EXPERTISE AND SERVICE PROVISION
QUALITY OF LABORATORIES

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

Expertise and service provision
Quality of laboratories
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Bernard China

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.

II. Aim

The aim of this PT was to evaluate the ability of the participating laboratories to identify the absence or presence of antibodies to capripox 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).

Table 1. The samples of the PT2020CAPXVIR panel

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

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.

Table 2. The final status of each sample

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	LSDV	LSDV field	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

Randomisation and panel composition

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

Table 3. PT2020CAPXVIR Panel composition for odd and even laboratories

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

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:

Table 6. PT2020CAPXSER panel composition for odd and even laboratories

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

The PT2020CAPXSER panel consisted of 10 serum samples of 500 µl.

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 EU Member State NRL's

Country	Name	Participation in serology survey	Participation in 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; Department of Virology	1	1
Portugal	Instituto Nacional de Investigaçao Agraria e Veterinaria (INIAV), Laboratório Nacional de Referência para a Saude animal	1	1
Romania	Institute for diagnosis and animal health	1	1
Slovakia	State veterinary and food institute, Veterinary institute in Zvolen	1	1
Slovenia	University of Ljubljana, Veterinary faculty/National Veterinary Institute, Institute of Microbiology and Parasitology, Department of Virology	1	1
Spain	Laboratorio Central De Veterinaria (LCV) (ALGETE) M.A.P.A.	1	1
The Netherlands	Wageningen Bioveterinary Research	1	1

Table 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.

Table 9 Results per sample

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	OK	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	OK	Negative

*: 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 (www.qcmd.org).

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

Method	Target gene	N	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	Pos	Pos	Pos	Pos	Pos	Pos	Pos	Neg/ NI	Neg	
97506	Pos	Pos	Pos	Pos	Pos	Pos	Pos	Pos	Neg	Neg	100
97600	Pos	Pos	Pos	Pos	Pos	Pos	Pos	Pos	Neg	Neg	100
97602	Pos	Neg	Pos	Pos	Pos	Pos	Pos	Pos	Neg	Neg	90
97604	Pos	Pos	Pos	Pos	Pos	Pos	Pos	Pos	Neg	Neg	100
97606	Pos	Pos	Pos	Pos	Pos	Pos	Pos	Pos	Neg	Neg	100
97608	Pos	Pos	Pos	Pos	Pos	Pos	Pos	Pos	Neg	Neg	100
97610	Pos	Pos	Pos	Pos	Pos	Pos	Pos	Pos	Neg	Neg	100
97612	Pos	Pos	Pos	Pos	Pos	Pos	Pos	Pos	Neg	Neg	100
97614	Pos	Pos	Pos	Pos	Pos	Pos	Pos	Pos	Neg	Neg	100
97616	Pos	Pos	Pos	Pos	Pos	NI	Pos	Pos	Neg	Neg	90
97618	Pos	Pos	Pos	Pos	Pos	Pos	Pos	Pos	Neg	Neg	100
97620	Pos	Pos	Pos	Pos	Pos	Pos	Pos	Pos	Neg	Neg	100
97620	Neg	Neg	Pos	Pos	Neg	Pos	Pos	Neg	Neg	Neg	**60**
97622	Pos	Pos	Pos	Pos	Pos	Pos	Pos	Pos	Neg	Neg	100
97624	Pos	Pos	Pos	Pos	Pos	Pos	Pos	Pos	Neg	Neg	100
97628	Neg	Neg	Pos	Pos	Pos	Neg	Pos	Pos	Neg	Neg	**70**
97630	Pos	Pos	Pos	Pos	Pos	Pos	Pos	Pos	Neg	Neg	100
97632	Pos	Pos	Pos	Pos	Pos	Pos	Pos	Pos	Neg	Neg	100
97634	Pos	Pos	Pos	Pos	Pos	Pos	Pos	Pos	Neg	Neg	100
97636	Pos	Pos	Pos	Pos	Pos	Pos	Pos	Pos	Neg	Neg	100
97601	Pos	Pos	Pos	Pos	Pos	Pos	Pos	Pos	Neg	Neg	100
97603	Pos	Pos	Pos	Pos	Pos	Pos	Pos	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	Pos	Pos	Pos	Pos	Pos	Pos	Pos	NI	Neg	100
97607	Pos	Pos	Pos	Pos	Pos	Pos	Pos	Pos	NI	Neg	100
97609	Pos	Pos	Pos	Pos	Pos	Pos	Pos	Pos	Neg	Neg	100
97611	Pos	Pos	Pos	Pos	Pos	Pos	Pos	Pos	Neg	Neg	100
97613	Pos	Pos	Pos	Pos	Pos	Pos	Pos	Pos	Neg	Neg	100

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

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

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%).

Table 14. Results and proficiency per sample for species differentiation

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

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

IV.4.1.2.2 Results per method

Table 15. Proficiency per method for species differentiation

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

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

Lab ID	VP1 GTPV	VP2 GTPV	TP1 LSDV	TP2 LSDV	TP3 LSDV	VP3 LSDV	TP4 LSDV	TP5 SPPV	VN1 Neg	TN1 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	GTPV/ LSDV	GTPV/ LSDV	GTPV/ LSDV	GTPV/ LSDV	GTPV/ LSDV	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	GTPV/ LSDV	GTPV/ LSDV	GTPV/ LSDV	GTPV/ LSDV	GTPV/ LSDV	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. Twenty-five 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

Sample ID	Expected result	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	N	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 l. 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

Lab	VP1	VP2	TP1	TP2	TP3	VP3	TP4	TP5	VN1	TN1	%
	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 field 1 (2,8%) CAPX pos- SPPV 1 (2,8%) CAPX neg
PT2020CAPXVIR_VP2	CAPX pos - GTPV field	21(58,3%) CAPX pos-GTPV (field) 3 (8,3%) CAPX pos - SPPV/GTPV 1 (2,8%) CAPX pos - SPPV field 8 (22,2%) CAPX pos 3 (8,3%) CAPX Negative
PT2020CAPXVIR_TP1	CAPX pos-LSDV field	21 (58,3%) CAPX Pos-LSDV field 5 (13,9%)CAPX Pos-LSDV 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
PT2020CAPXVIR_TP4	CAPX pos-LSDV field	24 (66,7%) CAPX pos-LSDV Field 4 (11,1%) CAPX pos-LSDV 8 (22,2,%) CAPX-Pos
PT2020CAPXVIR_TP5	CAPX pos-SPPV field	11 (30,6%) CAPX pos-SPPV Field 12 (33,3 %) CAPX pos-SPPV 10 (27,8%) CAPX Pos 3 (8,3 %) CAPX Pos-SPPV /GTPV
PT2020CAPXVIR_VN1	CAPX negative/ CAPX doubtful	35 (97,2%) CAPX Negative 1 (2,8%) NI
PT2020CAPXVIR_TN1	CAPX negative	36 (100%) CAPX negative

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).

Table 21. Results per sample

Sample status Laboratory	VP1 CAPX pos - GTPV field	VP2 CAPX pos - GTPV field	TP1 CAPX pos- LSDV field	TP2 CAPX pos- LSD field	TP3 CAPX pos- LSDV vaccine	VP3 CAPX pos- LSDV field	TP4 CAPX pos- LSDV field	TP5 CAPX pos- SPPV field	VN1 CAPX negative/NI	TN1 CAPX negative	% (NCR/NDR)
97506	CAPX pos - GTPV	CAPX pos - GTPV	CAPX pos- LSDV field	CAPX pos- LSD field	CAPX pos- LSDV vaccine	CAPX pos- LSDV field	CAPX pos- LSDV field	CAPX pos- SPPV field	CAPX negative	CAPX negative	100 (10/10)
97600	CAPX pos - GTPV	CAPX pos - GTPV	CAPX pos- LSDV field	CAPX pos- LSD field	CAPX pos- LSDV vaccine	CAPX pos- LSDV field	CAPX pos- LSDV field	CAPX pos- SPPV field	CAPX negative	CAPX negative	100 (10/10)
97602	CAPX pos - GTPV	CAPX pos - GTPV	CAPX pos- LSDV field	CAPX pos- LSD field	CAPX pos- LSDV vaccine	CAPX pos- LSDV field	CAPX pos- LSDV field	CAPX pos- SPPV field	CAPX negative	CAPX negative	100 (10/10)
97604	CAPX pos - GTPV	CAPX pos - GTPV	CAPX pos- LSDV field	CAPX pos- LSD field	CAPX pos-L SDV vaccine	CAPX pos- LSDV field	CAPX pos- LSDV field	CAPX pos- SPPV field	CAPX negative	CAPX negative	100 (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 - GTPV	CAPX pos - GTPV	CAPX pos- LSDV field	CAPX pos- LSD field	CAPX pos- LSDV vaccine	CAPX pos- LSDV field	CAPX pos- LSDV field	CAPX pos- SPPV field	CAPX negative	CAPX negative	100 (10/10)
97610	CAPX pos - GTPV	CAPX pos - GTPV	CAPX pos- LSDV field	CAPX pos- LSD field	CAPX pos -LSDV vaccine	CAPX pos- LSDV field	CAPX pos- LSDV field	CAPX pos- SPPV field	CAPX negative	CAPX negative	100 (10/10)
97612	CAPX pos- SPPV/GTPV	CAPX pos- SPPV/GTPV	CAPX pos- LSDV field	CAPX pos- LSD field	CAPX pos- LSDV vaccine	CAPX pos- LSDV field	CAPX pos- LSDV field	CAPX pos - SPPV/GTPV	CAPX negative	CAPX negative	100 (10/10)
97614	CAPX pos - GTPV	CAPX pos - GTPV	CAPX pos - LSDV	CAPX pos – LSDV - field	CAPX pos – LSDV - field	CAPX pos - LSDV	CAPX pos - LSDV	CAPX pos- SPPV field	CAPX negative	CAPX negative	90 (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 - GTPV	CAPX pos - GTPV	CAPX pos- LSDV field	CAPX pos- LSD field	CAPX pos- LSDV vaccine	CAPX pos- LSDV field	CAPX pos- LSDV field	CAPX pos - SPPV	CAPX negative	CAPX negative	100 (10/10)
97620	CAPX pos - GTPV	CAPX pos - GTPV	CAPX pos- LSDV field	CAPX pos- LSD field	CAPX pos- LSDV vaccine	CAPX pos- LSDV field	CAPX pos- LSDV field	CAPX pos- SPPV field	CAPX negative	CAPX negative	100 (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 - SPPV/GTPV	CAPX pos - SPPV/GTPV	CAPX pos- LSDV field	CAPX pos -LSDV field	CAPX pos- LSDV vaccine	CAPX pos- LSDV field	CAPX pos- LSDV field	CAPX pos- SPPV /GTPV	CAPX negative	CAPX negative	100 (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 - 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-SPPV	CAPX negative	CAPX negative	100 (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 - GTPV	CAPX pos - GTPV	CAPX pos-LSDV	CAPX pos-LSDV	CAPX pos-LSDV	CAPX pos-LSDV	CAPX pos-LSDV	CAPX pos-SPPV	CAPX negative	CAPX negative	100 (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 - 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-SPPV field	CAPX negative	CAPX negative	100 (10/10)
97611	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-SPPV	CAPX negative	CAPX negative	100 (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 - GTPV field	CAPX pos - GTPV	CAPX pos-LSDV	CAPX pos-LSDV	CAPX pos-LSDV	CAPX pos-LSDV field	CAPX pos-LSDV	CAPX pos-SPPV field	CAPX negative	CAPX negative	100 (10/10)
97619	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-SPPV	CAPX negative	CAPX negative	100 (10/10)
97621	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-SPPV	CAPX negative	CAPX negative	100 (10/10)
97623	CAPX pos - GTPV	CAPX pos - GTPV	CAPX pos-LSDV	CAPX pos-LSDV field	CAPX pos -LSDV field	CAPX pos-LSDV field	CAPX pos-LSDV field	CAPX pos-SPPV	CAPX negative	CAPX negative	90 (9/10)
97625	CAPX pos - SPPV field	CAPX pos - SPPV field	CAPX pos-LSDV field	CAPX pos-LSDV field	CAPX pos-LSDV Vaccine	CAPX pos-LSDV field	CAPX pos-LSDV field	CAPX pos-SPPV field	CAPX negative	CAPX negative	**80 (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 - SPPV	CAPX negative	CAPX negative	CAPX pos-LSDV field	CAPX pos-LSDV Vaccine	CAPX pos-SPPV	CAPX pos-LSDV field	CAPX pos-SPPV	CAPX negative	CAPX negative	**60 (6/10)**
97631	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-SPPV	CAPX negative	CAPX negative	100 (10/10)
97633	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-SPPV	CAPX negative	CAPX negative	100 (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.

Table 22. Results for ELISA

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)

97615	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)
97619	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)
97623	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)
97633	Pos	Pos	Pos	Pos	Pos	Pos	Pos	Pos	Neg	Neg	100 (10/10)
97635	Pos	Pos	Pos	Pos	Pos	Pos	Pos	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.

Table 23. Results for virus neutralization test

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)

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.

Table 24. Results for IPMA

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)

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	Pos	Pos	Pos	Pos	Pos	Pos	Pos	Neg	Neg	100 (10/10)
97635	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)

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_VP1 and 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. Additionaly, 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 and SPPV 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

value	sample	Labnr 97506	97600	97601	97602	97603	97604	97605
raw data	PT2020CAPXVIR_VP1	35,25	30,67	31,1	33,01	27,62	31,1	33,39
	PT2020CAPXVIR_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
	PT2020CAPXVIR_TP2	33,24	30,55	32,21	29,74	28,95	33,2	29,55
	PT2020CAPXVIR_TP3	31,26	29,32	30,35	27,18	27,29	30,3	28,55
	PT2020CAPXVIR_VP3	37,06	32,78	35,54	34,4	31,78	34,1	33,01
	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 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
Final results	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
	PT2020CAPXVIR_TP2	POS	POS	POS	POS	POS	POS	POS
	PT2020CAPXVIR_TP3	POS	POS	POS	POS	POS	POS	POS
	PT2020CAPXVIR_VP3	POS	POS	POS	POS	POS	POS	POS
	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
Information	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 extraction protocol (if yes, which?):	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
	Cut-off for positive:	Ct<45: Positive; 45≤Ct<50: Doubtful; Ct≥50: Negative		39		<35	40	No cut-off
	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 ct value from the positive extraction control

value	sample	Labnr 97606	97607	97608	97609	97610	97611	97612
raw data	PT2020CAPXVIR_VP1	33,13	30,50	32,31	30,5	29,26	27,7	29
	PT2020CAPXVIR_VP2	36,61	33,07	34,81	35,71	34,74	31,3	32
	PT2020CAPXVIR_TP1	29,28	26,54	27,31	26	27,04	26,4	24
	PT2020CAPXVIR_TP2	32,88	31,16	30,19	30,22	30,8	28,4	29
	PT2020CAPXVIR_TP3	30,09	28,90	28,67	28,63	29,72	27,2	27
	PT2020CAPXVIR_VP3	35,22	34,01	33,47	32,57	35,3	31,2	32
	PT2020CAPXVIR_TP4	34,02	32,24	32,2	32,29	31,43	31,1	30
	PT2020CAPXVIR_TP5	23,87	24,56	22,29	22,02	23,16	21,4	22
	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
Final results	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
	PT2020CAPXVIR_TP2	POS	POS	POS	POS	POS	POS	POS
	PT2020CAPXVIR_TP3	POS	POS	POS	POS	POS	POS	POS
	PT2020CAPXVIR_VP3	POS	POS	POS	POS	POS	POS	POS
	PT2020CAPXVIR_TP4	POS	POS	POS	POS	POS	POS	POS
	PT2020CAPXVIR_TP5	POS	POS	POS	POS	POS	POS	POS
	PT2020CAPXVIR_VN1	NEG	NI	NEG	NEG	NEG	NEG	NEG
	PT2020CAPXVIR_TN1	NEG	NEG	NEG	NEG	NEG	NEG	NEG
Information	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 RNA 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	n/a	N/A
	PCR Instrument used:	Bio-Rad CFX96 Touch	LightCycler96 (Roche)	Bio-Rad CFX 96	Rotor-Gene	RotorGene Q	Agilent ARIAmx	LightCycler 96 (Roche)
	Cut-off for positive:	< 40	No cut-off (Observation of a characteristic amplification curve)	40	ct 40	Ct 38	CT38	
	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	

value	sample	Labnr 97613	97614	97616	97617	97618	97619	97620
raw data	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
	PT2020CAPXVIR_TP2	29,63	30,2	31,5	32,65	30,91	32,4	31,88
	PT2020CAPXVIR_TP3	26,23	27,5	29,08	30,35	28,15	29,3	29,61
	PT2020CAPXVIR_VP3	30,53	31,8	35,73	34,34	32,18	34,3	36,4
	PT2020CAPXVIR_TP4	28,64	34,2	32,42	34,07	29,26	33	33,31
	PT2020CAPXVIR_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
Final results	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
	PT2020CAPXVIR_TP2	POS	POS	POS	POS	POS	POS	POS
	PT2020CAPXVIR_TP3	POS	POS	POS	POS	POS	POS	POS
	PT2020CAPXVIR_VP3	POS	POS	NI	POS	POS	POS	POS
	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
Information	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 TR, Babiuk SL, Parkyn GR, 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 RNA 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 yes, which?):	No	No	N/A	N/A	N/A	no	N/A
	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
	Cut-off for positive:			35	N/A	N/A	45	Ct < 45
	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.		

value	sample	97621	97622	97623	97624	97625	97627	97628
raw data	PT2020CAPXVIR_VP1	28,2	36,75 / 37,25	32,065	34,3	28,53	28,16	no Ct
	PT2020CAPXVIR_VP2	32,8	40,00 / 38,88	35,735	38,5	31,86	30,98	no Ct
	PT2020CAPXVIR_TP1	25,9	28,01 / 28,22	30,68	30,5	25,52	26,78	29,92
	PT2020CAPXVIR_TP2	29,3	32,30 / 32,32	33,235	34,6	29,31	29,31	35,56
	PT2020CAPXVIR_TP3	30,9	32,28 / 32,28	30,125	37,8	29,29	27,99	30,15
	PT2020CAPXVIR_VP3	34,5	36,00 / 36,84	35,795	38,8	33,67	32,1	no Ct
	PT2020CAPXVIR_TP4	32	33,08 / 33,21	34,65	38,1	31,36	31,59	33,22
	PT2020CAPXVIR_TP5	24,2	27,22 / 26,91	24,72	29,2	24,17	23,19	23,59
	PT2020CAPXVIR_VN1	No Ct	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	no Ct
Final results	PT2020CAPXVIR_VP1	POS	POS	POS	Pos	POS	POS	NEG
	PT2020CAPXVIR_VP2	POS	POS	POS	Pos	POS	POS	NEG
	PT2020CAPXVIR_TP1	POS	POS	POS	Pos	POS	POS	POS
	PT2020CAPXVIR_TP2	POS	POS	POS	Pos	POS	POS	POS
	PT2020CAPXVIR_TP3	POS	POS	POS	Pos	POS	POS	POS
	PT2020CAPXVIR_VP3	POS	POS	POS	Pos	POS	POS	NEG
	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
Information	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 extraction protocol (if yes, which?):	Proteinase treatment at 72 °C / 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
	Cut-off for positive:	Ct < 35: + (35 < Ct < 40: unconclusive)		None				
	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 AAAACGGTATATGGAATAGAGTTGGAA CaPV074R1 AAATGAAACCAATGGATGGGATA CaPV074P1FAM-TGGCTCATAGATTTCCA-MGB/NFQ	Sigma Forward primer-5`GGCGATGTCCATTCCCTG 3` Reverse primer-5` AGCATTTCATTTCCGTGAGGA-3` Probe MGB 5` CAATGGGTAAAAGATTTCTA-3`	
	Remark(s):							

value	sample	Labnr 97629	97630	97631	97632	97633	97634	97635	97636
raw data	PT2020CAPXVIR_VP1	31,76	32,4	27,31	31,8	27,65	30,78	30,13	27,51
	PT2020CAPXVIR_VP2	no Ct	38,01	29,13	34,6	32,16	33,36	33,32	30,72
	PT2020CAPXVIR_TP1	no Ct	26,2	24,2	26,1	25,46	23,95	26,97	21,21
	PT2020CAPXVIR_TP2	32,98	31,78	27,46	31,6	29,25	27,72	30,48	25,17
	PT2020CAPXVIR_TP3	32,59	28,73	28,04	28,1	26,27	26,41	29,17	22,79
	PT2020CAPXVIR_VP3	35,16	34,17	30,44	33,6	32,83	35,49	34,14	30,47
	PT2020CAPXVIR_TP4	36,09	32,2	30,82	31,1	31,19	29,56	32,13	29,24
	PT2020CAPXVIR_TP5	25,72	24,98	23,3	22,3	21,99	22,75	24,01	18,15
	PT2020CAPXVIR_VN1	no Ct	No ct	No ct	No Ct	No Ct	No Cq	No Ct	No Ct
	PT2020CAPXVIR_TN1	no Ct	No ct	No ct	No Ct	No Ct	No Cq	No Ct	No Ct
Final results	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
	PT2020CAPXVIR_TP2	POS	POS	POS	POS	POS	POS	POS	POS
	PT2020CAPXVIR_TP3	POS	POS	POS	POS	POS	POS	POS	POS
	PT2020CAPXVIR_VP3	POS	POS	POS	POS	POS	POS	POS	POS
	PT2020CAPXVIR_TP4	POS	POS	POS	POS	POS	POS	POS	POS
	PT2020CAPXVIR_TP5	POS	POS	POS	POS	POS	POS	POS	POS
	PT2020CAPXVIR_VN1	NEG	NEG	NEG	NEG	NEG	NEG	NEG	NEG
	PT2020CAPXVIR_TN1	NEG	NEG	NEG	NEG	NEG	NEG	NEG	NEG
Information	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
	Cut-off for positive:			38		up tp 35 cycles	38	≤38	
	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	
	Remark(s):								

Annex 1b: Raw Optional RT-PCR data

value	sample	Labnr 97605	Labnr 97607	Labnr 97620	Labnr 97621
raw data	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++
	PT2020CAPXVIR_TP3	29	30,29	No Ct	POS++
	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
Final results	PT2020CAPXVIR_VP1	POS	POS	NEG	POS
	PT2020CAPXVIR_VP2	NEG	POS	NEG	NEG
	PT2020CAPXVIR_TP1	POS	POS	POS	NEG
	PT2020CAPXVIR_TP2	POS	POS	POS	POS
	PT2020CAPXVIR_TP3	POS	POS	NEG	POS
	PT2020CAPXVIR_VP3	POS	POS	POS	POS
	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
Information	Name Extraction protocol / kit:	External lysis protocol	DNeasy® Blood & Tissue	QIAamp Viral RNA Mini Kit (250)	MagAttract 96 CADOR Pathogen Kit
	In-house modifications to extraction protocol (if yes, which?):	No cut-off	No		Proteinase treatment at 72 °C / Addition of carrier ARN
	PCR Instrument used:	MX3005p	Stratagene MX3005P (Agilent Technologies)	Stratagene Mx3005P	Verity (Applied Biosystems)
	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:	E3L	RPO30	EEV gene of LSD field strain	
	Remark(s):				

Annex 2: Raw Capripox Species Differentiation data

value	sample	Labar 37506	37600	37602	37603	37604	37607	37608
raw data	PT2020CAPXVIR_VP1	NEG	positive	35,01	172bp	POS	No Ct	sequencing
	PT2020CAPXVIR_VP2	NEG	positive	no Ct	172bp	POS	No Ct	sequencing
	PT2020CAPXVIR_TP1	29,6	positive	30,88	172bp	POS	28,52	27,04
	PT2020CAPXVIR_TP2	34,5	positive	35,29	172bp	POS	33,86	29,33
	PT2020CAPXVIR_TP3	32,2	positive	32,55	172bp	POS	31,305	30,05
	PT2020CAPXVIR_VP3	37,9	positive	no ct	172bp	POS	36,775	32,62
	PT2020CAPXVIR_TP4	35,9	positive	37,96	172bp	POS	34,33	32,23
	PT2020CAPXVIR_TP5	35,26	positive	26,33	151bp	POS	No Ct	sequencing
	PT2020CAPXVIR_VN1	NA	negative	no Ct	None	neg	No Ct	No Ct
	PT2020CAPXVIR_TN1	NA	negative	no Ct	None	neg	No Ct	No Ct
Final results	PT2020CAPXVIR_VP1	GTPV	GTPV	GTPV	GTPV/LSDV	GTPV	NEG	GTPV
	PT2020CAPXVIR_VP2	GTPV	GTPV	NEG	GTPV/LSDV	GTPV	NEG	GTPV
	PT2020CAPXVIR_TP1	LSDV	LSDV	LSDV	GTPV/LSDV	LSDV	LSDV	LSDV
	PT2020CAPXVIR_TP2	LSDV	LSDV	LSDV	GTPV/LSDV	LSDV	LSDV	LSDV
	PT2020CAPXVIR_TP3	LSDV	LSDV	LSDV	GTPV/LSDV	LSDV	LSDV	LSDV
	PT2020CAPXVIR_VP3	LSDV	LSDV	LSDV	GTPV/LSDV	LSDV	LSDV	LSDV
	PT2020CAPXVIR_TP4	LSDV	LSDV	LSDV	GTPV/LSDV	LSDV	LSDV	LSDV
	PT2020CAPXVIR_TP5	SPPV	SPPV	SPPV	SPPV	SPPV	NEG	SPPV
	PT2020CAPXVIR_VN1	NEG	NEG	NEG	NEG	NEG	ND	NEG
	PT2020CAPXVIR_TN1	NEG	NEG	NEG	NEG	NEG	NEG	NEG
Information	Protocol / SOP used :	Agianniotaki et al., 2016 / in-house Taqman assay	Lamien et al. 2011	Dual hybridisation probes	Lamien et al., 2011	6.3.51.4 Capripoxviruses GPCR seq	RT-PCR GPCR (specific LSDV - Under development)	Adedeji et al. , Transbound Emerg Dis. 2013;1-11. // Möller et al., Archives of Virology (2013) 164:2931-2941
	Producer Extraction protocol / kit:	Machery Nagel	Biosellal	ThermoFisher Scientific	Roche Diagnostics	Roche	Qiagen	Qiagen
	Name Extraction protocol / kit:	Blood	Biosellal Superball	MagMAX Core Nucleic Acid Purification Kit	MagNA Pure Compact Nucleic Acid Isolation Kit I	MagNA Pure 96 DNA and Viral NA Small Volume Kit	DNeasy® Blood & Tissue	QIAamp Viral RNA Mini Kit
	In-house modifications to extraction protocol (if yes, which?):	Addition of EC to Buffer B3	no		No		No	N/A
	PCR Instrument used:	Lightcycler 480	BioRad CFX96	RotorGene®	LightCycler 96 (Roche)	Eppendorf Mastercycler ProS	Stratagene MX3005P (Agilent Technologies)	GeneAmp 2720 Thermal Cycler
	Cut-off for positive:	Ct<45: Positive; 45≤Ct<50: Doubtful; Ct≥50: Negative			N/A		No cut-off (Observation of a characteristic amplification curve)	
	Methodology:	Roche		Real-time PCR - Dual hybridisation probes assay	Classical PCR amplification	Promega	Biorad	Sanger Sequencing
	Ref Methodology:	Taq Platinium		Lamien et al, 2011	Lamien et al., 2011	GoTaq Colorless Master mix	SsoAdvanced Universal Probes Supermix	
	The target of the RT primer:	LSDV GPCR (field strain)	GPCR	GPCR	RPO30	GPCR gene	GPCR	Adedeji et al. , Transbound Emerg Dis. 2013;1-11. // Möller et al., Archives of Virology (2013) 164:2931-2941
	remarks			As positive controles were used LSDV (Ct 25,66), GTPV (Ct 22,16) and SPPV (Ct 24,49) For samples 2006 and 2007 species was determined using protocols of Vidanovic et al. 2016. and Menacherow et al., 2014.	151bp product is genotyped as SPPV, 172bp product is genotyped as GTPV if collected from small ruminants and LSDV if collected from cattle DNA from known positive SPPV, GTPV and LSDV sample used as positive control with the results of 151bp for SPPV and 172bp for GTPV and LSDV.		For the V-CPX2009 sample, we have displayed ND because the choice NI is not available.	

value	sample	97609	97610	97611	97612	97613	97614	97617
raw data	PT2020CAPXVIR_VP1	gel-based (Lamien et al-ampicon sequencing)		33,1	No Ct	30,46	36,56	POS
	PT2020CAPXVIR_VP2	gel-based (Lamien et al-ampicon sequencing)		36,5	No Ct	36,63	40	POS
	PT2020CAPXVIR_TP1	27,2 (WT)		27,7	26	26,73	33,9	POS
	PT2020CAPXVIR_TP2	32,79 (WT)		29,6	30	35,9	34,7	POS
	PT2020CAPXVIR_TP3	29,66 (Vac)		28,1	27	31,28	35,7	POS
	PT2020CAPXVIR_VP3	36,59 (WT)		31,8	23	36,56	38,2	POS
	PT2020CAPXVIR_TP4	33,77 (WT)		31,5	31	34,98	38,5	POS
	PT2020CAPXVIR_TP5	gel-based (Lamien et al-ampicon sequencing plus Haegeman 2015 conv SPPV DIVA (ampicon sequencing) and SPPV WT Real Time PCR)		21,6	No Ct	33,95	33,7	POS
	PT2020CAPXVIR_VN1	not tested (Neg CapV)		No Ct	No Ct	No Ct	No Ct	N/A
	PT2020CAPXVIR_TN1	not tested (Neg CapV)		No Ct	No Ct	No Ct	No Ct	N/A
Final results	PT2020CAPXVIR_VP1	GTPV	GTPV	GTPV	ND	GTPV	GTPV	GTPV
	PT2020CAPXVIR_VP2	GTPV	GTPV	GTPV	ND	GTPV	GTPV	GTPV
	PT2020CAPXVIR_TP1	LSDV	LSDV	LSDV	LSDV	LSDV	LSDV	LSDV
	PT2020CAPXVIR_TP2	LSDV	LSDV	LSDV	LSDV	LSDV	LSDV	LSDV
	PT2020CAPXVIR_TP3	LSDV	LSDV	LSDV	LSDV	LSDV	LSDV	LSDV
	PT2020CAPXVIR_VP3	LSDV	LSDV	LSDV	LSDV	LSDV	LSDV	LSDV
	PT2020CAPXVIR_TP4	LSDV	LSDV	LSDV	LSDV	LSDV	LSDV	LSDV
	PT2020CAPXVIR_TP5	SPPV	SPPV	SPPV	ND	SPPV	SPPV	SPPV
	PT2020CAPXVIR_VN1	ND	NEG	NEG	NEG	NEG	ND	N/A
	PT2020CAPXVIR_TN1	ND	NEG	NEG	NEG	NEG	ND	N/A
Information	Protocol / SOP used :	Lamien et al/Haegeman 2015 SPPV DIVA conventional /Hageman 2015 SPPV WT Real Time PCR (Sybr)	Gelaye et al., PLOS 2013			Lamien et al 2011, J of Virol Methods 171(1):134-140	Lamien et.al.,2011 Journal of virological methods 171(1);134-140	Gelaye et al. 2015
	Producer Extraction protocol / 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 RNA mini kit	MagAttract 96 cador Pathogen Kit
	In-house modifications to extraction protocol (if yes, which?):	no	King Fisher Flex/Magattract cador Pathogen Kit	n/a		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
	Methodology:	gel-based PCR and sequencing analysis:	Qiagen/Quantitect	Melt Curve using SYBR Green chemisty	PCR real time	Dual Hybridization Probes Assay	Dual Hybridization Probe Assay	Nzytech
	Ref Methodology:	Lamien et al/Haegeman 2015 SPPV DIVA 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	RPO147	Target sequence of Lumpy Skin Disease viral genome	chemokine gene		RPO30
	remarks	No need for species differentiation regarding cattle samples. Only Field strain/Vaccine differentiation done by DIVA LSDV Real Time PCR GTPV 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		Results as Tm products and Ct values				

value	sample	Labnr 97618	97619	97620	97621	97623	97625	97628
raw data	PT2020CAPXVIR_VP1	GTPV Turkey	pos	172bp	POS+++		POS	no Ct
	PT2020CAPXVIR_VP2	GTPV Koppal	pos	172bp	POS+		POS	no Ct
	PT2020CAPXVIR_TP1	LSDV Isolate Pendik	pos	172bp	POS++		POS	25,33
	PT2020CAPXVIR_TP2	ND	pos	172bp	NEG		POS	31,09
	PT2020CAPXVIR_TP3	LSDV Isol 148-GP-RSA-1997	pos	172bp	POS++		POS	30,15
	PT2020CAPXVIR_VP3	ND	pos	172bp	POS+		POS	no Ct
	PT2020CAPXVIR_TP4	ND	pos	172bp	NEG		POS	31,72
	PT2020CAPXVIR_TP5	SPPVaccine Turkey	pos	151bp	POS+		POS	23,74
	PT2020CAPXVIR_VN1	ND	neg	neg	NEG		NEG	no Ct
	PT2020CAPXVIR_TN1	ND	neg	neg	NEG		NEG	no Ct
Final results	PT2020CAPXVIR_VP1	GTPV	GTPV	GTPV/LSDV	GTPV	GTPV	SPPV	NEG
	PT2020CAPXVIR_VP2	GTPV	GTPV	GTPV/LSDV	GTPV	GTPV	SPPV	NEG
	PT2020CAPXVIR_TP1	LSDV	LSDV	GTPV/LSDV	LSDV	LSDV	LSDV	GTPV/LSDV
	PT2020CAPXVIR_TP2	ND	LSDV	GTPV/LSDV	NEG	LSDV	LSDV	GTPV/LSDV
	PT2020CAPXVIR_TP3	LSDV	LSDV	GTPV/LSDV	LSDV	LSDV	LSDV	GTPV
	PT2020CAPXVIR_VP3	ND	LSDV	GTPV/LSDV	LSDV	ND	LSDV	NEG
	PT2020CAPXVIR_TP4	ND	LSDV	GTPV/LSDV	NEG	LSDV	LSDV	LSDV
	PT2020CAPXVIR_TP5	SPPV	SPPV	SPPV	SPPV	SPPV	SPPV	SPPV
	PT2020CAPXVIR_VN1	ND	NEG	NEG	NEG	ND	NEG	NEG
	PT2020CAPXVIR_TN1	ND	NEG	NEG	NEG	ND	NEG	NEG
Information	Protocol / SOP used :	in house	Tapputainen et al., 2014	Lamien CE, et al., Vet Microbiol. 2011; 149:30–9.	Menasherow S et al. Jounal of Virological Methods 199:95-101, 2014	NVR-SOP-46 (Lamien et al., 2011)	Classical PCR Method for Differential Diagnosis of Capripox: sheep&goat Pox/ Lumpy skin disease	ID Gene LSD DIVA Triplex
	Producer Extraction protocol / kit:	Promega	Roche	QIAGEN	Qiagen	ThermoFisher/ KingFisher Flex	QIAGEN	ThermoFisher
	Name Extraction protocol / kit:	Genomic DNA Purification Kit	The High Pure Viral Nucleic Acid Kit	QIAamp Viral RNA Mini Kit (250)	MagAttract 96 CADOR Pathogen Kit	LSI Magvet	DNA Mini Kit	PrepFiler Express Forensic DNA Extraction Kit
	In-house modifications to extraction protocol (if yes, which?):	No	no modification in ext protocol only in PCR HRM - replaced by Sanger sequencing of		Proteinase treatment at 72 °C / Addition of ARN carrier	None	no	N/A
	PCR Instrument used:	SimliAmp Thermal Cycler	CFX 96 Touch	Biometra T3 Thermocycler	Verity (Applied Biosystems)	AB7500 Fast		Rotor-Gene 6000
	Cut-off for positive:		45	/	Clear band at the right size and with the expected intensity + blasting in NCBI database	None		
	Methodology:	Sequencing of RPO 030 gene	Thermo Fisher Scientific	Invitrogen	Promega	Rotogene	APHL Seibersdorf(Lamien et.,) al 2011	DIVA PCR
	Ref Methodology:	Gelaye E. et al., 2015	SS III ONE-STEP HI FI	Platinum PCR Supermix	GoTaq® Hot Start Green Master Mix	-		
	The target of the RT primer:	RPO030 gene	G-protein-coupled chemokine receptor	RPO30 of LSDV, SPPV, GTPV		GPCR	SpGpRNApolF 5'-TCTATGTCTTGATATGTGGTGGTAG-3' SpGpRNApolR 5'-AGTGATTAGGTGGTGTATTATTTTCC-3'	
	remarks			Conventional PCR for species differentiation based on the PCR product length. Result is expresed as PCR product length in bp.			It is importamt to note that this PCR metod produce a product with similar size (172 bp) for GTPV and LSDV and the interpretation of the results needs to take into account the species from which the	

value	sample	97629	97631	97632	97633	97634	97636
raw data	PT2020CAPXVIR_VP1	32,88	No Ct		28,13	31	
	PT2020CAPXVIR_VP2	no Ct	No Ct		31,4	34,5	
	PT2020CAPXVIR_TP1	no Ct	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
	PT2020CAPXVIR_VP3	39,23	33,2		31,55	37,64	29,02
	PT2020CAPXVIR_TP4	31,93	31,93		28,84	31,72	27,59
	PT2020CAPXVIR_TP5	24,66	26,11		21,86	26	
	PT2020CAPXVIR_VN1	no Ct	No ct		No Ct	No Cq	
	PT2020CAPXVIR_TN1	no Ct	Neg.		No Ct	No Cq	
Final results	PT2020CAPXVIR_VP1	SPPV	GTPV	GTPV	GTPV	GTPV	ND
	PT2020CAPXVIR_VP2	NEG	GTPV	GTPV	GTPV	GTPV	ND
	PT2020CAPXVIR_TP1	NEG	LSDV	LSDV	LSDV	LSDV	LSDV
	PT2020CAPXVIR_TP2	LSDV	LSDV	LSDV	LSDV	LSDV	LSDV
	PT2020CAPXVIR_TP3	LSDV	LSDV	LSDV	LSDV	LSDV	LSDV
	PT2020CAPXVIR_VP3	SPPV	LSDV	LSDV	LSDV	LSDV	LSDV
	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
Information	Protocol / SOP used :	ID Gene LSD DIVA Triplex		Differentiation of SPPV from GTPV/LSD, based on Lamien et al. 2011; Genotyping of Capripoxvirus based on sequensing of RPO30 gene, based on Gelaye et al. 2015		Four qPCR test were used, first qPCR for field LSDV, second qPCR for vac LSDV, third qPCR for SPPV, and fourth qPCR for GTPV Vidanovic 2016, Vidanovic Unpublished	
	Producer Extraction protocol / kit:	ThermoFisher		Sacace Biotechnologies	Qiagen , Germany	LSI, Thermo	INDICAL Bioscience
	Name Extraction protocol / kit:	PrepFiler Express Forensic DNA Extraction Kit	Roche, High Pure Viral Nucleic Acid Kit	Viral Nucleic Acid Extraction Kit	Dna mini kit	Magvet universal kit	IndiSpin Pathogen Kit
	In-house modifications to extraction protocol (if yes, which?):	N/A			N/A		
	PCR Instrument used:	Rotor-Gene 6000	AB7500	SimpliAmp	rotor gene	AriaMx	
	Cut-off for positive:		38		up tp 35 cycles	40	
	Methodology:	DIVA PCR		conventional PCR and Sanger sequencing	FGBI ARRIAH		PCR-RT
	Ref Methodology:				A real time PCR kit for indentification of capripoxvirus DNA		
	The target of the RT primer:			RPO30	not disclosed		IDvet Genetics, ID Gene LSD DIVA Triplex

Annex 3: Raw Capripox field and vaccine strain differentiation data

value	sample	Labnr 97606	Labnr 97600	Labnr 97602	Labnr 97604	Labnr 97608	Labnr 97609	Labnr 97610
raw data	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
	PT2020CAPXVIR_TP3	32,2	33,21	no Ct	NA	30,05	29,66	NA
	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
Final results	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
	PT2020CAPXVIR_TP2	LSDV FIELD	LSDV FIELD	LSDV FIELD	LSDV FIELD	LSDV FIELD	LSDV FIELD	LSDV FIELD
	PT2020CAPXVIR_TP3	LSDV VACCINE	LSDV VACCINE	LSDV VACCINE	LSDV VACCINE	LSDV VACCINE	LSDV VACCINE	LSDV VACCINE
	PT2020CAPXVIR_VP3	LSDV FIELD	LSDV FIELD	LSDV FIELD	LSDV FIELD	LSDV FIELD	LSDV FIELD	LSDV FIELD
	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
	PT2020CAPXVIR_TN1	ND	NEG	ND	ND	ND	ND	NEG
Information	Protocol / SOP used :	Agianniotaki et al., 2016 and in-house Taqman assay	Confidential	Vidanovic 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
	Producer Extraction protocol / kit:	Machery Nagel	Biosellal	ThermoFisher Scientific	Roche	Qiagen	Qiagen	Qiagen/Magattract cador Pathogen Kit
	Name Extraction protocol / kit:	Blood	Biosellal Superball	MagMAX Core Nucleic Acid Purification Kit	MagNA Pure 96 DNA and Viral NA Small Volume Kit	QIAamp Viral RNA Mini Kit	Qiamp Cador Pathogen	King Fisher Flex/ Magattract cador Pathogen Kit
	In-house modifications to extraction protocol (if yes, which?):	Addition of EC to Buffer B3	no			N/A	NO	
	PCR Instrument used:	Lightcycler 480	ABI 7500Fast	RotorGeneQ	Eppendorf Mastercycler ProS	Bio-Rad CFX 96	RotorGene	Roto-Gene Q
	Cut-off for positive:	Ct<45: Positive; 45≤Ct<50: Doubtful; Ct≥50: Negative			Ct<45: Positive; 45≤Ct<50: Doubtful; Ct≥50: Negative	40		
	Methodology	Roche		Real-time PCR	GPCR sequencing + BLAST analysis	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/Quantitect
	Ref Methodology	Taq Platinium		Vidanovic et al, 2016	Gelaye E, et al.. 2015 + BLAST			
	Remark(s):							

value	sample	Labnr 97611	Labnr 97612	Labnr 97613	Labnr 97614	Labnr 97617	Labnr 97618	Labnr 97620
raw data	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)
	PT2020CAPXVIR_TP3	29,6	FAM - / 26 VIC	29,67	NA	Not done	32,19	32,54 (VIC)
	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	N/A		
	PT2020CAPXVIR_TN1	No Ct	-	No Ct	NA	N/A		
Final results	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	Not done	LSDV FIELD	LSDV FIELD
	PT2020CAPXVIR_TP2	LSDV FIELD	LSDV FIELD	LSDV FIELD	LSDV FIELD	Not done	LSDV FIELD	LSDV FIELD
	PT2020CAPXVIR_TP3	LSDV VACCINE	LSDV VACCINE	LSDV VACCINE	LSDV FIELD	Not done	LSDV VACCINE	LSDV VACCINE
	PT2020CAPXVIR_VP3	LSDV FIELD	LSDV FIELD	LSDV FIELD	NI	Not done	LSDV FIELD	LSDV FIELD
	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
Information	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
	Producer Extraction protocol / kit:	Qiagen QiaAmp Viral RNA Mini Kit	Roche	QIAGEN		Qiagen Biosprint 96	Invitrogen by Thermo Fisher Scientific	QIAGEN
	Name Extraction protocol / kit:		High Viral Nucleic Acid kit	IndiSpin Pathogen MiniKit		MagAttract 96 cador Pathogen Kit	Pure Link Genomic DNA Mini Kit	QIAamp Viral RNA Mini Kit (250)
	In-house modifications to extraction protocol (if yes, which?):	n/a		No		N/A	No	
	PCR Instrument used:	Agilent ARIA	LightCycler 96 (Roche)	RotorGeneQ		Bio-Rad C1000/CFX96	LC 480 Thermal Cycler	QuantStudio5
	Cut-off for positive:	CT38				N/A	No	Ct<40
	Methodology	Multiplex real time	PCR Real Time	Duplex real-time PCR with LNA TaqMan probes		Nzytech	Real-time PCR method	ID.Vet Genetics
	Ref Methodology	Thermofisher TaqMan Fast Virus 1-Step Master Mix	ID Gene LSD DIVA Triplex (Idvet)	Agianniotaki et al., 2017 J of Virol Methods 249; 48-57		NZYTaq 2x Master Mix;	Agianniotaki et al 2017	ID Gene LSD DIVA Triplex, Real time PCR
	Remark(s):							

value	sample	Labnr 97621			Labnr 97623	Labnr 97625	Labnr 97628	Labnr 97629
raw data	PT2020CAPXVIR_VP1	POS+++	No ct	NEG	NA	32,49	no Ct	no Ct
	PT2020CAPXVIR_VP2	POS+	No ct	NEG	NA	34,8	no Ct	no Ct
	PT2020CAPXVIR_TP1	POS+	39,1	NEG	NA	34,84	25,33	29,89
	PT2020CAPXVIR_TP2	NEG	36,8	NEG	NA	29,77	31,09	no Ct
	PT2020CAPXVIR_TP3	POS++	33,5	NEG	NA	28,96	no Ct	33,03
	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+	No ct	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
Final results	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
	PT2020CAPXVIR_TP3	LSDV VACCINE			LSDV FIELD	LSDV VACCINE	NEG	LSDV VACCINE
	PT2020CAPXVIR_VP3	LSDV FIELD			LSDV FIELD	LSDV FIELD	NEG	LSDV FIELD
	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
Information	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 / kit:	MagAttract 96 CADOR Pathogen Kit			none	DNA Mini Kit	PrepFiler Express Forensic DNA Extraction Kit	PrepFiler Express Forensic DNA Extraction Kit
	In-house modifications to extraction protocol (if yes, which?):	Proteinase treatment at 72 °C / Addition of carrier ARN			AB7500 Fast	no	N/A	N/A
	PCR Instrument used:	Verity (Applied Biosystems)			n/a		Rotor-Gene 6000	Rotor-Gene 6000
	Cut-off for positive:	Clear band at the right height and with the expected intensity + blasting in the NCBI database				Rotor-Gene		
	Methodology	Promega				Bernd Hoffman/QIAGEN	DIVA PCR	DIVA PCR
	Ref Methodology	Taq Platinium				QuantiTect Multiplex PCR NoROX Kit		
	Remark(s):							

value	sample	Labnr 97630	Labnr 97631	Labnr 97632	Labnr 97633	Labnr 97634	Labnr 97636
raw data	PT2020CAPXVIR_VP1	No ct	0		30,65		
	PT2020CAPXVIR_VP2	No ct	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
	PT2020CAPXVIR_TP3	30,18	27,45	28,9	29,13	23,13	23,13
	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 ct	0	21,5			
	PT2020CAPXVIR_VN1	No ct	0	No Ct			
	PT2020CAPXVIR_TN1	No ct	0	No Ct	23,88		
Final results	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
	PT2020CAPXVIR_TP2	LSDV FIELD	LSDV FIELD	LSDV FIELD	LSDV FIELD	LSDV FIELD	LSDV FIELD
	PT2020CAPXVIR_TP3	LSDV VACCINE	LSDV VACCINE	LSDV VACCINE	LSDV VACCINE	LSDV VACCINE	LSDV VACCINE
	PT2020CAPXVIR_VP3	LSDV FIELD	LSDV FIELD	LSDV FIELD	LSDV FIELD	LSDV FIELD	LSDV FIELD
	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
Information	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 / kit:			Viral Nucleic Acid Extraction Kit	Dna mini kit	Magvet universal kit	IndiSpin Pathogen Kit
	In-house modifications to extraction protocol (if yes, which?):				N/A		
	PCR Instrument used:	Smartcycler II Cepheid	AS7500		rotor gene	AriaMx	Rotor Gene 3000
	Cut-off for positive:		38		up tp 35 cycles	40	
	Methodology	ID GENE LSD DIVA Triplex			FGBI ARRIAH		
	Ref Methodology				A real time PCR kit for indentification of capripoxvirus DNA		
	Remark(s):						

Annex 4: Raw ELISA data

value	sample	Labnr 97506	97600	97602	97604	97605	97607	97608	97609	97610	97611	97612	97613	97614
OD	positive kit control (mean)	1,054	0,896	0,83	0,863	1,022	0,807	0,739	0,039	1,07	0,836	0,849	0,844	1,011
	negative kit control (mean)	0,051	0,045	0,047	0,057	0,063	0,045	0,057	0,068	0,047	0,058	0,052	0,052	0,05
	PT2020CAPXSER_SERP1	0,592	0,615	0,571	0,522	0,667	0,466	0,575	0,551	0,606	0,512	0,601	0,591	0,727
	PT2020CAPXSER_SERP2	0,8	0,718	0,701	0,817	0,669	0,584	0,799	0,802	0,688	0,645	0,69	0,689	0,769
	PT2020CAPXSER_SERP3	1,167	1,095	0,985	0,983	1,105	0,879	0,994	1,091	1,084	0,874	1,099	1,005	1,182
	PT2020CAPXSER_SERP4	1,177	0,986	1,021	0,988	1,024	0,778	0,721	0,985	1,01	0,798	0,957	0,993	1,09
	PT2020CAPXSER_SERP5	2,349	1,801	1,678	1,911	1,812	1,563	0,572	1,992	2,067	1,734	1,656	1,888	2,281
	PT2020CAPXSER_SERP6	2,196	1,72	1,603	1,927	1,753	1,432	0,77	1,965	2,012	1,706	1,654	1,811	2,199
	PT2020CAPXSER_SERP7	0,994	0,762	0,707	0,96	0,801	0,692	0,965	0,924	0,807	0,764	0,754	0,827	0,98
	PT2020CAPXSER_SERP4	1,142	0,991	0,965	1,048	1,079	0,794	0,659	0,947	0,974	0,865	0,981	0,931	1,149
	PT2020CAPXSER_SERN1	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
	PT2020CAPXSER_SERN2	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
Normalized data	positive kit control (mean)		100		100%	100	100	100			100	100%		
	negative kit control (mean)		0		0%	0	0	0			0	0%		
	PT2020CAPXSER_SERP1	53,954	67	66,90	57%	63	55	76	59,37%	54,64	58,435	69%	68,02	70,484
	PT2020CAPXSER_SERP2	74,638	79	83,50	94%	63	71	108,9	88,51%	62,66	75,456	80%	80,48	74,857
	PT2020CAPXSER_SERP3	111,29	123	119,80	115%	109	109	137,4	122,11%	101,37	104,987	131%	120,39	117,855
	PT2020CAPXSER_SERP4	112,297	110	214,40	116%	100	96	97,4	109,81%	94,13	95,22	114%	118,85	108,277
	PT2020CAPXSER_SERP5	229,151	206	208,30	230%	182	199	75,6	226,64%	197,46	215,603	201%	231,96	232,275
	PT2020CAPXSER_SERP6	213,873	197	198,70	233%	176	182	104,6	223,51%	192,08	211,96	201%	222,18	223,738
	PT2020CAPXSER_SERP7	94,046	84	84,20	112%	77	85	133,1	102,73%	74,29	90,76	88%	97,92	96,825
	PT2020CAPXSER_SERP4	108,796	111	117,20	123%	106	98	88,3	105,40%	90,62	103,753	117%	111,04	114,42
	PT2020CAPXSER_SERN1	0,289	0	1,46	2%	0	1	-1,8	0,17%	0,68	0,472	-1%	-0,06	(-) 0,104
	PT2020CAPXSER_SERN2	0,319	0	0,19	1%	0	1	-1,8	0,47%	0,39	0,428	0%	-0,07	0,312
Final results	positive kit control (mean)		POS	POS		POS	POS	POS			POS	POS	POS	
	negative kit control (mean)		NEG	NEG		NEG	NEG	NEG			NEG	NEG	NEG	
	PT2020CAPXSER_SERP1	POS	POS	POS	POS	POS	POS	POS	POS	POS	POS	POS	POS	POS
	PT2020CAPXSER_SERP2	POS	POS	POS	POS	POS	POS	POS	POS	POS	POS	POS	POS	POS
	PT2020CAPXSER_SERP3	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_SERP5	POS	POS	POS	POS	POS	POS	POS	POS	POS	POS	POS	POS	POS
	PT2020CAPXSER_SERP6	POS	POS	POS	POS	POS	POS	POS	POS	POS	POS	POS	POS	POS
	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
Information	Name ELISA kit producer:	ID-VET	IDVet	ID Vet	IDvet	ID VET	IDVET	ID.vet	ID.vet	IDEXX	IDVet	IDVet	ID.Vet	ID.vet
	Name ELISA kit:	ID screen Capripox Double antigen	ID Screen Capripox Double Antigen Multispecies	ID Screen Capripox double antigen multispecies	ID Screen Capripox Double Antigen Multi-species	Capripox Double Antigen Multi-species	ID Screen Capripox Double antigen multi-species	ID Screen® Capripox Double Antigen	ID Screen Capripox Double Antigen Multi-species	IDScreen Capripox Double Antigen	ID Screen, Capripox Double Antigen Multi Species	ID Screen Capripox Double Antigen Multi-species	Capripox Double Antigen Multi-species	Capripox double antigen multi-species
	Short or long incubation protocol *(if applicable)* :		short			short		short			short		short	short
	Formula to calculate the normalized data:	S/P%= (OD(Sample)-OD(NC))/(OD(PC)-OD(NC))*100	S/P%=(ODSample-ODNC)/(ODPC-ODNC)x100	S/P%=[(Odsample-Odneg)/(Odpos-Odneg)]*100	(ODsample-ODNC)/(ODPC-ODNC)*100	S/P%=((ODsample-ODnc)/(ODpc-ODnc)) *100	S/P%= ((ODsample - OD NC) / (OD PC - OD NC))*100	S/P% = (OD Probe - OD nK)/(OD pK - OD nK) * 100	S/P%=(ODsample-ODnc)x100/(ODpc-ODnc)	S/P%=((ODs - ODn)/(ODp - ODn))*100	((OD Sample-OD Negative)/(OD Positive- OD Negative))*100	S/P%= [(Odsample-Odneg)/(Odpos-Odneg)]*100	S/P% = ((OD sample - OD NC) / (OD PC - OD NC) * 100	S/P % = (OD sample - OD NC) / (OD PC - OD NC) x 100
	Used Cut-off :	30%	NEG = S/P% <30%; POS = S/P%≥30%	30%	30%	S/P% <30% Negativ, S/P%> 30% Positiv	S/P% < 30% NEGATIVE	S/P%≥ 30% = Pos; S/P% < 30% = Neg	S/P%<30% negative, S/P%>=30% positive	S/P%>=30 Positive	>=30% positive, <30% negative	S/P%>=30% POS	POS, if S/P% >= 30, NEG, if S/P% < 30	30%
	Batch ELISA kit:	E83	E83-01	E83	E83	E83	G66	G66	E83	E83	Lot E83	E83	E83	E83

value	sample	Labnr 97615	97616	97617	97618	97619	97620	97621	97622	97623	97628
OD	positive kit control (mean)	0,795	0,856	0,775	1,131	0,988	0,767	0,919	0,950 / 0,928 / 0,937	0,986	0,882
	negative kit control (mean)	0,05	0,054	0,032	0,052	0,058	0,048	0,0495	0,053 / 0,061 / 0,050	0	0,052
	PT2020CAPXSER_SERP1	0,563	0,628	0,596	0,628	0,673	0,547	0,744	0,661 / 0,671 /0,645	0,612	0,588
	PT2020CAPXSER_SERP2	0,733	0,646	0,689	0,824	0,687	0,773	0,944	0,848 / 0,868 / 0,737	0,661	0,778
	PT2020CAPXSER_SERP3	1,024	0,992	0,986	1,118	1,089	1,051	1,288	1,192 / 1,133 / 1,158	1,107	1,085
	PT2020CAPXSER_SERP4	0,991	1,044	1,31	1,006	0,955	0,989	1,262	1,174 / 1,150 / 1,036	1,083	1,056
	PT2020CAPXSER_SERP5	1,8835	1,83	1,795	1,93	1,891	1,881	2,159	2,047 / 2,108 / 1,765	1,786	2,171
	PT2020CAPXSER_SERP6	1,845	1,717	1,836	1,765	1,693	1,774	2,042	1,947 / 1,986 / 1,705	1,655	2,109
	PT2020CAPXSER_SERP7	0,801	0,716	0,88	0,892	0,791	0,858	1,166	0,911 / 0,917 / 0,704	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	0,054 / 0,055 / 0,056	0,055	0,047
	PT2020CAPXSER_SERN2	0,049	0,054	0,071	0,055	0,071	0,048	0,054	0,056 / 0,055 / 0,056	0,055	0,049
Normalized data	positive kit control (mean)	N/A		100		100%	100	100	100 / 100 / 100	100	
	negative kit control (mean)	N/A		0		0%	0	0	0 / 0 / 0	0	
	PT2020CAPXSER_SERP1	68,792	72	75,9	S/P% = 53,38%	66,10%	69,47	80	68 / 70 / 67	59,58	64,5394
	PT2020CAPXSER_SERP2	91,611	74	88,4	S/P% = 71,54%	67,55%	100,9	103	89 / 93 / 77	64,92	87,4172
	PT2020CAPXSER_SERP3	130,738	117	128,4	S/P% = 98,79%	110,95%	139,56	142	127 / 124 / 125	113,05	124,383
	PT2020CAPXSER_SERP4	126,309	123	172	S/P% = 88,41%	96,45%	130,94	139	125 / 126 / 111	110,45	120,891
	PT2020CAPXSER_SERP5	246,107	222	237,3	S/P% = 174,05%	197,15%	255	243	222 / 236 / 193	186,15	255,148
	PT2020CAPXSER_SERP6	240,939	207	242,8	S/P% = 158,75%	175,90%	240,12	229	211 / 222 / 187	172,05	247,682
	PT2020CAPXSER_SERP7	100,805	83	114,1	S/P% = 77,85%	78,85%	112,72	128	96 / 99 / 74	76,24	93,9193
	PT2020CAPXSER_SERP4	126,913	121	128	S/P% = 94,25%	92,50%	130,38	144	118 / 120 / 108	110,45	120,65
	PT2020CAPXSER_SERN1	<0.001	0	-0,7	S/P% = 1,29%	-0,40%	0,9	1	0 / -1 / 1	-0,3877	0,769
	PT2020CAPXSER_SERN2	<0.001	0	5,2	S/P% = 0,28%	1,30%	0,067	1	0 / -1 / 1	-0,34475	0,808
Final results	positive kit control (mean)	POS	POS	POS		POS	POS	POS			
	negative kit control (mean)	NEG	NEG	NEG		NEG	NEG	NEG			
	PT2020CAPXSER_SERP1	POS	POS	POS	POS	POS	POS	POS	POS	POS	POS
	PT2020CAPXSER_SERP2	POS	POS	POS	POS	POS	POS	POS	POS	POS	POS
	PT2020CAPXSER_SERP3	POS	POS	POS	POS	POS	POS	POS	POS	POS	POS
	PT2020CAPXSER_SERP4	POS	POS	POS	POS	POS	POS	POS	POS	POS	POS
	PT2020CAPXSER_SERP5	POS	POS	POS	POS	POS	POS	POS	POS	POS	POS
	PT2020CAPXSER_SERP6	POS	POS	POS	POS	POS	POS	POS	POS	POS	POS
	PT2020CAPXSER_SERP7	POS	POS	POS	POS	POS	POS	POS	POS	POS	POS
	PT2020CAPXSER_SERP4	POS	POS	POS	POS	POS	POS	POS	POS	POS	POS
	PT2020CAPXSER_SERN1	NEG	NEG	NEG	NEG	NEG	NEG	NEG	NEG	NEG	NEG
	PT2020CAPXSER_SERN2	NEG	NEG	NEG	NEG	NEG	NEG	NEG	NEG	NEG	NEG
Information	Name ELISA kit producer:	ID Vet	IDVet	IDVet	ID.vet	ID vet	ID.vet	ID VET	ID-Vet	IDVet	Idvet France
	Name ELISA kit:	ID Screen Capripox Double Antigen Multi-species	ID Screen Capripox Double Antigen Multi-species	ID Screen Capripox Double Antigen Multi-species	ID Screen Capripox Double Antigen Multi-species/CPVD	ID Screen Capripox Double Antigen Multi-species	ID Screen Capripox double antigen Multi-species	ID Screen Capripox Double Antigen MULTI-SPECIES	ID Screen Capripox double antigen multi species	IDScreen Capripox double antigen multi-species	ID Screen Capripox Antigen Multi-species
	Short or long incubation protocol *(if applicable)* :	short		N/A							
	Formula to calculate the normalized data:	S/P% = ((OD Sampe - OD Neg Ctrl) / (OD Pos Ctrl - OD Neg Ctrl))x 100	S/P ratio = test sample OD - negative control OD/positive control OD - negative	S/P% = (OD sample-OD NC)/(OD PC-OD NC)x100	S/P% = (OD sample - OD negativ control/OD pozitiv control - OD negativ control) x 100	S/P % = (ODsample - OD NC / OD PC - OD NC) x 100	S/P%=(ODsample-ODnc)/(ODpc-ODnc)*100	((OD sample - OD NC)/(OD PC - OD NC)) *100	S/P % = (Odsample-Odnc)/(Odpc-Odnc)*100		S/P%=((OD sample - OD NC) / (OD PC -OD NC)) * 100
	Used Cut-off :	Positive >= to 30, Negative < 30	30%	0,254	NEGATIVE = S/P% < 30%.POSITIVE S/P%≥ 30%	30,00%	S/P greather or equal to 30%	POS >= 30%; NEG < 30%	S/P % <30%=negative; S/P % >= 30%=positive	30%	less than 30% are considered negative
	Batch ELISA kit:	E83	E83	E83	G66	Lot E83	E83	E83	E83	E89	???

value	sample	Labnr 97629	97630	97632	97633	97634	97635	97636
OD	**positive kit control (mean)**	0,882	0,697	0,789	0,981	0,845	0,755	1,13
	negative kit control (mean)	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
	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_SERN1	0,048	0,073	0,054	0,055	0,092	0,06	0,051
	PT2020CAPXSER_SERN2	0,048	0,049	0,054	0,057	0,108	0,063	0,054
Normalized data	**positive kit control (mean)**			100		100		
	negative kit control (mean)			0		0		
	PT2020CAPXSER_SERP1	58,2781	64,81	108,1	59,30%	72,618	82,3782235	72
	PT2020CAPXSER_SERP2	83,4437	56,2	105,2	70,50%	89,908	88,53868195	69
	PT2020CAPXSER_SERP3	117,279	112,53	164,5	117,40%	149,824	142,5501433	132
	PT2020CAPXSER_SERP4	112,824	103,99	153,7	112,30%	126,253	129,7994269	117
	PT2020CAPXSER_SERP5	237,568	188,95	246,7	182,70%	221,313	255,3008596	193
	PT2020CAPXSER_SERP6	230,223	186,62	237	170,00%	208,045	231,3753582	179
	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
Final results	**positive kit control (mean)**			POS		POS		
	negative kit control (mean)			NEG		NEG		
	PT2020CAPXSER_SERP1	POS	POS	POS	POS	POS	POS	POS
	PT2020CAPXSER_SERP2	POS	POS	POS	POS	POS	POS	POS
	PT2020CAPXSER_SERP3	POS	POS	POS	POS	POS	POS	POS
	PT2020CAPXSER_SERP4	POS	POS	POS	POS	POS	POS	POS
	PT2020CAPXSER_SERP5	POS	POS	POS	POS	POS	POS	POS
	PT2020CAPXSER_SERP6	POS	POS	POS	POS	POS	POS	POS
	PT2020CAPXSER_SERP7	POS	POS	POS	POS	POS	POS	POS
	PT2020CAPXSER_SERP4	POS	POS	POS	POS	POS	POS	POS
	PT2020CAPXSER_SERN1	NEG	NEG	NEG	NEG	NEG	NEG	NEG
	PT2020CAPXSER_SERN2	NEG	NEG	NEG	NEG	NEG	NEG	NEG
Information	**Name ELISA kit producer:**	Idvet France	ID.VET	ID Vet	IDVET	ID-VET	IDVet	ID vet
	Name ELISA kit:	ID Screen Capripox Antigen Multi-species	ID screen Capripox Double Antigen Multi-species ELISA	ID Screen Capripox Double Antigen Multi-species	ID Screen Capripox Double Antigen Multi-species	ID Screen Capripox double antigen Multispecies	ID Screen Double Antigen Multi Species	ID Screen Capripox Double Antigen Multi-species
	Short or long incubation protocol *(if applicable)* :	short		short				
	Formula to calculate the normalized data:	S/P%=((OD sample - OD NC) / (OD PC - OD NC)) * 100		As per kit instructions (kit manual)	S/P%= (ODS - OD (K-)/ OD (K+))x100%	S/P% = (ODSample - ODNc /ODPc - ODNc) x 100	S/P %= ODSample- ODNc /ODPc- ODNc x 100	S/P%= OD sample - OD neg control/OD pos control - ODneg control*100
	Used Cut-off :	S/P%: -less than 30% are considered negative	Sp% < 30% NEG; Sp% > 30% POS	30	S/P%<30% - negative, S/P%>30% - positive	40	S/P %≥ 30%	no
	Batch ELISA kit:	E83	E83	D15	E83	E83	E83	E 83

Annex 5: Raw VN1 with Antibody Titer data

value	sample	Labnr 97600	97612	97618	97623	97633
Antibody titer	**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
	PT2020CAPXSER_SERP3	60	1:160	1:40	1/4	1/64
	PT2020CAPXSER_SERP4	120	1:320	1:160	1/4	1/32
	PT2020CAPXSER_SERP5	120	1:320	1:160	1/3	1/64
	PT2020CAPXSER_SERP6	60	1:160	1:160	1/6	1/32
	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
Final results	**positive kit control (mean)**	POS	POS	POS		POS
	negative kit control (mean)	NEG	NEG	NEG		NEG
	PT2020CAPXSER_SERP1	POS	POS	POS	NEG	POS
	PT2020CAPXSER_SERP2	POS	POS	POS	POS	POS
	PT2020CAPXSER_SERP3	POS	POS	POS	NEG	POS
	PT2020CAPXSER_SERP4	POS	POS	POS	NEG	POS
	PT2020CAPXSER_SERP5	POS	POS	POS	NEG	POS
	PT2020CAPXSER_SERP6	POS	POS	POS	POS	POS
	PT2020CAPXSER_SERP7	POS	POS	POS	NEG	POS
	PT2020CAPXSER_SERP4	POS	POS	POS	POS	POS
	PT2020CAPXSER_SERN1	NEG	NEG	NEG	NEG	NEG
	PT2020CAPXSER_SERN2	NEG	NEG	NEG	NEG	NEG
Information	**Protocol/SOP used**	yes after OIE manual	In house protocol	Pirbright Protocol/IDAH SOP 39"Serum neutralization test for the detection of Capripoxvirus	NVR-SOP-42	Home method
	Name (+Reference) cell type used	ESH	OA3.Ts (ATCC CRL-6546)	OA3Ts	MDBK	LSDV "Dagestan/2015"
	Starting dilution of PT serum samples tested:	5	01:05	01:05	43862	0,5
	Virus 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	VN83	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	
	Dilutions of PT serum 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

value	sample	Labnr 97506	97618
Antibody titer	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
	PT2020CAPXSER_SERP3	300-50	1:50 and 1:300
	PT2020CAPXSER_SERP4	300-300	1:50 and 1:300
	PT2020CAPXSER_SERP5	300-300	1:50 and 1:300
	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
Final results	positive kit control (mean)		POS
	negative kit control (mean)		NEG
	PT2020CAPXSER_SERP1	POS	POS
	PT2020CAPXSER_SERP2	POS	POS
	PT2020CAPXSER_SERP3	POS	POS
	PT2020CAPXSER_SERP4	POS	POS
	PT2020CAPXSER_SERP5	POS	POS
	PT2020CAPXSER_SERP6	POS	POS
	PT2020CAPXSER_SERP7	POS	POS
	PT2020CAPXSER_SERP4	POS	POS
	PT2020CAPXSER_SERN1	NEG	NEG
	PT2020CAPXSER_SERN2	NEG	NEG
Information	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
	Virus dose used in test (TCID50): e.g. 100 TCID50	100TCID50	100 TCID50
	Positive control serum used:	R6F 45dpi (LSDV) and S6F (SPPV)	Antiserum EURL Capripox
	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.

Table A1. Repeatability of the SERP4 sample per laboratory for the ELISA data.

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%

The data were also represented as boxplots (figure A1).

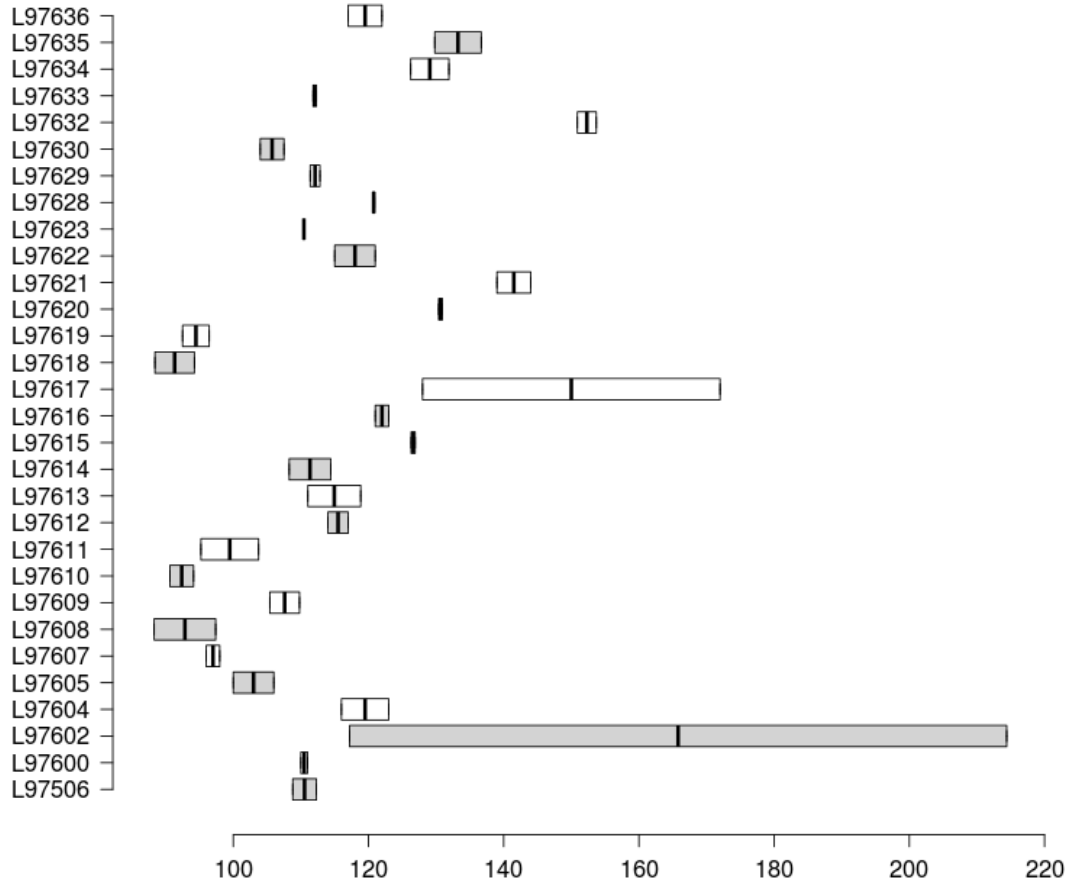
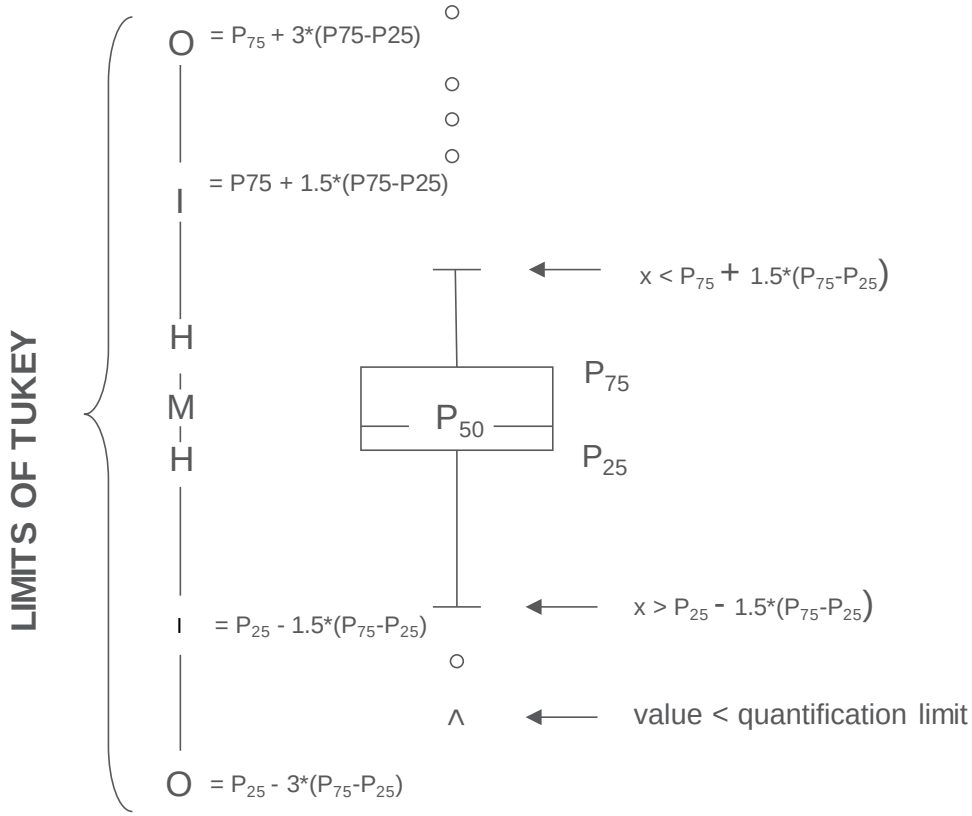


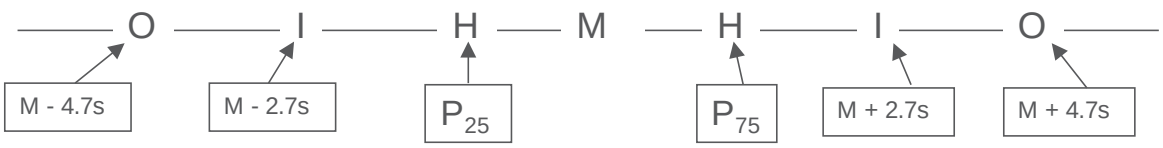
Figure A1. Representation in boxplot of the dispersion of the ELISA value for PT2020CPAXSER_P4.

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





Corresponding limits in case of normal distribution

END