640	1:5 - 1:160	1/2 to 1/256	
	Expected antibody titer in positive control serum:	120	1:20	1:20		

Annex 6: Raw IPMA data

		Labnr					
value	sample	97506	97618				
	positive kit control (mean)		1:50 and 1:300				
	negative kit control (mean)		0				
	PT2020CAPXSER_SERP1	<50 (LSDV)- 300 (SPPV)	1:50 and 1:300				
	PT2020CAPXSER_SERP2	300-50	1:50 and 1:300				
iter	PT2020CAPXSER_SERP3	300-50	1:50 and 1:300				
dy 1	PT2020CAPXSER_SERP4	300-300	1:50 and 1:300				
ibo	PT2020CAPXSER_SERP5	300-300	1:50 and 1:300				
Ant	PT2020CAPXSER_SERP6	300-300	1:50 and 1:300				
	PT2020CAPXSER_SERP7	<50 - 300	01:50				
	PT2020CAPXSER_SERP4	300-300	1:50 and 1:300				
	PT2020CAPXSER_SERN1	<50 - <50	0				
	PT2020CAPXSER_SERN2	<50 - <50	0				
	positive kit control (mean)		POS				
	negative kit control <u>(mean)</u>		NEG				
-	PT2020CAPXSER_SERP1	POS	POS				
	PT2020CAPXSER_SERP2	POS	POS				
ults	PT2020CAPXSER_SERP3	POS	POS				
res	PT2020CAPXSER_SERP4	POS	POS				
la	PT2020CAPXSER_SERP5	POS	POS				
Ē	PT2020CAPXSER_SERP6	POS	POS				
	PT2020CAPXSER_SERP7	POS	POS				
	PT2020CAPXSER_SERP4	POS	POS				
	PT2020CAPXSER_SERNI	NEG	NEG				
	PT2020CAPXSER_SERN2	NEG	NEG				
	Protocol/SOP used	In house	EURL Capripoxviruses Protocol/IDAH SOP 40"Detection of Capripoxvirus antibodies by IPMA"				
	Name (+ reference) cell type used:	OA3.T ATCC6546	OA3Ts				
	Starting dilution of PT serum samples tested:	1/50	1:50				
	¥irus dose used in test (TCID50): e.g. 100 TCID50	100TCID50	100 TCID50				
ation	Positive control serum used:	R6F 45dpi (LSDV) and S6F (SPPV)	Antiserum EURL Capripox				
Informa	Secondary antibody (•reference) used for staining:	Anti bovine IgG (g) whole molecule -peroxidase produced in rabbit (Sigma A5295)	Anti-Bovine Ig G Peroxidase antibody produced in rabbit A 5295 SIGMA				
	Cut-off for positive:	NA	≥ 1:50				
	Name (+ reference) virus strain used:	LSDV Neethling or B1/10	SPPV, EURL Capripox and Neethling, Pirbright				
	Dilutions of PT serum samples tested:	1/50, 1/300	1:50 - 1:300				
	Expected antibody titer in positive control serum:	positive in 1/400 dilution	1:50				
	Dilution of secondary antibody used:	1/1000	1:1000				

Annex 7: Quantitative data analysis

For serology the sample PT2020CAPXSER_SERP4 was proposed 2 times in the panel. Therefore a repeatability study can be done.

Lab ID	SERP4R1	SERP4R2	mean	SD	CV
97506	112.297	108.796	110.5465	2.475581	2.24%
97600	110	111	110.5	0.707107	0.64%
97602	214.4	117.2	165.8	68.73078	41.45%
97604	116	123	119.5	4.949747	4.14%
97605	100	106	103	4.242641	4.12%
97607	96	98	97	1.414214	1.46%
97608	97.4	88.3	92.85	6.434672	6.93%
97609	109.8	105.4	107.6	3.11127	2.89%
97610	94.13	90.62	92.375	2.481945	2.69%
97611	95.220	103.753	99.4865	6.034214	6.07%
97612	114.00	117.00	115.5	2.12132	1.84%
97613	118.85	111.04	114.945	5.522504	4.80%
97614	108.277	114.42	111.3485	4.343757	3.90%
97615	126.309	126.913	126.611	0.427092	0.34%
97616	123	121	122	1.414214	1.16%
97617	172.0	128.0	150	31.12031	20.75%
97618	88.41	94.25	91.33	4.129504	4.52%
97619	96.45	92.5	94.475	2.793072	2.96%
97620	130.94	130.38	130.66	0.39598	0.30%
97621	139	144	141.5	3.535534	2.50%
97622	121	115	118	4.242641	3.60%
97623	110.45	110.45	110.45	0	0.00%
97628	120.891	120.65	120.7705	0.170413	0.14%
97629	112.824	111.379	112.1015	1.021769	0.91%
97630	103.99	107.5	105.745	2.481945	2.35%
97632	153.7	150.9	152.3	1.979899	1.30%
97633	112.3	111.8	112.05	0.353553	0.32%
97634	126.253	131.90	129.0755	3.992134	3.09%
97635	129.799	136.676	133.2378	4.862625	3.65%
97636	117	122	119.5	3.535534	2.96%

Table A1. Repeatability of the SERP4 sam	mple per laboratory for the ELISA data.
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The data were also represented as boxplots (figure A1).



Figure A1. Representation in boxplot of the dispersion of the ELISA value for PT2020CPAXSER_P4.

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 6 participants:

- a rectangle ranging from percentile 25 (P₂₅) to percentile 75 (P₇₅)
- a central line representing the median of the results (P₅₀)
- a lower limit showing the smallest value x > P₂₅ 1.5 * (P₇₅ P₂₅)
- an upper limit representing the largest value x < P₇₅ + 1.5 * (P₇₅ P₂₅)
- all points outside this interval are represented by a dot.



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