

**EXPERTISE AND SERVICE PROVISION  
QUALITY OF LABORATORIES**

**EXTERNAL QUALITY ASSESSMENT  
IN VETERINARY DIAGNOSIS**

**FINAL GLOBAL REPORT**

**Proficiency Testing in Veterinary Diagnosis  
Capripox virus  
Serology and Virology  
SURVEY 2021/4**

**Sciensano/PT VET Capripox/3-E**

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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'. 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 ruminants (Serology component of the PT: PT2021CAPXSER) and/or to assess the ability of the participating laboratories to detect capripox (CAPX) virus DNA in different matrices (Virology component of the PT: PT2021CAPXVIR).

## **III. Materials and methods**

### **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, blood and tissue homogenate samples using their primary diagnostic assay(s) for molecular diagnosis of capripox virus infection. Furthermore, within this component, participants could submit additional results on capripox virus species differentiation and field or vaccine strain differentiation. The procedures for the assays must be fully described in the SOPs of the participating laboratories.

All participants received a supporting document containing a background of the samples (species from which the sample is taken and type of sample).

### **2. Reference samples**

Twenty-eight laboratories received the PT2021CAPXSER panel containing 10 aliquots of serum and the PT2021CAPXVIR panel containing 10 aliquots of cell culture supernatant, blood or tissue homogenate samples. One NRL received the PT2021CAPXSER panel and 6 NRLs received the PT2021CAPXVIR 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, Scientific Directorate Infectious Diseases in Animals, Sciensano.

#### **2.1 PT2021CAPXVIR panel : Reference cell culture supernatant, blood and tissue homogenate samples**

##### **Origin of the samples**

Replicates of 3 reference cell culture supernatants containing detectable capripox virus DNA (n = 3; coded PT2021CAPXVIR\_VP1, PT2021CAPXVIR\_VP2 and PT2021CAPXVIR\_VP3) were used as well as replicates of 2 reference blood samples, either free from detectable capripox virus DNA (n = 1; coded PT2021CAPXVIR\_BN1) or containing detectable capripox virus DNA (n = 1; coded PT2021CAPXVIR\_BP1). The remaining 5 reference samples were tissue homogenate samples, either free from detectable capripox virus DNA (n=1; coded PT2021CAPXVIR\_TN1) or containing detectable capripox virus DNA (n=2; coded PT2021CAPXVIR\_TP1 and PT2021CAPXVIR\_TP2). PT2021CAPXVIR\_TP1 and PT2021CAPXVIR\_TP2 were both represented in the panel twice.

In total, 350 aliquots were distributed to 35 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, the status was determined based on the background of the sample and the results obtained during pre-verification, hereby using the real-time PCR for Capripox D5R (Haegeman et al. 2013) and DIVA tests (Agianniotaki et al. 2016; Haegeman et al. 2016; Chibssa et al. 2018).

Table 1: The origin of the samples in the PT2021CAPXVIR panel

Sample ID	Origin	Background	Strain	Status
PT2021CAPXVIR_VP1	Cell culture	GTPV culture	Gorgon Field strain	Capx Positive GTPV Field strain
PT2021CAPXVIR_VP2	Cell culture	LSDV Culture	Bulgarian field strain	Capx Positive LSDV Field strain
PT2021CAPXVIR_VP3	Cell culture	SPPV culture	Kenyan field strain	Capx Positive SPPV Field strain
PT2021CAPXVIR_BP1	Blood	LSDV spiked blood	Israeli field strain	Capx Positive LSDV Field strain
PT2021CAPXVIR_TP1	Bovine tissue	LSDV infected cow	Israeli field strain	Capx Positive LSDV Field strain
PT2021CAPXVIR_TP2	Ovine tissue	SPPV infected sheep	Moroccan field strain	Capx Positive SPPV Field strain
PT2021CAPXVIR_BN1	Blood	Uninfected and Unvaccinated	N/A	Capx negative
PT2021CAPXVIR_TN1	Bovine tissue	Uninfected and Unvaccinated	N/A	Capx negative

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 DNA. Moreover, 3 additional aliquots of each reference sample were tested once the PT deadline has passed using the real-time PCR for capripox D5R (Haegeman et al., 2013) in order to confirm the stability and status of the samples (post-PT verification).

For the **detection of capripox virus DNA**, the samples PT2021CAPXVIR\_TN1 and PT2021CAPXVIR\_BN1 were considered as capripox virus negative samples and the samples PT2021CAPXVIR\_VP1, PT2021CAPXVIR\_VP2, PT2021CAPXVIR\_VP3, PT2021CAPXVIR\_TP1, PT2021CAPXVIR\_TP2, and PT2021 CAPXVIR\_BP1 as positive samples. For samples PT2021CAPXVIR\_VP1, PT2021CAPXVIR\_VP2 and PT2021CAPXVIR\_VP3, it is possible that no value could be obtained for the internal control in the real-time PCR due to its origin (cell culture medium). Therefore in addition to a positive result, a non-interpretable (doubtful) result was also accepted.

For the **capripox virus species differentiation**, the samples PT2021CAPXVIR\_TN1 and PT2021CAPXVIR\_BN1 were considered negative samples, the sample PT2021CAPXVIR\_VP3 and PT2021CAPXVIR\_TP2 as SPPV positive samples (where SPPV or SPPV/GTPV results were considered acceptable), the samples PT2021CAPXVIR\_VP2, PT2021CAPXVIR\_TP1 and PT2021CAPXVIR\_BP1 as LSDV positive samples (where LSDV or GTPV/LSDV results were considered acceptable) and sample PT2021CAPXVIR\_VP1 as GTPV positive samples (SPPV/GTPV or GTPV/LSDV were considered acceptable).

Finally, for the **field or vaccine strain differentiation**, the samples PT2021CAPXVIR\_TN1 and PT2021CAPXVIR\_BN1 were considered as negative samples, the samples PT2021CAPXVIR\_VP2, PT2021CAPXVIR\_VP3, PT2021CAPXVIR\_TP1, PT2021CAPXVIR\_TP2, and PT2021 CAPXVIR\_BP1 as field strains. For PT2021CAPXVIR\_VP1, which contained GTPX DNA, no DIVA test is available and therefore 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 capripox viruses, based on the pre-PT verification.

Table 2: The final status of each sample in the PT2021CAPXVIR panel

Sample ID	Final status pan-capripox	Final status Species differentiation	Final status DIVA	Final diagnostic
PT2021CAPXVIR_VP1	Positive	GTPV	GTPV field type	Capx Positive GTPV (Field) type strain
PT2021CAPXVIR_VP2	Positive	LSDV	LSDV field type	Capx Positive LSDV Field type strain
PT2021CAPXVIR_VP3	Positive	SPPV	SPPV field type	Capx Positive SPPV Field type strain
PT2021CAPXVIR_BP1	Positive	LSDV	LSDV field type	Capx Positive LSDV Field type strain
PT2021CAPXVIR_TP1	Positive	LSDV	LSDV field type	Capx Positive LSDV Field type strain
PT2021CAPXVIR_TP2	Positive	SPPV	SPPV field type	Capx Positive SPPV Field type strain
PT2021CAPXVIR_BN1	Negative	Negative	Negative	Capx Negative
PT2021CAPXVIR_TN1	Negative	Negative	Negative	Capx Negative

## Randomization and panel composition

The samples were randomized differently for each laboratory, an overview of the randomization can be found in the preliminary report.

The PTCAPXVIR2021 panel was constituted of 10 samples of 600 µl.

## Stability

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

### **2.2 PT2021CAPXSER panel : Reference serum samples**

Replicates of 10 reference serum samples, either free from detectable antibodies to capripox viruses (n=2; coded PT2021CAPXSER\_SERN1 and PT2021CAPXSER\_SERN2) or containing detectable antibodies to capripox viruses (n=8; coded PT2021CAPXSER\_SERP1, PT2021CAPXSER\_SERP2, PT2021CAPXSER\_SERP3, PT2021CAPXSER\_SERP4, PT2021CAPXSER\_SERP5, PT2021CAPXSER\_SERP6, PT2021CAPXSER\_SERP7 and PT2021CAPXSER\_SERP8) were used. In total, 290 aliquots were distributed to 29 participating laboratories. PT2021CAPXSER\_SERP7 and PT2021CAPXSER\_SERP8 were dilutions of respectively 1/2 and 1/5 of PT2021CAPXSER\_SERP6. The participants received 10 aliquots: 1 aliquot from each serum reference sample. The serum samples were randomized for each participant.

For each serum sample, the status was determined based on the background of the animals from which the samples originated and the results obtained during pre-verification, hereby using the ELISA ID Screen® Capripox Double Antigen Multi-species (ID.Vet), the immunoperoxidase monolayer assay (IPMA)

(Haegeman et al. 2020) and the virus neutralization test with the serum titrated against a constant titer of capripox virus (VN).

Table 3: Origin of the samples in the PTCAPX2021 panel

Sample ID	Origin	Background	Status
PT2021CAPXSER_SERP1	Ovine	SPPV Infected	Positive
PT2021CAPXSER_SERP2	Bovine	LSDV Infected	Positive
PT2021CAPXSER_SERP3	Ovine	SPPV Infected	Positive
PT2021CAPXSER_SERP4	Ovine	Vaccinated + SPPV infected	Positive
PT2021CAPXSER_SERP5	Bovine	Vaccinated + LSDV infected	Positive
PT2021CAPXSER_SERP6	Bovine	LSDV Infected	Positive
PT2021CAPXSER_SERP7	Bovine	LSDV Infected	Positive
PT2021CAPXSER_SERP8	Bovine	LSDV Infected	Positive
PT2021CAPXSER_SERN1	Ovine	Commercial serum	Negative
PT2021CAPXSER_SERN2	Bovine	Commercial serum	Negative

After aliquoting the different reference serum samples, a homogeneity check was performed on 10 aliquots of each sample using the ELISA ID Screen® Capripox Double Antigen Multi-species (ID.Vet), IPMA and VN. For each sample, the same qualitative result was obtained for all 10 aliquots of the same reference serum sample for each test. When performing IPMA with heterologous virus by the EURL, a difference in status was found for samples PT2021CAPXSER\_SERP1, PT2021CAPXSER\_SERP3 and PT2021CAPXSER\_SERP4. The IPMA status of these samples was therefore considered as positive or doubtful. All serum samples were considered as reliable samples to evaluate the ability of laboratories to identify the absence or presence of antibodies to capripox viruses in serum. In addition, 3 more aliquots of each serum sample were tested after the PT in order to confirm their stability and status (post PT verification) using the ELISA ID Screen® Capripox Double Antigen Multi-species (ID.Vet), IPMA and VN.

The reference serum samples PT2021CAPXSER\_SERN1 and PT2021CAPXSER\_SERN2 were considered as negative samples, and the reference serum samples, PT2021CAPXSER\_SERP2, PT2021CAPXSER\_SERP5, PT2021CAPXSER\_SERP6, PT2021CAPXSER\_SERP7 and PT2021CAPXSER\_SERP8 as positive samples. The reference serum PT2021CAPXSER\_SERP1, PT2021CAPXSER\_SERP3 and PT2021CAPXSER\_SERP4 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. 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 capripox viruses using the pre-PT results.

Table 4: Final status of each sample in the PTCAPX2021 panel

Sample ID	Status
PT2021CAPXSER_SERP1	Positive
PT2021CAPXSER_SERP2	Positive
PT2021CAPXSER_SERP3	Positive
PT2021CAPXSER_SERP4	Positive
PT2021CAPXSER_SERP5	Positive
PT2021CAPXSER_SERP6	Positive
PT2021CAPXSER_SERP7	Positive
PT2021CAPXSER_SERP8	Positive
PT2021CAPXSER_SERN1	Negative
PT2021CAPXSER_SERN2	Negative

## Randomization and panel composition

The samples were randomized differently for each laboratory, an overview of the randomization can be found in the preliminary report.

The PTCAPXSER2021 panel was constituted of 10 samples of 500µl.

## Stability

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

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

#### **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.

#### **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.

#### **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

### 1. The participants

Twenty-four NRL's of European Union Member states and 10 laboratories from third countries and 1 private laboratory participated in the capripox virus proficiency test.

Table 5: NRL's from EU member states

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	Statens Serum Institut	1	1
Finland	Finnish Food Authority, Virology Unit	0	1
France	LNR poxviroses des ruminants, UMR Cirad-Inra ASTRE, "Ani ma, santé, Terri toires, 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 molecular 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 6: the non EU member states participants

Country	Name	Participation in serology survey	Participation in virology survey
Albania	Food Safety and Veterinary Insitute, Dep of Animal Health, Molecular Biology	0	1
Belarus	Belarusian State Veterinary Centre	0	1
Georgia	Laboratory of the Ministry of Agriculture (LMA) of Georgia	0	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
South-Korea	Foreign Animal Disease Division Animal and Plant Quarantine Agency	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

## **2. Survey timeline**

Transfer of the samples from NRL to QL: 19/04/2021

Randomization of the samples by QL: from 20/04 to 30/04

Sending samples to participants: from 04/05/2021 to 11/05/2021. The samples were sent on dry ice.

Deadline for the results encoding: 14/06/2021

Preliminary report: 06/09/2021

The preliminary report is available at:

[https://www.wiv-isp.be/QML/activities/PT%20VET/fr/originaux/rapports\\_annee.htm](https://www.wiv-isp.be/QML/activities/PT%20VET/fr/originaux/rapports_annee.htm)

Final report: 30/09/2021

## **3. Compliance with the procedure**

All participating laboratories have provided a duly dated copy of the results, except laboratory 97637, who did not report results for the PT2021.

## 4. Qualitative data analysis

### 4.1 Virology

#### 4.1.1 Pan-capripox real-time PCR

##### Results per sample

Thirty-three laboratories encoded results, 30 laboratories encoded 1 dataset and 3 laboratories encoded 2 dataset. In total, 36 datasets were encoded. Raw data can be found in Annex 1.

Table 7: Results per sample

Sample ID	Repetition	Expected results	Positive	Negative	ND	Status*
PT2021CAPXVIR_VP1	1	Positive	35	0	1	Frequently detected
PT2021CAPXVIR_VP2	1	Positive	35	1	0	Frequently detected
PT2021CAPXVIR_VP3	1	Positive	35	0	1	Frequently detected
PT2021CAPXVIR_BP1	1	Positive	36	0	0	Frequently detected
PT2021CAPXVIR_TP1	2	Positive	72	0	0	Frequently detected
PT2021CAPXVIR_TP2	2	Positive	70	0	2	Frequently detected
PT2021CAPXVIR_BN1	1	Negative	36	0	0	Negative
PT2021CAPXVIR_TN1	1	Negative	36	0	0	Negative

ND: Not done

\*: 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](http://www.qcmd.org)).

##### Summary of the results

Table 8: summary of the results

Parameter	N	%
Number of results	360	100
Number of correct results	355	98.6
False positive	0	0
False negative	1	0.3
ND	4	1.1

ND : Not Done

## Methods used

Table 9: Proficiency per method

Kit or reference	Target gene	N	NR	NCR	FN	ND
In-house	other	2	20	20	0	0
Bio-T kit Lumpy Skin Disease (BioSellal)	UD	1	10	6	0	4
Bowden et al., 2008.	P32	16	160	160	0	0
ID.VET - ID GENE® CAPRIPOX VIRUS TRIPLEX (ID.VET)	UD	2	20	20	0	0
Haegeman et al. 2013	D5R/E3L	4	40	40	0	0
In house	D5R	1	10	10	0	0
Babiuk et al 2008	P32	1	10	10	0	0
Bowden et al. 2008; Babiuk et al. 2008; SOP from Pirbright	P32	1	10	10	0	0
In house	P32	2	20	19	1	0
Bowden et al. 2008 Stubbs et al. 2012	P32	1	10	10	0	0
In house	RPO30	1	10	10	0	0
UD	UD	1	10	10	0	0
Wolff et al. 2021,	P32	1	10	10	0	0
Path-ID (Life Technologies)	P32	1	10	10	0	0
Balinsky et al., 2008	ORF 068	1	10	10	0	0
<b>Total</b>		<b>36</b>	<b>360</b>	<b>355</b>	<b>1</b>	<b>4</b>

N: number of laboratories; NR: number of results; NCR: Number of correct results; FN: false negative; ND: Not done UD: Undisclosed

## Results per laboratory

For the detection of capripox virus DNA) in the PT panel : 32 out of 33 participating laboratories provided qualitative results that were in full agreement with the assigned status of the 10 reference samples (100% of agreement), whereas LAB97604 misclassified 1 aliquot (90% of agreement).

Among the 3 laboratories that performed a second optional RT-PCR, 2 NRLs (LAB97605 and LAB97607) were in full agreement (100% agreement) with the assigned status of the 10 reference samples. One laboratory (LAB97612) classified only 6 out of 10 samples and these were in full agreement with the assigned status of the samples.

Table 10: Results per laboratory

Laboratory	VP1	VP2	VP3	BP1	TP1 (rep 1)	TP1 (Rep 2)	TP2 (rep 1)	TP2 (Rep 2)	BN1	TN1	NCR/ NR%
	Pos	Pos	Pos	Pos	Pos	Pos	Pos	Pos	Neg	Neg	
97506	Pos	Pos	Pos	Pos	Pos	Pos	Pos	Pos	Neg	Neg	10/10 100
97600	Pos	Pos	Pos	Pos	Pos	Pos	Pos	Pos	Neg	Neg	10/10 100
97601	Pos	Pos	Pos	Pos	Pos	Pos	Pos	Pos	Neg	Neg	10/10 100
97602	Pos	Pos	Pos	Pos	Pos	Pos	Pos	Pos	Neg	Neg	10/10 100
97603	Pos	Pos	Pos	Pos	Pos	Pos	Pos	Pos	Neg	Neg	10/10 100

97604	Pos	Neg	Pos	Pos	Pos	Pos	Pos	Pos	Neg	Neg	9/10 90
97605 (1)	Pos	Neg	Pos	Pos	Pos	Pos	Pos	Pos	Neg	Neg	10/10 100
97605 (2)	Pos	Neg	Pos	Pos	Pos	Pos	Pos	Pos	Neg	Neg	10/10 100
97606	Pos	Pos	Pos	Pos	Pos	Pos	Pos	Pos	Neg	Neg	10/10 100
97607 (1)	Pos	Pos	Pos	Pos	Pos	Pos	Pos	Pos	Neg	Neg	10/10 100
97607 (2)	Pos	Pos	Pos	Pos	Pos	Pos	Pos	Pos	Neg	Neg	10/10 100
97608	Pos	Pos	Pos	Pos	Pos	Pos	Pos	Pos	Neg	Neg	10/10 100
97609	Pos	Pos	Pos	Pos	Pos	Pos	Pos	Pos	Neg	Neg	10/10 100
97610	Pos	Pos	Pos	Pos	Pos	Pos	Pos	Pos	Neg	Neg	10/10 100
97611	Pos	Pos	Pos	Pos	Pos	Pos	Pos	Pos	Neg	Neg	10/10 100
97612 (1)	Pos	Pos	Pos	Pos	Pos	Pos	Pos	Pos	Neg	Neg	10/10 100
97612 (2)	ND	Pos	ND	Pos	Pos	Pos	ND	ND	Neg	Neg	6/6 100
97613	Pos	Pos	Pos	Pos	Pos	Pos	Pos	Pos	Neg	Neg	10/10 100
97614	Pos	Pos	Pos	Pos	Pos	Pos	Pos	Pos	Neg	Neg	10/10 100
97616	Pos	Pos	Pos	Pos	Pos	Pos	Pos	Pos	Neg	Neg	10/10 100
97617	Pos	Pos	Pos	Pos	Pos	Pos	Pos	Pos	Neg	Neg	10/10 100
97618	Pos	Pos	Pos	Pos	Pos	Pos	Pos	Pos	Neg	Neg	10/10 100
97619	Pos	Pos	Pos	Pos	Pos	Pos	Pos	Pos	Neg	Neg	10/10 100
97620	Pos	Pos	Pos	Pos	Pos	Pos	Pos	Pos	Neg	Neg	10/10 100
97621	Pos	Pos	Pos	Pos	Pos	Pos	Pos	Pos	Neg	Neg	10/10 100
97622	Pos	Pos	Pos	Pos	Pos	Pos	Pos	Pos	Neg	Neg	10/10 100
97624	Pos	Pos	Pos	Pos	Pos	Pos	Pos	Pos	Neg	Neg	10/10 100
97625	Pos	Pos	Pos	Pos	Pos	Pos	Pos	Pos	Neg	Neg	10/10 100
97627	Pos	Pos	Pos	Pos	Pos	Pos	Pos	Pos	Neg	Neg	10/10 100
97630	Pos	Pos	Pos	Pos	Pos	Pos	Pos	Pos	Neg	Neg	10/10 100
97631	Pos	Pos	Pos	Pos	Pos	Pos	Pos	Pos	Neg	Neg	10/10 100
97632	Pos	Pos	Pos	Pos	Pos	Pos	Pos	Pos	Neg	Neg	10/10 100
97633	Pos	Pos	Pos	Pos	Pos	Pos	Pos	Pos	Neg	Neg	10/10 100
97634	Pos	Pos	Pos	Pos	Pos	Pos	Pos	Pos	Neg	Neg	10/10 100

97638	Pos	Pos	Pos	Pos	Pos	Pos	Pos	Pos	Neg	Neg	10/10 100
97642	Pos	Pos	Pos	Pos	Pos	Pos	Pos	Pos	Neg	Neg	10/10 100

Pos: positive; Neg: negative; NCR: Number correct results; NR: number of results ND: Not Done; %: percentage success

#### 4.1.2 Species differentiation

##### Results per sample

Twenty-three laboratories encoded results for the species differentiation. Out of 218 encoded results, 215 were considered as successful by the EURL for diseases caused by Capripox viruses (98.6%). Raw data can be found in Annex 1.

Table 11: results per sample for species differentiation

Sample ID	# in panel	Expected results	GTPV	GTPV/ LSDV	LSDV	SPPV	Neg	ND	NR	%
PT2021CAPXVIR_VP1	1	GTPV	17	4	1	0	0	1	23	91.3
PT2021CAPXVIR_VP2	1	LSDV	0	5	15	1	1	1	23	86.9
PT2021CAPXVIR_VP3	1	SPPV	0	0	0	22	0	1	23	95.6
PT2021CAPXVIR_BP1	1	LSDV	0	5	16	0	0	2	23	91.3
PT2021CAPXVIR_TP1	2	LSDV	0	10	32	0	0	4	46	91.3
PT2021CAPXVIR_TP2	2	SPPV	0	0	0	44	0	2	46	95.6
PT2021CAPXVIR_BN1	1	Negative	0	0	0	0	23	0	23	100
PT2021CAPXVIR_TN1	1	Negative	0	0	0	0	23	0	23	100

ND: Not determined; NR: number of results; %: percentage success

##### Results per method

Table 12: Proficiency per method for species differentiation

Protocol	Method	N	NR	NCR	%
Agianniotaki et al. 2016	RT-qPCR	1	10	10	100
UD	PCR + sequencing	1	10	9	90
UD	RT-qPCR	1	6	6	100
Wolf et al. 2021	RT-qPCR	1	10	10	100
Lamien et al. 2010	PCR + gel	1	6	6	100
Gelaye et al., 2015	Sequencing	2	16	16	100
Gelaye et al 2017	RT-qPCR	1	10	10	100
Lamien et al 2011	RT-qPCR	8	80	79	98.7
In-house	RT-qPCR	3	30	29	96.6
Vidanovic et al. 2016	RT-qPCR	1	10	10	100
Vidanovic et al (unpublished) Lamien et al. 2011 Gelaye et al., 2015	RT-qPCR	1	10	10	100
ID gene LSD DIVA triplex LSDV (ID.VET)	UD	1	10	10	100
Batra et al., 2014	PCR + sequencing	1	10	10	100
<b>Total</b>		<b>23</b>	<b>218</b>	<b>215</b>	<b>98.6</b>

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



## Results per laboratory

For the differentiation of capripox virus species: Eighteen out of 23 participating laboratories provided qualitative results that were in full agreement with the assigned status of the 10 reference samples (100% of agreement), whereas LAB97604, LAB97613 and LAB97614 misclassified 1 aliquot (90% of agreement). LAB97614 already misclassified this aliquot in their primary PCR. LAB97618 did the analysis of 7 out of 10 samples and LAB97607 and LAB97613 did the analysis on 6 out of 10 samples. For the samples tested, these laboratories provided qualitative results that were in full agreement with the assigned status of the samples.

Table 13: Result of the species differentiation per participating laboratory

Laboratory	VP1	VP2	VP3	BP1	TP1 (rep 1)	TP1 (Rep 2)	TP2 (rep 1)	TP2 (Rep 2)	BN1	TN1	% (NCR/ NR)
	GTPV	LSDV	SPPV	LSDV	LSDV	LSDV	SPPV	SPPV	Neg	Neg	
97506	GTPV	LSDV	SPPV	LSDV	LSDV	LSDV	SPPV	SPPV	Neg	Neg	100 (10/10)
97600	GTPV	LSDV	SPPV	LSDV	LSDV	LSDV	SPPV	SPPV	Neg	Neg	100 (10/10)
97602	GTPV	LSDV	SPPV	LSDV	LSDV	LSDV	SPPV	SPPV	Neg	Neg	100 (10/10)
97603	GTPV/ LSDV	GTPV/ LSDV	SPPV	GTPV/ LSDV	GTPV/ LSDV	GTPV/ LSDV	SPPV	SPPV	Neg	Neg	100 (10/10)
97604	GTPV	Neg	SPPV	LSDV	LSDV	LSDV	SPPV	SPPV	Neg	Neg	90 (9/10)
97607	ND	LSDV	ND	LSDV	LSDV	LSDV	ND	ND	Neg	Neg	100 (6/6)
97608	GTPV	LSDV	SPPV	LSDV	LSDV	LSDV	SPPV	SPPV	Neg	Neg	100 (10/10)
97609	GTPV	ND	SPPV	ND	ND	ND	SPPV	SPPV	Neg	Neg	100 (6/6)
97610	GTPV	LSDV	SPPV	LSDV	LSDV	LSDV	SPPV	SPPV	Neg	Neg	100 (10/10)
97611	GTPV	LSDV	SPPV	LSDV	LSDV	LSDV	SPPV	SPPV	Neg	Neg	100 (10/10)
97613	LSDV	LSDV	SPPV	LSDV	LSDV	LSDV	SPPV	SPPV	Neg	Neg	90 (9/10)
97614	GTPV	SPPV	SPPV	LSDV	LSDV	LSDV	SPPV	SPPV	Neg	Neg	90 (9/10)
97617	GTPV/ LSDV	GTPV/ LSDV	SPPV	GTPV/ LSDV	GTPV/ LSDV	GTPV/ LSDV	SPPV	SPPV	Neg	Neg	100 (10/10)
97618	GTPV	LSDV	SPPV	ND	ND	ND	SPPV	SPPV	Neg	Neg	100 (7/7)
97619	GTPV	LSDV	SPPV	LSDV	LSDV	LSDV	SPPV	SPPV	Neg	Neg	100 (10/10)
97620	GTPV/ LSDV	GTPV/ LSDV	SPPV	GTPV/ LSDV	GTPV/ LSDV	GTPV/ LSDV	SPPV	SPPV	Neg	Neg	100 (10/10)
97621	GTPV/ LSDV	GTPV/ LSDV	SPPV	GTPV/ LSDV	GTPV/ LSDV	GTPV/ LSDV	SPPV	SPPV	Neg	Neg	100 (10/10)
97625	GTPV	LSDV	SPPV	LSDV	LSDV	LSDV	SPPV	SPPV	Neg	Neg	100 (10/10)
97631	GTPV	LSDV	SPPV	LSDV	LSDV	LSDV	SPPV	SPPV	Neg	Neg	100 (10/10)
97632	GTPV	GTPV/ LSDV	SPPV	GTPV/ LSDV	GTPV/ LSDV	GTPV/ LSDV	SPPV	SPPV	Neg	Neg	100 (10/10)
97633	GTPV	LSDV	SPPV	LSDV	LSDV	LSDV	SPPV	SPPV	Neg	Neg	100 (10/10)
97634	GTPV	LSDV	SPPV	LSDV	LSDV	LSDV	SPPV	SPPV	Neg	Neg	100 (10/10)

97638	GTPV	LSDV	SPPV	LSDV	LSDV	LSDV	SPPV	SPPV	Neg	Neg	100 (10/10)
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ND: Not done; NCR: Number of correct results; NR: Number of results; %: Percentage succes.

### 4.1.3 DIVA PCR

In this section, laboratories could report whether the samples contained field type or vaccine type strains. Twenty-two laboratories encoded results. For sample PT2021CAPXVIR\_VP1 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. Raw data can be found in Annex 1.

#### Results per sample

Table 14: Result of the DIVA per sample

Sample ID	# in panel	Expected result	Encoded result
PT2021CAPXVIR_VP1	1	GTPV (field)	4 GTPV field 17 ND results 1 NI result
PT2021CAPXVIR_VP2	1	LSDV field	20 LSDV field results 1 ND Result <b>1 negative result</b>
PT2021CAPXVIR_VP3	1	SPV field	8 SPPV field results 14 ND results
PT2021CAPXVIR_BP1	1	LSDV field	21 LSDV Field results 1 ND result
PT2021CAPXVIR_TP1	2	LSDV field	42 LSDV Field results 2 ND result
PT2021CAPXVIR_TP2	2	SPPV field	14 SPPV field 28 ND results <b>2 SPPV vaccine results</b>
PT2021CAPXVIR_BN1	1	NEGATIVE	22 negative results
PT2021CAPXVIR_TN1	1	NEGATIVE	22 negative results

ND: Not done

#### Results per method

Table 15: Proficiency per method for DIVA

Method	N	NR	NCR	%
Vidanovic et al. 2016	1	7	7	100
UD	1	7	6	85.7
Wolf et al. 2021	1	7	7	100
Agiannotaki et al. 2017	1	10	10	100
Haegeman et al. 2015	1	10	8	80
Agianniotaki et al. 2017	4	28	28	100
Agianniotaki et al. 2017 and Chibssa et al. 2018	3	30	30	100
ID.VET - ID GENE® LSD DIVA TRIPLEX (ID.VET)	4	36	36	100
Chibssa et al. 2018	1	6	6	100
Hoffmann/Qiagen	1	10	10	100

Vidanovic et al. 2016	1	7	7	100
Moller et al 2019	1	7	7	100
Vidanovic et al. 2021				
Vidanovic et al. 2021	1	7	7	100
UD	1	10	10	100
<b>Total</b>	<b>22</b>	<b>182</b>	<b>179</b>	<b>98.4</b>

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

### Results per laboratory

For the differentiation between capripox virus field and vaccine strain: Twenty out of twenty-two participating laboratories provided qualitative results that were in full agreement with the assigned status of the samples they analyzed (100% of agreement). Six laboratories (LAB97506, LAB97600, LAB97609, LAB97613, LAB97625 and LAB97633) analysed all 10 samples. Thirteen laboratories analysed 7 samples (LAB97602, LAB97608, LAB97611, LAB97612, LAB97618, LAB97620, LAB97621, LAB97630, LAB97631, LAB97632, LAB97634, LAB97638 and LAB97642) and did not analyse the SPPV (and GTPV) samples. One other laboratory did not analyse the LSDV samples (LAB97617) and analysed a total of 6 samples.

One laboratories (LAB97604) analyzed the LSDV samples and negative samples and misclassified 1 (PTCAPXVIR2021\_VP2) of the LSDV samples, resulting in a level of agreement of 85%. One laboratory (LAB97610) analyzed all aliquots and misclassified PTCAPXVIR2021\_TP1 and PTCAPXVIR2021\_TP2 (replicates), resulting in a level of agreement of 80%.

All results for PTCAPXVIR2021 were considered correct, since no DIVA test is available for GTPV at the moment.

Table 16: Result of the DIVA per participating laboratory

Laboratory	VP1	VP2	VP3	BP1	TP1 (rep 1)	TP1 (Rep 2)	TP2 (rep 1)	TP2 (Rep 2)	BN1	TN1	% (NCR/ NDR)
	GTPV/ ND	LSDV Field	SPPV Field	LSDV Field	LSDV Field	LSDV Field	SPPV Field	SPPV Field	Neg	Neg	
97506	GTPV	LSDV Field	SPPV Field	LSDV Field	LSDV Field	LSDV Field	SPPV Field	SPPV Field	Neg	Neg	100 (10/10)
97600	ND	LSDV Field	SPPV Field	LSDV Field	LSDV Field	LSDV Field	SPPV Field	SPPV Field	Neg	Neg	100 (10/10)
97602	ND	LSDV Field	ND	LSDV Field	LSDV Field	LSDV Field	ND	ND	Neg	Neg	100 (7/7)
97604	ND	Neg	ND	LSDV Field	LSDV Field	LSDV Field	ND	ND	Neg	Neg	85 (6/7)
97608	ND	LSDV Field	ND	LSDV Field	LSDV Field	LSDV Field	ND	ND	Neg	Neg	100 (7/7)
97609	ND	LSDV Field	SPPV Field	LSDV Field	LSDV Field	LSDV Field	SPPV Field	SPPV Field	Neg	Neg	100 (10/10)
97610	GTPV Field	LSDV Field	SPPV Field	LSDV Field	LSDV Field	LSDV Field	SPPV Vaccine	SPPV Vaccine	Neg	Neg	80 (8/10)
97611	ND	LSDV Field	ND	LSDV Field	LSDV Field	LSDV Field	ND	ND	Neg	Neg	100 (7/7)
97612	ND	LSDV Field	ND	LSDV Field	LSDV Field	LSDV Field	ND	ND	Neg	Neg	100 (7/7)
97613	ND	LSDV Field	SPPV Field	LSDV Field	LSDV Field	LSDV Field	SPPV Field	SPPV Field	Neg	Neg	100 (10/10)
97617	GTPV Field	ND	SPPV Field	ND	ND	ND	SPPV Field	SPPV Field	Neg	Neg	100 (6/6)
97618	ND	LSDV Field	ND	LSDV Field	LSDV Field	LSDV Field	ND	ND	Neg	Neg	100 (7/7)
97620	ND	LSDV Field	ND	LSDV Field	LSDV Field	LSDV Field	ND	ND	Neg	Neg	100 (7/7)
97621	ND	LSDV Field	ND	LSDV Field	LSDV Field	LSDV Field	ND	ND	Neg	Neg	100 (7/7)

97625	NI	LSDV Field	SPPV Field	LSDV Field	LSDV Field	LSDV Field	SPPV Field	SPPV Field	Neg	Neg	100 (10/10)
97630	ND	LSDV Field	ND	LSDV Field	LSDV Field	LSDV Field	ND	ND	Neg	Neg	100 (7/7)
97631	ND	LSDV Field	ND	LSDV Field	LSDV Field	LSDV Field	ND	ND	Neg	Neg	100 (7/7)
97632	ND	LSDV Field	ND	LSDV Field	LSDV Field	LSDV Field	ND	ND	Neg	Neg	100 (7/7)
97633	GTPV Field	LSDV Field	SPPV Field	LSDV Field	LSDV Field	LSDV Field	SPPV Field	SPPV Field	Neg	Neg	100 (10/10)
97634	ND	LSDV Field	ND	LSDV Field	LSDV Field	LSDV Field	ND	ND	Neg	Neg	100 (7/7)
97638	ND	LSDV Field	ND	LSDV Field	LSDV Field	LSDV Field	ND	ND	Neg	Neg	100 (7/7)
97642	ND	LSDV Field	ND	LSDV Field	LSDV Field	LSDV Field	ND	ND	Neg	Neg	100 (7/7)

ND: Not Done; NI: Doubtful, NCR: Number of correct results; NR: Number of results; %: percentage of correct results.

#### 4.1.4 Final Diagnostic

Thirty-three laboratories encoded results. The encoded results depend on the different tests performed by each participating laboratory. Not all laboratories performed species differentiation or DIVA PCR.

#### Results per sample

Table 17: Final diagnostic results per sample

Sample ID	Expected result	Encoded result
PT2021CAPXVIR_VP1	CAPX Pos-GTPV (field)	21 Capx Pos – GTPV (field) 2 Capx pos-SPPV/GTPV 1 Capx Pos – LSDV/GTPV 9 Capx pos
PT2021CAPXVIR_VP2	CAPX Pos-LSDV field	21 Capx pos- LSDV field 3 Capx pos - LSDV 8 Capx pos 1 Capx neg
PT2021CAPXVIR_VP3	CAPX Pos-SPVV field	15 Capx pos- SSPV field 7 Capx pos – SPPV 3 Capx Pos-SPPV/GTPV 8 Capx pos
PT2021CAPXVIR_BP1	CAPX Pos-LSDV field	22 Capx pos- LSDV field 3 Capx pos - LSDV 8 Capx pos
PT2021CAPXVIR_TP1	CAPX Pos-LSDV field	44 Capx pos- LSDV field 6 Capx pos - LSDV 18 Capx pos
PT2021CAPXVIR_TP2	CAPX Pos-SPPV field	26 Capx pos- SSPV field 14 Capx pos – SPPV 6 Capx Pos-SPPV/GTPV 16 Capx pos 4 Capx pos – SPPV vaccine
PT2021CAPXVIR_BN1	NEGATIVE	33 negative results
PT2021CAPXVIR_TN1	NEGATIVE	33 negative results

### Results per laboratory

For the final diagnostic interpretation of the detection of capripox virus DNA in cell culture supernatant, blood and tissue homogenate: Thirty out of thirty-three laboratories provided qualitative results that were in full agreement with the assigned status of the 10 reference samples and hence reached 100% of agreement. LAB97604 misclassified 1 aliquot (90% of agreement). Two laboratories (LAB97610 and LAB97618) misclassified 2 aliquots, reaching an agreement of 80%.

Table 18: Result of the final diagnostic per participating laboratory

Sample status	VP1 Pos - GTPV field	VP2 Pos - LSDV field	VP3 Pos- SPPV field	BP1 Pos- LSD field	TP1 (Rep 1) Pos- LSDV field	TP1 (Rep 2) Pos- LSDV field	TP2 (Rep 1) Pos- SPPV field	TP1 (Rep 2) Pos- SPPV field	BN1 Neg	TN1 Neg	% (NCR/ NR)
Laboratory											
97506	GTPV	LSDV Field	SPPV Field	LSDV Field	LSDV Field	LSDV Field	SPPV Field	SPPV Field	Neg	Neg	100 (10/10)
97600	GTPV	LSDV Field	SPPV Field	LSDV Field	LSDV Field	LSDV Field	SPPV Field	SPPV Field	Neg	Neg	100 (10/10)
97601	Pos	Pos	Pos	Pos	Pos	Pos	Pos	Pos	Neg	Neg	100 (10/10)
97602	GTPV	LSDV Field	SPPV	LSDV Field	LSDV Field	LSDV Field	SPPV	SPPV	Neg	Neg	100 (10/10)
97603	Pos	Pos	SPPV	Pos	Pos	Pos	SPPV	SPPV	Neg	Neg	100 (10/10)
97604	GTPV	Neg	SPPV	LSDV Field	LSDV Field	LSDV Field	SPPV	SPPV	Neg	Neg	90 (9/10)
97605	Pos	Pos	Pos	Pos	Pos	Pos	Pos	Pos	Neg	Neg	90 (9/10)
97606	Pos	Pos	Pos	Pos	Pos	Pos	Pos	Pos	Neg	Neg	100 (10/10)
97607	SPPV/ GTPV	LSDV	SPPV/ GTPV	LSDV	LSDV	LSDV	SPPV/ GTPV	SPPV/ GTPV	Neg	Neg	100 (10/10)
97608	GTPV	LSDV Field	SPPV	LSDV Field	LSDV Field	LSDV Field	SPPV	SPPV	Neg	Neg	100 (10/10)

97609	GTPV	LSDV Field	SPPV Field	LSDV Field	LSDV Field	LSDV Field	SPPV Field	SPPV Field	Neg	Neg	100 (10/10)
97610	GTPV	LSDV Field	SPPV Field	LSDV Field	LSDV Field	LSDV Field	SPPV Vaccine	SPPV Vaccine	Neg	Neg	800 (8/10)
97611	GTPV	LSDV Field	SPPV	LSDV Field	LSDV Field	LSDV Field	SPPV	SPPV	Neg	Neg	100 (10/10)
97612	SPPV/ GTPV	LSDV	SPPV/ GTPV	LSDV	LSDV	LSDV	SPPV/ GTPV	SPPV/ GTPV	Neg	Neg	100 (10/10)
97613	GTPV	LSDV Field	SPPV Field	LSDV Field	LSDV Field	LSDV Field	SPPV Field	SPPV Field	Neg	Neg	100 (10/10)
97614	GTPV	LSDV	SPPV	LSDV	LSDV	LSDV	SPPV	SPPV	Neg	Neg	90 (9/10)
97616	Pos	Pos	Pos	Pos	Pos	Pos	Pos	Pos	Neg	Neg	100 (10/10)
97617	GTPV Field	LSDV Field	SPPV Field	LSDV Field	LSDV Field	LSDV Field	SPPV Field	SPPV Field	Neg	Neg	100 (10/10)
97618	GTPV	LSDV Field	SPPV Field	LSDV Field	LSDV Field	LSDV Field	SPPV Vaccine	SPPV Vaccine	Neg	Neg	80 (8/10)
97619	GTPV	LSDV Field	SPPV Field	LSDV Field	LSDV Field	LSDV Field	SPPV Field	SPPV Field	Neg	Neg	100 (10/10)
97620	GTPV	LSDV Field	SPPV Field	LSDV Field	LSDV Field	LSDV Field	SPPV Field	SPPV Field	Neg	Neg	100 (10/10)
97621	GTPV	LSDV Field	SPPV Field	LSDV Field	LSDV Field	LSDV Field	SPPV Field	SPPV Field	Neg	Neg	100 (10/10)
97622	Pos	Pos	Pos	Pos	Pos	Pos	Pos	Pos	Neg	Neg	100 (10/10)
97624	Pos	Pos	Pos	Pos	Pos	Pos	Pos	Pos	Neg	Neg	100 (10/10)

97625	GTPV	LSDV Field	SPPV Field	LSDV Field	LSDV Field	LSDV Field	SPPV Field	SPPV Field	Neg	Neg	100 (10/10)
97627	Pos	Pos	Pos	Pos	Pos	Pos	Pos	Pos	Neg	Neg	100 (10/10)
97630	LSDV/ GTPV	LSDV Field	SPPV/ GTPV	LSDV Field	LSDV Field	LSDV Field	SPPV/ GTPV	SPPV/ GTPV	Neg	Neg	100 (10/10)
97631	GTPV	LSDV Field	SPPV	LSDV Field	LSDV Field	LSDV Field	SPPV	SPPV	Neg	Neg	100 (10/10)
97632	GTPV	LSDV Field	SPPV Field	LSDV Field	LSDV Field	LSDV Field	SPPV Field	SPPV Field	Neg	Neg	100 (10/10)
97633	GTPV	LSDV Field	SPPV Field	LSDV Field	LSDV Field	LSDV Field	SPPV Field	SPPV Field	Neg	Neg	100 (10/10)
97634	GTPV	LSDV Field	SPPV Field	LSDV Field	LSDV Field	LSDV Field	SPPV Field	SPPV Field	Neg	Neg	100 (10/10)
97638	GTPV	LSDV Field	SPPV Field	LSDV Field	LSDV Field	LSDV Field	SPPV Field	SPPV Field	Neg	Neg	100 (10/10)
97642	Pos	LSDV Field	Pos	LSDV Field	LSDV Field	LSDV Field	Pos	Pos	Neg	Neg	100 (10/10)

NCR: Number of correct results; NR: Number of results.

## 4.2 Serology

The PT2021CAPXSER panel was composed of 8 positive samples and 2 negative samples.

### 4.2.1 ELISA

Twenty-eight laboratories encoded results. All the laboratories used the same ELISA kit (ID-Vet ID Screen Capripox Double Antigen Multispecies from ID.VET). All 28 participating laboratories provided qualitative results that were in full agreement with the assigned status of the reference samples and hence reached 100% agreement. Raw data can be found in Annex 1.

Table 19: Result of the ELISA per participating laboratory

Laboratory	SERP1	SERP2	SERP3	SERP4	SERP5	SERP6	SERP7	SERP8	SERN1	SERN2	%
	Pos	Pos	Pos	Pos	Pos	Pos	Pos	Pos	Neg	Neg	
97506	Pos	Pos	Pos	Pos	Pos	Pos	Pos	Pos	Neg	Neg	100
97600	Pos	Pos	Pos	Pos	Pos	Pos	Pos	Pos	Neg	Neg	100
97602	Pos	Pos	Pos	Pos	Pos	Pos	Pos	Pos	Neg	Neg	100
97604	Pos	Pos	Pos	Pos	Pos	Pos	Pos	Pos	Neg	Neg	100
97605	Pos	Pos	Pos	Pos	Pos	Pos	Pos	Pos	Neg	Neg	100
97607	Pos	Pos	Pos	Pos	Pos	Pos	Pos	Pos	Neg	Neg	100
97608	Pos	Pos	Pos	Pos	Pos	Pos	Pos	Pos	Neg	Neg	100
97609	Pos	Pos	Pos	Pos	Pos	Pos	Pos	Pos	Neg	Neg	100
97610	Pos	Pos	Pos	Pos	Pos	Pos	Pos	Pos	Neg	Neg	100
97611	Pos	Pos	Pos	Pos	Pos	Pos	Pos	Pos	Neg	Neg	100
97612	Pos	Pos	Pos	Pos	Pos	Pos	Pos	Pos	Neg	Neg	100
97613	Pos	Pos	Pos	Pos	Pos	Pos	Pos	Pos	Neg	Neg	100
97614	Pos	Pos	Pos	Pos	Pos	Pos	Pos	Pos	Neg	Neg	100
97615	Pos	Pos	Pos	Pos	Pos	Pos	Pos	Pos	Neg	Neg	100
97616	Pos	Pos	Pos	Pos	Pos	Pos	Pos	Pos	Neg	Neg	100
97617	Pos	Pos	Pos	Pos	Pos	Pos	Pos	Pos	Neg	Neg	100
97618	Pos	Pos	Pos	Pos	Pos	Pos	Pos	Pos	Neg	Neg	100
97619	Pos	Pos	Pos	Pos	Pos	Pos	Pos	Pos	Neg	Neg	100
97620	Pos	Pos	Pos	Pos	Pos	Pos	Pos	Pos	Neg	Neg	100
97621	Pos	Pos	Pos	Pos	Pos	Pos	Pos	Pos	Neg	Neg	100
97622	Pos	Pos	Pos	Pos	Pos	Pos	Pos	Pos	Neg	Neg	100
97630	Pos	Pos	Pos	Pos	Pos	Pos	Pos	Pos	Neg	Neg	100
97631	Pos	Pos	Pos	Pos	Pos	Pos	Pos	Pos	Neg	Neg	100



97632	Pos	Pos	Pos	Pos	Pos	Pos	Pos	Pos	Neg	Neg	100
97633	Pos	Pos	Pos	Pos	Pos	Pos	Pos	Pos	Neg	Neg	100
97634	Pos	Pos	Pos	Pos	Pos	Pos	Pos	Pos	Neg	Neg	100
97638	Pos	Pos	Pos	Pos	Pos	Pos	Pos	Pos	Neg	Neg	100
97642	Pos	Pos	Pos	Pos	Pos	Pos	Pos	Pos	Neg	Neg	100

#### 4.2.2 Virus Neutralization

Five laboratories encoded results for the VN (dilution antibody) tests. All laboratories provided qualitative results that were in full agreement with the assigned status of the reference serum samples and hence reached 100% agreement. Raw data can be found in Annex 1.

Table 20: Result of the virus neutralization test per participating laboratory

Laboratory	SERP1	SERP2	SERP3	SERP4	SERP5	SERP6	SERP7	SERP8	SERN1	SERN2	%
	Pos	Pos	Pos	Pos	Pos	Pos	Pos	Pos	Neg	Neg	
97506	Pos	Pos	Pos	Pos	Pos	Pos	Pos	Pos	Neg	Neg	100
97600	Pos	Pos	Pos	Pos	Pos	Pos	Pos	Pos	Neg	Neg	100
97612	Pos	Pos	Pos	Pos	Pos	Pos	Pos	Pos	Neg	Neg	100
97618	Pos	Pos	Pos	Pos	Pos	Pos	Pos	Pos	Neg	Neg	100
97633	Pos	Pos	Pos	Pos	Pos	Pos	Pos	Pos	Neg	Neg	100

#### 4.2.3 Immunoperoxidase 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. Raw data can be found in Annex 1.

Table 21: Result of the IPMA test per participating laboratory

Laboratory	SERP1	SERP2	SERP3	SERP4	SERP5	SERP6	SERP7	SERP8	SERN1	SERN2	%
	Pos	Pos	Pos	Pos	Pos	Pos	Pos	Pos	Neg	Neg	
97506	Pos	Pos	Pos	Pos	Pos	Pos	Pos	Pos	Neg	Neg	100
97618	Pos	Pos	Pos	Pos	Pos	Pos	Pos	Pos	Neg	Neg	100

#### 4.2.4 Final diagnosis

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 22. Final diagnostic

Laboratory	SERP1	SERP2	SERP3	SERP4	SERP5	SERP6	SERP7	SERP8	SERN1	SERN2	%
	Pos	Pos	Pos	Pos	Pos	Pos	Pos	Pos	Neg	Neg	
97506	Pos	Pos	Pos	Pos	Pos	Pos	Pos	Pos	Neg	Neg	100

97600	Pos	Pos	Pos	Pos	Pos	Pos	Pos	Pos	Neg	Neg	100
97601	Pos	Pos	Pos	Pos	Pos	Pos	Pos	Pos	Neg	Neg	100
97602	Pos	Pos	Pos	Pos	Pos	Pos	Pos	Pos	Neg	Neg	100
97604	Pos	Pos	Pos	Pos	Pos	Pos	Pos	Pos	Neg	Neg	100
97605	Pos	Pos	Pos	Pos	Pos	Pos	Pos	Pos	Neg	Neg	100
97608	Pos	Pos	Pos	Pos	Pos	Pos	Pos	Pos	Neg	Neg	100
97609	Pos	Pos	Pos	Pos	Pos	Pos	Pos	Pos	Neg	Neg	100
97610	Pos	Pos	Pos	Pos	Pos	Pos	Pos	Pos	Neg	Neg	100
97611	Pos	Pos	Pos	Pos	Pos	Pos	Pos	Pos	Neg	Neg	100
97612	Pos	Pos	Pos	Pos	Pos	Pos	Pos	Pos	Neg	Neg	100
97613	Pos	Pos	Pos	Pos	Pos	Pos	Pos	Pos	Neg	Neg	100
97614	Pos	Pos	Pos	Pos	Pos	Pos	Pos	Pos	Neg	Neg	100
97615	Pos	Pos	Pos	Pos	Pos	Pos	Pos	Pos	Neg	Neg	100
97616	Pos	Pos	Pos	Pos	Pos	Pos	Pos	Pos	Neg	Neg	100
97617	Pos	Pos	Pos	Pos	Pos	Pos	Pos	Pos	Neg	Neg	100
97618	Pos	Pos	Pos	Pos	Pos	Pos	Pos	Pos	Neg	Neg	100
97619	Pos	Pos	Pos	Pos	Pos	Pos	Pos	Pos	Neg	Neg	100
97620	Pos	Pos	Pos	Pos	Pos	Pos	Pos	Pos	Neg	Neg	100
97621	Pos	Pos	Pos	Pos	Pos	Pos	Pos	Pos	Neg	Neg	100
97622	Pos	Pos	Pos	Pos	Pos	Pos	Pos	Pos	Neg	Neg	100
97630	Pos	Pos	Pos	Pos	Pos	Pos	Pos	Pos	Neg	Neg	100
97631	Pos	Pos	Pos	Pos	Pos	Pos	Pos	Pos	Neg	Neg	100
97632	Pos	Pos	Pos	Pos	Pos	Pos	Pos	Pos	Neg	Neg	100
97633	Pos	Pos	Pos	Pos	Pos	Pos	Pos	Pos	Neg	Neg	100
97634	Pos	Pos	Pos	Pos	Pos	Pos	Pos	Pos	Neg	Neg	100
97638	Pos	Pos	Pos	Pos	Pos	Pos	Pos	Pos	Neg	Neg	100
97642	Pos	Pos	Pos	Pos	Pos	Pos	Pos	Pos	Neg	Neg	100

## V. Discussion

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

### 1. Virology component of the PT

For the **detection of capripox virus DNA** by real-time PCR (RT-PCR) in the PT panel : 32 out of 33 participating laboratories provided qualitative results that were in full agreement with the assigned status of the 10 reference samples (100% of agreement), whereas LAB97604 misclassified 1 sample (90% of agreement).

LAB97604 misclassified PTCAPXVIR\_VP2 (Neg instead of Pos) using an in house RT-qPCR method with ORF 074 (P32) as target gene.

Three participating laboratories performed a **secondary PCR**. LAB97605 and LAB97607 provided qualitative results that were identical in both tests. All of the samples were classified in agreement with the assigned status of the reference samples. Lab 97605 used the methods described by Bowden et al. 2008 as primary PCR and Haegeman et al. 2013 as secondary PCR, whereas lab 97607 used the Bowden et al. 2008 as primary PCR and an in-house RT-PCR targeting RPO30 as secondary PCR. LAB97612 classified all samples correctly in their primary PCR. In the secondary PCR, using the Bio-T kit Lumpy Skin Disease (BioSella) kit, they only classified the 6 bovine samples present in the panel and classified all correctly.

For the **differentiation of capripox virus species**: Seventeen out of 23 participating laboratories provided qualitative results that were in full agreement with the assigned status of the 10 reference samples (100% of agreement). Three laboratories misclassified 1 sample and reached a level of agreement of 90%. These labs were lab 97604, 97613 and 97614 and misclassified respectively PTCAPXVIR\_VP2 (neg instead of LSDV), PTCAPXVIR\_VP1 (LSDV instead of GTPV) and PTCAPXVIR\_VP2 (SPPV instead of LSDV). LAB 97618 classified 7 out of 10 samples and 2 laboratories (LAB97607 and LAB97609) classified 6 out of ten samples. These laboratories classified all the samples in agreement with the assigned status of the reference samples.

LAB97604 already misclassified PTCAPXVIR\_VP2 as negative in their primary PCR. LAB97613 and LAB97614 classified respectively PTCAPXVIR\_VP1 and PTCAPXVIR\_VP2 correctly in their final diagnosis, suggesting that the error occurred during the entry on the results.

For the **differentiation between capripox virus field and vaccine strain**: Twenty out of twenty-two participating laboratories provided qualitative results that were in full agreement with the assigned status of the samples they analyzed (100% of agreement). Six laboratories (LAB97506, LAB97600, LAB97609, LAB97613, LAB97625 and LAB97633) analyzed all 10 samples. Thirteen laboratories analyzed 7 samples (LAB97602, LAB97608, LAB97611, LAB97612, LAB97618, LAB97620, LAB97621, LAB97630, LAB97631, LAB97632, LAB97634, LAB97638 and LAB97642) and did not analyse the SPPV (and GTPV) samples. One other laboratory did not analyse the LSDV samples (LAB97617) and analyzed a total of 6 samples. LAB97604 analyzed all LSDV samples and misclassified (PTCAPXVIR\_VP2 as neg instead of LSDV field), resulting in a level of agreement of 85%. This was the same sample that was already misclassified in the primary PCR and the species differentiation. Finally, 1 lab (LAB97610) misclassified 2 samples. These 2 samples were replicates of PTCAPXVIR\_TP2 and both were misclassified as SPPV vaccine instead of SPPV field, using an in house protocol for the DIVA.

For the **final diagnostic interpretation of the detection of capripox virus DNA in cell culture supernatant, blood and tissue homogenate**: Thirty out of thirty-three laboratories provided qualitative results that were in full agreement with the assigned status of the 10 reference samples.

LAB97604 misclassified PTCAPXVIR\_VP2 as negative instead of Pos/LSDV/LSDV field strain. The sample was misclassified as negative in the primary and was misclassified in all following tests as well.

LAB 97610 misclassified both repetitions of PTCAPXVIR\_TP1 as SPPV vaccine instead of SPPV field.

LAB97618 misclassified the same samples as LAB97610 as SPPV vaccine instead of SPPV field. However LAB97618 only performed the differentiation between field strain and vaccine strain for the LSDV samples. Therefore, it can be assumed that the errors occurred during the entry of the results in the toolkit and not during the analysis of the samples.

## **2. Serology component of the PT**

For the **detection of specific antibodies to capripox virus** in reference serum samples, using ELISA and in some cases TN1 with antibody titer or IPMA, 28 out of 28 laboratories provided qualitative results that were in full agreement with the assigned status of the reference serum samples.

In accordance, the final diagnostic interpretation was 100% successful for 28 out of 28 laboratories.

## **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 European Union Reference Laboratory for disease caused by capripox viruses of the Scientific Directorate Infectious Diseases in Animals of Sciensano (see III.3.3.).

For the detection of capripox virus nucleic acid in the cell culture supernatant, blood or tissue homogenate samples, all participating laboratories achieved a satisfactory performance based on the final diagnostic.

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

## VII. Annex 1 Raw data (not under accreditation)

### Raw data Primary PCR

value	sample	Labnr						Labnr				
		97506	97600	97601	97602	97603	97604	97605	97606	97607	97608	97609
raw data	PT2020CAPXVIR_VP1	26,77	26,46	27,13	25,33	24,14	27,1	24,22	26,44	25,63	24,96	26,94
	PT2020CAPXVIR_VP2	34,06	33,4	34,1	31,7	31,09	no ct	31,64	35,62	31,66	32,5	33,78
	PT2020CAPXVIR_VP3	29,53	30,39	30,45	28,82	26,91	31,4	27,99	29,55	28,89	28,53	31,08
	PT2020CAPXVIR_BP1	33,55	32,83	36,06	32,48	33,53	31,5	31,46	36,46	34,09	31,69	34,7
	PT2020CAPXVIR_TP1 (1)	31,24	29,58	30,46	26,2	29,26	36	26,72	30,57	29,32	27,56	28,54
	PT2020CAPXVIR_TP1 (2)	31,21	33,34	31,09	25,93	29,07	35,2	27,08	29,22	28,9	27,56	28,37
	PT2020CAPXVIR_TP2 (1)	29,19	27,84	28,92	24,61	26,56	31,4	24,75	27,91	28,6	25,24	25,47
	PT2020CAPXVIR_TP2 (2)	30,68	33,88	29,87	25,02	27,09	36,7	24,74	28,24	28,04	25,36	25,42
	PT2020CAPXVIR_BN1	no ct	no ct	no ct	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	no ct	no ct	no ct
Final results	PT2020CAPXVIR_VP1	Pos	Pos	Pos	Pos	Pos	Pos	Pos	Pos	Pos	Pos	Pos
	PT2020CAPXVIR_VP2	Pos	Pos	Pos	Pos	Pos	Pos	Pos	Pos	Pos	Pos	Pos
	PT2020CAPXVIR_VP3	Pos	Pos	Pos	Pos	Pos	Pos	Pos	Pos	Pos	Pos	Pos
	PT2020CAPXVIR_BP1	Pos	Pos	Pos	Pos	Pos	Pos	Pos	Pos	Pos	Pos	Pos
	PT2020CAPXVIR_TP1 (1)	Pos	Pos	Pos	Pos	Pos	Pos	Pos	Pos	Pos	Pos	Pos
	PT2020CAPXVIR_TP1 (2)	Pos	Pos	Pos	Pos	Pos	Pos	Pos	Pos	Pos	Pos	Pos
	PT2020CAPXVIR_TP2 (1)	Pos	Pos	Pos	Pos	Pos	Pos	Pos	Pos	Pos	Pos	Pos
	PT2020CAPXVIR_TP2 (2)	Pos	Pos	Pos	Pos	Pos	Pos	Pos	Pos	Pos	Pos	Pos
	PT2020CAPXVIR_BN1	Neg	Neg	Neg	Neg	Neg	Neg	Neg	Neg	Neg	Neg	Neg
	PT2020CAPXVIR_TN1	Neg	Neg	Neg	Neg	Neg	Neg	Neg	Neg	Neg	Neg	Neg
Information	Protocol / SOP used :	Haegeman et al. (2013)	Bowden et al. 2008 as modified by FLI	UD	Bowden et al. 2008.	Bowden et al. 2008; Babiuk et al. 2008; SOP from Pirbright	6.3.51.1 Capripoxviruses gPCR	Bowden et al. 2008	Bowden et al. 2008	Bowden et al. 2008	Wolff et al. 2021	Bowden et al. 2008
	Producer Extraction protocol / kit:	Machery Nagel	Biosellal	GPSolutions Spain	Invitrogen/Thermo Fisher Scientific	Roche	Roche	Roche	Qiagen	Qiagen	Qiagen	Indical Bioscience
	Name Extraction protocol / kit:	UD	Biosellal Superball	GPSpin Vrial DNA/RNA Extraction Kit	MagMAX Core Nucleic Acid Purification Kit	Magna Pure Compact Nucleic Acid Isolation Kit I	MagNA Pure 24 total NA Kit	Roche-MP 96/ Viral NA SV	QIAamp Viral RNA Mini Kit	Qiagen DNAeasy Blood and tissue	QIAamp Viral RNA Mini Kit	Indispin pathogen kit
	In-house modifications to extraction protocol (if yes, which?):	Addition of EC to buffer B3	no	no	no	no	no	no	no	no	no	no
	RT-PCR protocol/kit	Fast Start DNA Polymerase	QuantiTect Multiplex PCR Kit NoRox	Virotype MIX IC DNA PCR Kit	Quanti Fast Pathogen +IC kit (Qiagen)	Path-ID qPCR Master Mix (Thermo Fisher Scientific)	Home made	Other	Home made	QuantiFast Probe PCR kit	QuantiFast/PerfeCTa qPCR ToughMix	QIAGEN: Quantifast pathogen IC PCR Kit
	Target gene	D5R	P32	UD	P32	P32	P32	P32	ORF074 (P32)	P32	P32	P32
	PCR Instrument used:	Roche - Roche Light Cyclser 480	Quantstudio 6Pro	Bio-Rad - Bio-Rad CFX 96	RotorGene Q	LightCycler 96 (Roche)	Bio-Rad - Bio-Rad CFX 96	Agilent Technologies - Stratagene Mx3005p	Bio-Rad - Bio-Rad CFX96 Touch	Light Cyclser96 Roche	Bio-Rad - Bio-Rad CFX 96	Rotor gene Q
	Cut-off for positive:	45	45	40	40	35	38	No cut-off	40	No cut-off	40	37
	Remark(s):	N/A	N/A	N/A	N/A	N/A	N/A	N/A	N/A	N/A	N/A	N/A

value	sample	Labnr									
		97610	97611	97612	97613	97614	97616	97617	97618	97620	97621
raw data	PT2020CAPXVIR_VP1	26,59	23	27,4	22,39	27	23,88	24,14	20,74	29,06	23,2
	PT2020CAPXVIR_VP2	33,67	29,5	33,6	29,69	33	30,31	31,38	30,25	34,93	29,7
	PT2020CAPXVIR_VP3	31,16	26,3	31,4	25,19	30	27,33	28,29	24,7	32,96	26,4
	PT2020CAPXVIR_BP1	33,89	29,1	33,8	30,23	32	30,62	30,43	30,86	36,26	29,6
	PT2020CAPXVIR_TP1 (1)	27,89	25,8	30,2	25,28	28	26,95	26,02	27,26	29,5	26,5
	PT2020CAPXVIR_TP1 (2)	29,56	25,3	30,4	25,66	29	27,19	24,72	26,74	29,62	26,7
	PT2020CAPXVIR_TP2 (1)	26,39	23,9	29	23,48	26	25,22	25,96	26,01	27,24	27,3
	PT2020CAPXVIR_TP2 (2)	25,94	24	27,6	24	26	25,13	26,76	25,32	26,64	24,9
	PT2020CAPXVIR_BN1	no ct	no ct	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	no ct	no ct
Final results	PT2020CAPXVIR_VP1	Pos	Pos	Pos	Pos	Pos	Pos	Pos	Pos	Pos	Pos
	PT2020CAPXVIR_VP2	Pos	Pos	Pos	Pos	Pos	Pos	Pos	Pos	Pos	Pos
	PT2020CAPXVIR_VP3	Pos	Pos	Pos	Pos	Pos	Pos	Pos	Pos	Pos	Pos
	PT2020CAPXVIR_BP1	Pos	Pos	Pos	Pos	Pos	Pos	Pos	Pos	Pos	Pos
	PT2020CAPXVIR_TP1 (1)	Pos	Pos	Pos	Pos	Pos	Pos	Pos	Pos	Pos	Pos
	PT2020CAPXVIR_TP1 (2)	Pos	Pos	Pos	Pos	Pos	Pos	Pos	Pos	Pos	Pos
	PT2020CAPXVIR_TP2 (1)	Pos	Pos	Pos	Pos	Pos	Pos	Pos	Pos	Pos	Pos
	PT2020CAPXVIR_TP2 (2)	Pos	Pos	Pos	Pos	Pos	Pos	Pos	Pos	Pos	Pos
	PT2020CAPXVIR_BN1	Neg	Neg	Neg	Neg	Neg	Neg	Neg	Neg	Neg	Neg
	PT2020CAPXVIR_TN1	Neg	Neg	Neg	Neg	Neg	Neg	Neg	Neg	Neg	Neg
Information	Protocol / SOP used :	Bowden et al. 2008	Haegeman et al 2013	in house	Babiuk et al. 2008	UD	Path-ID qPCR	Bowden et al. 2008	Bowden et al. 2008	Bowden et al. 2008	Bowden et al. 2008
	Producer Extraction protocol / kit:	Indical Bioscience	Qiagen	Qiagen	Indical Bioscience	Qiagen	Qiagen	Indical Bioscience	Invitrogen/ Thermo Fisher Scientific	Qiagen	Qiagen
	Name Extraction protocol / kit:	Indical IndiMag Pathogen Kit	QIAamp Viral RNA Mini Kit	Qiagen Biosprint 96 DNA Blood kit	Indispin pathogen kit	QIAamp Viral RNA Mini Kit	QIAamp DNA mini kit	UD	Pure Link Genomic DNA Mini Kit (Invitrogen)	QIAamp Viral RNA Mini Kit	MagAttract 96 cador Pathogen Kit
	In-house modifications to extraction protocol (if yes, which?):	no	no	Qiagen Biosprint 96 One-For-All Vet - Animal tissues	No	no	no	No	no	no	no
	RT-PCR protocol/kit	Home Made - Home made	Mater mix ThermoFisher. TaqMan Fast Virus 1-Step Master Mix	ID.VET - ID GENE® CAPRIPox VIRUS TRIPLEX	Path-ID qPCR	UD	Path-ID qPCR Master Mix from Applied Biosystems.	UD	Invitrogen: Platinum Quantitative PCR SuperMix-UDG with ROX	Qiagen-QuantiTect Virus Kit	Path-ID qPCR Master Mix (Thermo Fisher Scientific)
	Target gene	P32	E3L	Other	P32	D5R	P32	P32	P32	P32	P32
	PCR Instrument used:	RotorgeneQ	Agilent Technologies - Agilent AriaMx	Roche - Roche Light Cycler 480	RotorGeneQ	Applied Biosystems - ABI 7500 Fast	Roche Lightcycler96	Bio-Rad - Bio-Rad CFX 96	Applied Biosystems - ABI 7900 HT Fast Real-Time PCR System	Agilent Technologies - Stratagene Mx3005p	Applied Biosystems - ABI 7500 Fast
	Cut-off for positive:	35	38	39	40	UD	35	40	UD	45	35
	Remark(s):	N/A	N/A	N/A	N/A	N/A	N/A	N/A	N/A	N/A	N/A

value	sample	97622	97624	97625	97627	Labnr 97630	97631	97632	97633	97634	97638	97642	
raw data	PT2020CAPXVIR_VP1	25,8	29	21,7	21,84	25,26	26,77	25,8	22	23,89	19,87	26,02	
	PT2020CAPXVIR_VP2	33,5	32	27,89	32,22	29,44	34,76	32,9	30	30,05	25,75	32,76	
	PT2020CAPXVIR_VP3	30	30	25,22	24,14	28,39	29,98	28,7	26	26,87	23,92	29,23	
	PT2020CAPXVIR_BP1	33,7	37	28,79	26,8	30,62	32,79	33,7	30	29,4	25,88	33,24	
	PT2020CAPXVIR_TP1 (1)	31,3	32	25,41	24,12	32,4	27,28	26,5	24	26,23	23,58	27,75	
	PT2020CAPXVIR_TP1 (2)	30,1	34	28,54	24,47	30,93	27,22	27,9	24	26,98	23,6	27,26	
	PT2020CAPXVIR_TP2 (1)	31,1	33	23,81	23,31	31,33	25,33	27,2	23	25,07	23,79	25,77	
	PT2020CAPXVIR_TP2 (2)	29,1	35	24,71	22,4	27,34	29,47	26,4	23	25,02	23,7	25,64	
	PT2020CAPXVIR_BN1	no ct	no ct	no ct	no ct	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	no ct	no ct	no ct	no ct
Final results	PT2020CAPXVIR_VP1	Pos	Pos	Pos	Pos	Pos	Pos	Pos	Pos	Pos	Pos	Pos	
	PT2020CAPXVIR_VP2	Pos	Pos	Pos	Pos	Pos	Pos	Pos	Pos	Pos	Pos	Pos	
	PT2020CAPXVIR_VP3	Pos	Pos	Pos	Pos	Pos	Pos	Pos	Pos	Pos	Pos	Pos	
	PT2020CAPXVIR_BP1	Pos	Pos	Pos	Pos	Pos	Pos	Pos	Pos	Pos	Pos	Pos	
	PT2020CAPXVIR_TP1 (1)	Pos	Pos	Pos	Pos	Pos	Pos	Pos	Pos	Pos	Pos	Pos	
	PT2020CAPXVIR_TP1 (2)	Pos	Pos	Pos	Pos	Pos	Pos	Pos	Pos	Pos	Pos	Pos	
	PT2020CAPXVIR_TP2 (1)	Pos	Pos	Pos	Pos	Pos	Pos	Pos	Pos	Pos	Pos	Pos	
	PT2020CAPXVIR_TP2 (2)	Pos	Pos	Pos	Pos	Pos	Pos	Pos	Pos	Pos	Pos	Pos	
	PT2020CAPXVIR_BN1	Neg	Neg	Neg	Neg	Neg	Neg	Neg	Neg	Neg	Neg	Neg	Neg
	PT2020CAPXVIR_TN1	Neg	Neg	Neg	Neg	Neg	Neg	Neg	Neg	Neg	Neg	Neg	Neg
Information	Protocol / SOP used :	00-14-0959	Bowden et al. 2008	Bowden 2008	ID gene Capripox Virus Triplex	Bowden et al. 2008	Bowden et al. 2008	Bowden et al. 2008 Stubbs et al. 2012	in-house	Bowden 2008	Instruction of the kit	Bowden 2008	
	Producer Extraction protocol / kit:	Roche	Qiagen	Qiagen	ID VET	Indical Bioscience	Roche	Sacace Biotechnologies	Qiagen	Biosellal	Indical Bioscience	Promega	
	Name Extraction protocol / kit:	MagNa Pure 96 DNA (Roche)	Qiagen DNA minikit	QIAmp DNA mini kit	ID Gene Spin Universal Extraction Kit	Indispin pathogen kit	Roche-High Viral Nucleic Acid kit	Sacace-viral Nucleic acid Extraction	Qiagen DNA minikit	Biosellal Superball	Indispin pathogen kit	axwell RSC Viral Total Nucleic Acid Purification Kit	
	In-house modifications to extraction protocol (if yes, which?):	no	no		no	no	no	no	no	no	no	no	
	RT-PCR protocol/kit	Roche Light Cycler FastStart DNA Master HybProbe	UD	ThermoFisher Path-ID	ID.VET - ID GENE® CAPRIPOX VIRUS TRIPLEX	Qiagen Quanti Tect Probe RT-PCR Kit	Qiagen-quantitect probe PCR kit	Path-ID Multiplex One-Step RT-PCR Kit	Home made	Home made	ID.VET - ID GENE® CAPRIPOX VIRUS TRIPLEX	Home made	
	Target gene	P32	P32	UD	UD	UD	UD	P32	UD	P32	UD	P32	
	PCR Instrument used:	Roche - Roche Light Cycler 480	Applied Biosystems - ABI 7500	UD	Applied Biosystems - ABI 7500 Fast	Applied Biosystems - ABI QuantStudio5	Applied Biosystems - ABI 7500	Applied Biosystems - ABI QuantStudio5	Other - Other	Agilent Technologies - Agilent AriaMx	Applied Biosystems - ABI QuantStudio5	Bio-Rad - Bio-Rad CFX96 Touch	
	Cut-off for positive:	40	UD	UD	UD	UD	38	38	36	40	UD	40	
Remark(s):	N/A	Samples were not frozen at the time of arrival	N/A	N/A	N/A	N/A	N/A	N/A	N/A	N/A	N/A		

## Raw data Secondary PCR

value	sample	Labnr		
		97605	97607	97612
raw data	PT2020CAPXVIR_VP1	26,77	26,46	27,13
	PT2020CAPXVIR_VP2	34,06	33,4	34,1
	PT2020CAPXVIR_VP3	29,53	30,39	30,45
	PT2020CAPXVIR_BP1	33,55	32,83	36,06
	PT2020CAPXVIR_TP1 (1)	31,24	29,58	30,46
	PT2020CAPXVIR_TP1 (2)	31,21	33,34	31,09
	PT2020CAPXVIR_TP2 (1)	29,19	27,84	28,92
	PT2020CAPXVIR_TP2 (2)	30,68	33,88	29,87
	PT2020CAPXVIR_BN1	no ct	no ct	no ct
	PT2020CAPXVIR_TN1	no ct	no ct	no ct
Final results	PT2020CAPXVIR_VP1	Pos	Pos	ND
	PT2020CAPXVIR_VP2	Pos	Pos	Pos
	PT2020CAPXVIR_VP3	Pos	Pos	ND
	PT2020CAPXVIR_BP1	Pos	Pos	Pos
	PT2020CAPXVIR_TP1 (1)	Pos	Pos	Pos
	PT2020CAPXVIR_TP1 (2)	Pos	Pos	Pos
	PT2020CAPXVIR_TP2 (1)	Pos	Pos	ND
	PT2020CAPXVIR_TP2 (2)	Pos	Pos	ND
	PT2020CAPXVIR_BN1	Neg	Neg	Neg
	PT2020CAPXVIR_TN1	Neg	Neg	Neg
Information	Protocol / SOP used :	Haegeman et al. 2013	RT-PCR RPO30	IZS TE B456.1 SOP021
	Producer Extraction protocol / kit: Name Extraction	Roche	Qiagen	Roche
	protocol / kit: In-house modifications to extraction protocol (if yes, w hich?): RT-PCR protocol/kit	Roche-MP 96/ Viral NA SV	Qiagen DNAeasy Blood and tissue	Roche-The High Pure Viral Nucleic Acid Kit
		No	No	No
	Target gene	UD	Sso Advanced Universal Probes Supermix	Bio-T kit Lumpy Skin Disease (BioSellal)
	PCR Instrument used:	E3L	RPO30	UD
	Cut-off for positive:	Agilent Technologies - Stratagene Mx3005p	Agilent Technologies - Stratagene Mx3005p	Roche - Roche Light Cyclcr 480
	Remark(s):	No cut-off	No cut-off (Observation of a characteristic amplification curve)	40
	N/A	N/A	N/A	



## Raw data Species differentiation

value	sample	Labnr									
		97506	97600	97602	97603	97604	97607	97608	97609	97610	97611
raw data	PT2020CAPXVIR_VP1	45	14,67	26,3	172			25,64			74,8
	PT2020CAPXVIR_VP2	35,83	16,84	34,74	172		34,1	32,78			75,8
	PT2020CAPXVIR_VP3	45	17,05	32,94	151			28,79			75
	PT2020CAPXVIR_BP1	34,64	16,05	34,37	172		37,05	33,1			75,8
	PT2020CAPXVIR_TP1 (1)	32,27	15,65	29,37	172		31,42	27,71			75,8
	PT2020CAPXVIR_TP1 (2)	32,24	21,81	30,1	172		31,47	28,07			76
	PT2020CAPXVIR_TP2 (1)	45	15,9	27,81	151			25,92			74,8
	PT2020CAPXVIR_TP2 (2)	45	15,71	28,31	151			25,6			74,8
	PT2020CAPXVIR_BN1	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	GTPV	GTPV	GTPV	GTPV/ LSDV	GTPV	ND	GTPV	GTPV	GTPV	GTPV
	PT2020CAPXVIR_VP2	LSDV	LSDV	LSDV	GTPV/ LSDV	Neg	LSDV	LSDV	ND	LSDV	LSDV
	PT2020CAPXVIR_VP3	SPPV	SPPV	SPPV	SPPV	SPPV	ND	SPPV	SPPV	SPPV	SPPV
	PT2020CAPXVIR_BP1	LSDV	LSDV	LSDV	GTPV/ LSDV	LSDV	LSDV	LSDV	ND	LSDV	LSDV
	PT2020CAPXVIR_TP1 (1)	LSDV	LSDV	LSDV	GTPV/ LSDV	LSDV	LSDV	LSDV	ND	LSDV	LSDV
	PT2020CAPXVIR_TP1 (2)	LSDV	LSDV	LSDV	GTPV/ LSDV	LSDV	LSDV	LSDV	ND	LSDV	LSDV
	PT2020CAPXVIR_TP2 (1)	SPPV	SPPV	SPPV	SPPV	SPPV	ND	SPPV	SPPV	SPPV	SPPV
	PT2020CAPXVIR_TP2 (2)	SPPV	SPPV	SPPV	SPPV	SPPV	ND	SPPV	SPPV	SPPV	SPPV
	PT2020CAPXVIR_BN1	Neg	Neg	Neg	Neg	Neg	Neg	Neg	Neg	Neg	Neg
	PT2020CAPXVIR_TN1	Neg	Neg	Neg	Neg	Neg	Neg	Neg	Neg	Neg	Neg
Information	Protocol / SOP used :	Agianniotaki et al. 2016 / in-house Taqman assay	Lamien et al. 2011	Lamien et al 2011.	Lamien et al. 2011	6.3.51.4.Capripoxv ruses GPCR seq	RT-PCR GPCR (specific LSDV - Under development)	Wolf et al. 2021	Lamien et al. 2010	UD	Galaye et al. 2017
	Producer Extraction protocol / kit:	Machery Nagel	Biosellal	Thermo fisher	Roche	Roche	QIAGEN	QIAGEN	Indical	UD	QIAGEN
	Name Extraction protocol / kit:	UD	Biosellal Superball	MagMax Core Nucleic acid purification kit	Magna Pure Compact Nucleic Acid Isolation Kit I	MagNA Pure 24 total NA Kit	DNeasy® Blood & Tissue	UD	Indimag Pathogen/Manual Indispin Pathogen	UD	AiaAmp Viral RNA Mini Ki
	In-house modifications to extraction protocol (if yes, which?):	Addition of EC to buffer B3	no	no	No	No	No	UD	no	UD	Galaye et al 2017 adapted in-house for Quantitect SYBR Green Master mix.
	RT-PCR protocol/kit	Roche	UD	Quanti Fast Pathogen +IC kit (Qiagen)	Taq DNA Polymerase kit (Qiagen)	Home made	Sso Advanced Universal Probes Supermix	UD	UD	UD	Other - Other
	Methodology:	Roche - Roche Light Cyclser 480	Roche - 2 probes system	UD	Classical PCR amplification	Le Goff C et al. 2009	Real-time PCR	UD	Conventional PCR (RPO30)	UD	Melt curve SYBR Green
	PCR Instrument used:	Roche - Roche Light Cyclser 480	Bio-Rad - Bio-Rad CFX 96	RotorGeneQ	Bio-Rad - Other	UD	Agilent Technologies - Stratagene Mx3005p	Bio-Rad - Bio-Rad CFX 96	Applied Biosystems - ABI Verity	UD	Agilent Technologies - Agilent AriaMx
	Cut-off for positive:	45	N/A	40	151bp for SPPV 172bp for GTPV and LSDV	UD	No cut-off	40	presence of absence of 21 bp deletion	UD	37
remarks	N/A	N/A	N/A	N/A	N/A	N/A	N/A	N/A	UD	N/A	

value	sample	labnr				Labnr											
		97613	97614	97617	97618	97620	97621	97625	97631	97632	97633	97634	97638				
raw data	PT2020CAPXVIR_VP1	24,33	25			172					28	27	no ct				
	PT2020CAPXVIR_VP2	33,4	16			172			36,59		35	29,32	23,68				
	PT2020CAPXVIR_VP3	39,38	20			151			34,66	30,3	29	30,85	no ct				
	PT2020CAPXVIR_BP1	32,52	18			172			35,64		32	31,12	24,21				
	PT2020CAPXVIR_TP1 (1)	28,35	21			172			28,29		27	28,23	22,61				
	PT2020CAPXVIR_TP1 (2)	28,8	16			172			29,7		27	26,53	22,41				
	PT2020CAPXVIR_TP2 (1)	39,9	13			151			29,14		26	29,04					
	PT2020CAPXVIR_TP2 (2)	36,61	16			151			32,97		26	29,53					
	PT2020CAPXVIR_BN1	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	LSDV	GTPV	GTPV/ LSDV	GTPV	GTPV/ LSDV	GTPV/ LSDV	GTPV	GTPV	GTPV	GTPV	GTPV	GTPV	GTPV	GTPV	GTPV	GTPV
	PT2020CAPXVIR_VP2	LSDV	SPPV	GTPV/ LSDV	ND	GTPV/ LSDV	GTPV/ LSDV	LSDV	LSDV	GTPV/ LSDV	GTPV/ LSDV	GTPV/ LSDV	LSDV	LSDV	LSDV	LSDV	
	PT2020CAPXVIR_VP3	SPPV	SPPV	SPPV	SPPV	SPPV	SPPV	SPPV	SPPV	SPPV	SPPV	SPPV	SPPV	SPPV	SPPV	SPPV	SPPV
	PT2020CAPXVIR_BP1	LSDV	LSDV	GTPV/ LSDV	ND	GTPV/ LSDV	GTPV/ LSDV	LSDV	LSDV	GTPV/ LSDV	GTPV/ LSDV	GTPV/ LSDV	LSDV	LSDV	LSDV	LSDV	LSDV
	PT2020CAPXVIR_TP1 (1)	LSDV	LSDV	GTPV/ LSDV	ND	GTPV/ LSDV	GTPV/ LSDV	LSDV	LSDV	GTPV/ LSDV	GTPV/ LSDV	GTPV/ LSDV	LSDV	LSDV	LSDV	LSDV	LSDV
	PT2020CAPXVIR_TP1 (2)	LSDV	LSDV	GTPV/ LSDV	ND	GTPV/ LSDV	GTPV/ LSDV	LSDV	LSDV	GTPV/ LSDV	GTPV/ LSDV	GTPV/ LSDV	LSDV	LSDV	LSDV	LSDV	LSDV
	PT2020CAPXVIR_TP2 (1)	SPPV	SPPV	SPPV	SPPV	SPPV	SPPV	SPPV	SPPV	SPPV	SPPV	SPPV	SPPV	SPPV	SPPV	SPPV	SPPV
	PT2020CAPXVIR_TP2 (2)	SPPV	SPPV	SPPV	SPPV	SPPV	SPPV	SPPV	SPPV	SPPV	SPPV	SPPV	SPPV	SPPV	SPPV	SPPV	SPPV
	PT2020CAPXVIR_BN1	Neg	Neg	Neg	Neg	Neg	Neg	Neg	Neg	Neg	Neg	Neg	Neg	Neg	Neg	Neg	Neg
PT2020CAPXVIR_TN1	Neg	Neg	Neg	Neg	Neg	Neg	Neg	Neg	Neg	Neg	Neg	Neg	Neg	Neg	Neg	Neg	
Information	Protocol / SOP used :	Lamien et al. 2011	UD	Lamien et al. 2011	in house	Lamien et al. 2011	Lamien et al. 2011	Lamien 2011	Vidanovic et al. 2016	Vidanovic et al (unpublished protocol)	Lamien et al. 2011 (Differentiation of SPPV from GTPV/LSD)	Gelaye et al. 2015 (Genotyping of Capripoxvirus based on sequencing of RPO30 gene)	in house	UD	Instruction of the kit		
	Producer Extraction protocol / kit:	Indical	QIAGEN	Indical	ThermoFisher Scientific	QIAGEN	QIAGEN	QIAGEN	UD	Sacace	Other	IDEXX	QIAGEN	UD	Indical		
	Name Extraction protocol / kit:	UD	UD	UD	UD	UD	UD	UD	UD	Sacace Viral Nucleic Acid Extraction Kit	Sacace Viral Nucleic Acid Extraction Kit	Sacace Viral Nucleic Acid Extraction Kit	UD	UD	UD		
	In-house modifications to extraction protocol (if yes, which?):	No	no	No	No	no	no	no	no	No	No	No	no	UD	UD		
	RT-PCR protocol/kit	Home made	Home made	Home made	Other - Other	Invitrogen-Platinum PCR Supermix	Go Taq Hot Start Green Master Mix (Promega)	Litech Made in the Russian Federation	Home made	Path-ID multiplex One-Step RT-PCR Kit	Path-ID multiplex One-Step RT-PCR Kit	Path-ID multiplex One-Step RT-PCR Kit	Home made	UD	UD		
	Methodology:	Dual Hybridization Probes Assay	UD	UD	Sequencing of RPO 030 gene/Gelaye E. et al. 2015	target: RPO30 of LSDV SPPV GTPV	Conventional PCR RPO30	UD	Manual	Primer-probe set for specific detection of SPPV (RT PCR)	Conventional PCR	Conventional PCR and sequencing	UD	UD	PCR		
	PCR Instrument used:	RotorGeneQ	Roche - Roche Light Cycler 480	UD	SimliAmp Thermal Cycler/Applied Biosystems Genetic Analyzer 3130	Applied Biosystems ProFlex	Applied Biosystems - ABI Verity	UD	Applied Biosystems - ABI 7500	Applied Biosystems - ABI QuantStudio5	Applied Biosystems - ABI Verity	Applied Biosystems - ABI Verity	UD	UD	UD		
Cut-off for positive:	40	UD	UD	UD	172= 172 bp (conventional PCR); 151= 151 bp (conventional PCR)	UD	UD	38	38	N/A	N/A	36	UD	UD			
remarks	N/A	N/A	N/A	N/A	N/A	N/A	N/A	N/A	N/A	N/A	N/A	N/A	N/A	N/A	N/A	N/A	

# Raw data DIVA

value	sample	Labnr 97506	Labnr 97600	Labnr 97602	Labnr 97604	Labnr 97608	Labnr 97609	Labnr 97610	Labnr 97611	Labnr 97612	Labnr 97613	Labnr 97617		
raw data	PT2020CAPXVIR_VP1	45		45		40				40				
	PT2020CAPXVIR_VP2	35,83	35,15	33,99		32,78	38,54			33	32,75			
	PT2020CAPXVIR_VP3	45	33,59	45		40		31,99		27,3	40			
	PT2020CAPXVIR_BP1	34,64	35,31	35,05		33,1	35,92		27,4	31,6	31,81			
	PT2020CAPXVIR_TP1 (1)	32,27	32,38	29,5		27,71	31,71		31,6	28,2	28,23			
	PT2020CAPXVIR_TP1 (2)	32,34	36,18	30,18		28,07	31,29		30	29,2	27,87			
	PT2020CAPXVIR_TP2 (1)	45	33,31	45		40		28,35		40				
	PT2020CAPXVIR_TP2 (2)	45	33,95	45		40		24,75		40				
	PT2020CAPXVIR_BN1	50	45	45		40	40			40				
PT2020CAPXVIR_TN1	50	45	45		40	40			40					
Final results	PT2020CAPXVIR_VP1	GTPV	ND	ND	ND	ND	ND	ND	GTPV Field	ND	ND	GTPV Field	GTPV Field	
	PT2020CAPXVIR_VP2	LSDV Field	LSDV Field	LSDV Field	Neg	LSDV Field	LSDV Field	LSDV Field	LSDV Field	LSDV Field	LSDV Field	LSDV Field	ND	ND
	PT2020CAPXVIR_VP3	SPPV Field	SPPV Field	ND	ND	SPPV Field	SPPV Field	SPPV Field	SPPV Field	ND	ND	SPPV Field	SPPV Field	SPPV Field
	PT2020CAPXVIR_BP1	LSDV Field	LSDV Field	LSDV Field	LSDV Field	LSDV Field	LSDV Field	LSDV Field	LSDV Field	LSDV Field	LSDV Field	LSDV Field	ND	ND
	PT2020CAPXVIR_TP1 (1)	LSDV Field	LSDV Field	LSDV Field	LSDV Field	LSDV Field	LSDV Field	LSDV Field	LSDV Field	LSDV Field	LSDV Field	LSDV Field	ND	ND
	PT2020CAPXVIR_TP1 (2)	LSDV Field	LSDV Field	LSDV Field	LSDV Field	LSDV Field	LSDV Field	LSDV Field	LSDV Field	LSDV Field	LSDV Field	LSDV Field	ND	ND
	PT2020CAPXVIR_TP2 (1)	SPPV Field	SPPV Field	ND	ND	ND	SPPV Field	SPPV Field	SPPV Vaccine	ND	ND	SPPV Field	SPPV Field	SPPV Field
	PT2020CAPXVIR_TP2 (2)	SPPV Field	SPPV Field	ND	ND	ND	SPPV Field	SPPV Field	SPPV Vaccine	ND	ND	SPPV Field	SPPV Field	SPPV Field
	PT2020CAPXVIR_BN1	Neg	Neg	Neg	Neg	Neg	Neg	Neg	Neg	Neg	Neg	Neg	Neg	Neg
PT2020CAPXVIR_TN1	Neg	Neg	Neg	Neg	Neg	Neg	Neg	Neg	Neg	Neg	Neg	Neg	Neg	
Information	Protocol / SOP used :	Agianniotaki et al. 2016 and in-house Taqman assay	confidential (from FLI Riems)	Vidanovic et al. 2016.	6.3.51.4 Capripoxviruses GPCR seq	Wolf et al. 2021	DIVA LSDV RT PCR//	SPPV WT sybr green/SPPV DIVA conventional PCR	Gelaye et al. 2015	Agianniotaki et al 2017	in house	Agianniotaki et al. 2017	Chibssa et al. 2018	Chibssa et al. 2018
	Producer Extraction protocol / kit:	Machery Nagel	Biosellal	ThermoFisher Scientific	Roche	QIAGEN	Indical	Indical	Indical	QIAGEN	QIAGEN	Indical	Indical	Indical
	In-house modifications to extraction protocol (if yes, which?):	Addition of EC to buffer B3	no	no	No	UD	no	no	no	no	no	No	No	No
	RT-PCR protocol / kit	Roche - Roche Light Cyclor 480	Quantitec multiplex PCR kit (Qiagen)	Quanti Fast Pathogen +IC kit (Qiagen)	home made	UD	UD	UD	UD	Thermofisher Taqman Fast Virus 1-Step Master Mix	ID.VET - ID GENE® LSD DIVA TRIPLEX	Home made	home made	home made
	Méthodologie	Taq Platinum	UD	UD	Gelaye E et al. 2015	LSDvac-ORF008; LSD field ORF126	Angianniotaki et al. 2017	Haegeman et al. 2015	Gelaye et al. 2015	Multiplex real time	PCR Real Time	Duplex real-time PCR with LNA TaqMan probes	conventional PCR	UD
	Machine PCR utilisée	Roche - Roche Light Cyclor 480	Bio-Rad - Bio-Rad CFX 96	RotorGeneQ	UD	Bio-Rad - Bio-Rad CFX 96	RotorgeneQ	Applied Biosystems - ABI Verity	RotorgeneQ	Agilent Technologies - Agilent AriaMx	Roche - Roche Light Cyclor 480	UD	Applied Biosystems AB Proflex	UD
	Remark(s):	N/A	N/A	N/A	N/A	N/A	N/A	N/A	N/A	N/A	N/A	N/A	N/A	N/A

value	sample	Labnr 97618	Labnr 97620	Labnr 97621	Labnr 97625	Labnr 97630	Labnr 97631	Labnr 97632	Labnr 97633	Labnr 97634	Labnr 97638	Labnr 97642
raw data	PT2020CAPXVIR_VP1			0	45				28	45		0
	PT2020CAPXVIR_VP2	35,3	32	35,3	29,38	27,18	36,56	33	31	31,32	23,68	32,68
	PT2020CAPXVIR_VP3			35	25,95				29	45		0
	PT2020CAPXVIR_BP1	36,93	35,54	36,6	29,97	29,84	35,64	35,4	32	31,12	24,21	33,32
	PT2020CAPXVIR_TP1 (1)	32,06	28,8	31,7	25,83	32,56	28,29	28,2	25	28,33	22,61	28,19
	PT2020CAPXVIR_TP1 (2)	32,52	28,36	31	26,78	30,21	29,7	29,2	25	27,53	22,41	28,05
	PT2020CAPXVIR_TP2 (1)			0	24,91				26	45		38,99
	PT2020CAPXVIR_TP2 (2)			35	26,35				25	45		39,56
	PT2020CAPXVIR_BN1			35	45		38			45		0
PT2020CAPXVIR_TN1			35	45		38			45		0	
Final results	PT2020CAPXVIR_VP1	ND	ND	ND	ND	ND	ND	ND	GTPV Field	ND	ND	ND
	PT2020CAPXVIR_VP2	LSDV Field	LSDV Field	LSDV Field	LSDV Field	LSDV Field	LSDV Field	LSDV Field	LSDV Field	LSDV Field	LSDV Field	LSDV Field
	PT2020CAPXVIR_VP3	ND	ND	ND	SPPV Field	ND	ND	ND	SPPV Field	ND	ND	ND
	PT2020CAPXVIR_BP1	LSDV Field	LSDV Field	LSDV Field	LSDV Field	LSDV Field	LSDV Field	LSDV Field	LSDV Field	LSDV Field	LSDV Field	LSDV Field
	PT2020CAPXVIR_TP1 (1)	LSDV Field	LSDV Field	LSDV Field	LSDV Field	LSDV Field	LSDV Field	LSDV Field	LSDV Field	LSDV Field	LSDV Field	LSDV Field
	PT2020CAPXVIR_TP1 (2)	LSDV Field	LSDV Field	LSDV Field	LSDV Field	LSDV Field	LSDV Field	LSDV Field	LSDV Field	LSDV Field	LSDV Field	LSDV Field
	PT2020CAPXVIR_TP2 (1)	ND	ND	ND	SPPV Field	ND	ND	ND	SPPV Field	ND	ND	ND
	PT2020CAPXVIR_TP2 (2)	ND	ND	ND	SPPV Field	ND	ND	ND	SPPV Field	ND	ND	ND
	PT2020CAPXVIR_BN1	Neg	Neg	Neg	Neg	Neg	Neg	Neg	Neg	Neg	Neg	Neg
PT2020CAPXVIR_TN1	Neg	Neg	Neg	Neg	Neg	Neg	Neg	Neg	Neg	Neg	Neg	
Information	Protocol / SOP used :	DIVA GREECE Agianniotaki et al 2017	manufacturer Instructions	Agianniotaki et al. 2017	Hoffmann	Id gene LSD DIVA Triplex	Vidanovic et al. 2016	Moller et al. 2019/ Vidanovic et al. 2021	in house	Vidanovic 2021	Instruction of the kit	Agianniotaki et al. 2017
	Producer Extraction protocol / kit:	ThermoFisher Scientific	QIAGEN	QIAGEN	QIAGEN	Indical	UD	Sacace	QIAGEN	UD	Indical	Maxwell RSC
	In-house modifications to extraction protocol (if yes, which?):	no	no	no	UD	IndiSpin Pahtogen kit	no	No	no	no	no	no
	RT-PCR protocol / kit	IVA Greece/kit PlatinumTM Taq DNA Polymerase (Invitrogen)	ID.VET - ID GENE® LSD DIVA TRIPLEX	Path-ID qPCR Master Mix (Thermo Fisher Scientific)	Applied Biosystems TaqMan Fast Universal PCR	ID.VET - ID GENE® LSD DIVA TRIPLEX	Home made	Path-ID multiplex One- Step RT-PCR Kit	Home Made - Home made	Other - Other	ID.VET - ID GENE® LSD DIVA TRIPLEX	Home Made - Home made
	Méthodologie	Real-time PCR method Agianniotaki et al 2017	UD	Duplex PCR LSD field / LSD vaccine	UD	UD	Manual	Primer-probe set specific for LSDV vaccine strains	UD	UD	UD	UD
	Machine PCR utilisée	Roche - Roche Light Cycler 480	Applied Biosystems - ABI QuantStudio5	Applied Biosystems - ABI 7500 Fast	UD	Applied Biosystems - ABI QuantStudio5	Applied Biosystems - ABI 7500	Applied Biosystems - ABI QuantStudio5	UD	Agilent Technologies - Agilent AriaMx	Applied Biosystems - ABI QuantStudio5	Bio-Rad - Bio- Rad CFX96 Touch
	Remark(s):	N/A	N/A	N/A	N/A	N/A	N/A	N/A	N/A	N/A	N/A	N/A

# Raw data ELISA

value	sample	Labnr										
		97506	97600	97602	97604	97605	97607	97608	97609	97610	97611	
OD	<b>positive kit control (mean)</b>	0,621	0,732	0,8245	0,709	0,771	0,718	0,773	0,9	0,74	0,074	
	<b>negative kit control (mean)</b>	0,046	0,052	0,0505	0,082	0,056	0,048	0,057	0,048	0,054	0,808	
	PT2021CAPXSER_SERN1	0,047	0,052	0,063	0,083	0,055	0,053	0,083	0,0501	0,055	0,058	
	PT2021CAPXSER_SERN2	0,046	0,053	0,056	0,095	0,06	0,054	0,07	0,0498	0,056	0,058	
	PT2021CAPXSER_SERP1	1,406	1,196	1,042	1,209	1,4	1,272	1,412	1,607	1,231	1,294	
	PT2021CAPXSER_SERP2	0,802	0,636	0,777	0,707	0,856	0,757	0,85	1,042	0,907	0,728	
	PT2021CAPXSER_SERP3	0,739	0,435	0,472	0,521	0,465	0,468	0,487	0,721	0,47	0,532	
	PT2021CAPXSER_SERP4	1,624	1,415	1,728	1,467	1,563	1,434	1,705	2,021	1,632	1,728	
	PT2021CAPXSER_SERP5	0,801	0,647	0,642	0,679	0,772	0,702	0,796	0,918	0,889	0,76	
	PT2021CAPXSER_SERP6	1,842	1,607	1,623	1,527	1,691	1,635	1,835	2,491	1,751	1,766	
PT2021CAPXSER_SERP7	1,659	1,431	1,675	1,428	1,605	1,489	1,611	2,296	1,697	1,509		
PT2021CAPXSER_SERP8	1,417	1,228	1,282	1,197	1,42	1,246	1,361	1,897	1,591	1,497		
	PT2021CAPXSER_SERN1	0,209	0	1,615	0,159	-0,140	0,746	3,631	0,246	0,146	-2,180	
	PT2021CAPXSER_SERN2	-0,104	0,147	2,071	2,073	0,559	0,896	1,816	0,211	0,292	-2,180	
	PT2021CAPXSER_SERP1	236,628	168,235	128,101	179,745	187,972	182,687	189,246	182,981	171,574	166,213	
	PT2021CAPXSER_SERP2	131,442	85,882	93,863	99,681	111,888	105,821	110,754	116,667	124,344	89,101	
	PT2021CAPXSER_SERP3	120,619	56,324	54,457	70,016	57,203	62,687	60,056	78,991	60,641	62,398	
	PT2021CAPXSER_SERP4	277,693	200,441	216,731	220,893	210,769	206,866	230,168	231,573	230,029	225,341	
	PT2021CAPXSER_SERP5	131,338	87,5	76,421	95,215	100,140	97,612	103,212	102,113	121,720	93,460	
	PT2021CAPXSER_SERP6	312,546	228,676	203,165	230,463	228,671	236,866	248,324	286,737	247,376	230,518	
PT2021CAPXSER_SERP7	280,633	202,794	209,884	214,673	216,643	215,075	217,039	263,850	239,504	195,504		
PT2021CAPXSER_SERP8	238,437	172,941	159,109	177,831	190,769	178,806	182,123	217,019	224,052	193,869		
	PT2021CAPXSER_SERN1	NEG	NEG	NEG	NEG	NEG	NEG	NEG	NEG	NEG	NEG	
	PT2021CAPXSER_SERN2	NEG	NEG	NEG	NEG	NEG	NEG	NEG	NEG	NEG	NEG	
	PT2021CAPXSER_SERP1	POS	POS	POS	POS	POS	POS	POS	POS	POS	POS	
	PT2021CAPXSER_SERP2	POS	POS	POS	POS	POS	POS	POS	POS	POS	POS	
	PT2021CAPXSER_SERP3	POS	POS	POS	POS	POS	POS	POS	POS	POS	POS	
	PT2021CAPXSER_SERP4	POS	POS	POS	POS	POS	POS	POS	POS	POS	POS	
	PT2021CAPXSER_SERP5	POS	POS	POS	POS	POS	POS	POS	POS	POS	POS	
	PT2021CAPXSER_SERP6	POS	POS	POS	POS	POS	POS	POS	POS	POS	POS	
PT2021CAPXSER_SERP7	POS	POS	POS	POS	POS	POS	POS	POS	POS	POS		
PT2021CAPXSER_SERP8	POS	POS	POS	POS	POS	POS	POS	POS	POS	POS		
Information	<b>Name ELISA kit producer:</b>	ID.VET	ID.VET	ID.VET	ID.VET	ID.VET	ID.VET	ID.VET	ID.VET	ID.VET	ID.VET	
	<b>Name ELISA kit:</b>	ID SCREEN® CAPRIPOX DOUBLE ANTIGEN MULT- SPECIES	ID SCREEN® CAPRIPOX DOUBLE ANTIGEN MULT- SPECIES	ID SCREEN® CAPRIPOX DOUBLE ANTIGEN MULT- SPECIES	ID SCREEN® CAPRIPOX DOUBLE ANTIGEN MULT- SPECIES	ID SCREEN® CAPRIPOX DOUBLE ANTIGEN MULT- SPECIES	ID SCREEN® CAPRIPOX DOUBLE ANTIGEN MULT- SPECIES	ID SCREEN® CAPRIPOX DOUBLE ANTIGEN MULT- SPECIES	ID SCREEN® CAPRIPOX DOUBLE ANTIGEN MULT- SPECIES	ID SCREEN® CAPRIPOX DOUBLE ANTIGEN MULT- SPECIES	ID SCREEN® CAPRIPOX DOUBLE ANTIGEN MULT- SPECIES	ID SCREEN® CAPRIPOX DOUBLE ANTIGEN MULT- SPECIES
	<b>Short or long incubation protocol (if applicable):</b>	/	short	short	/	short	/	short	/	/	/	
	<b>Formula to calculate the normalized data:</b>	ELISA Indirect	ELISA Indirect	ELISA Indirect	ELISA Indirect	ELISA Indirect	ELISA Indirect	ELISA Indirect	ELISA Indirect	ELISA Indirect	ELISA Indirect	
	<b>Used Cut-off :</b>	30,00	30,00	30,00	30,00	30,00	30,00	30,00	30,00	30,00	30,00	
	<b>Batch ELISA kit:</b>	E83	H35	H35	H35	H35	H35	H35	H35	H35	H35	
<b>Remark(s)</b>	N/A	N/A	N/A	N/A	N/A	N/A	N/A	N/A	N/A	N/A		

value		Labnr										
sample		97612	97613	97614	97615	97616	97617	97618	97619	97620	97621	97622
OD	positive kit control (mean)	0,943	0,883	0,926	0,749	0,888	0,817	1,17	0,882	0,825	0,706	0,927
	negative kit control (mean)	0,044	0,081	0,0475	0,053	0,051	0,063	0,075	0,051	0,0485	0,062	0,077
	PT2021CAPXSER_SERN1	0,044	0,056	0,049	0,058	0,055	0,076	1,27	0,055	0,055	0,051	0,073
	PT2021CAPXSER_SERN2	0,045	0,05	0,06	0,053	0,053	0,07	1,55	0,053	0,052	0,052	0,079
	PT2021CAPXSER_SERP1	1,569	1,29	1,739	1,487	1,572	1,563	179,54	1,454	1,466	1,33	1,473
	PT2021CAPXSER_SERP2	0,931	0,887	1,011	0,895	0,83	1,094	86,94	0,95	1,081	0,787	0,838
	PT2021CAPXSER_SERP3	0,585	0,559	0,745	0,534	0,514	0,63	52,51	0,549	0,752	0,429	0,527
	PT2021CAPXSER_SERP4	1,932	1,657	1,994	1,766	1,765	1,908	192,5	1,757	1,933	1,503	1,699
	PT2021CAPXSER_SERP5	0,861	0,79	0,968	0,823	0,751	1,044	87,58	0,915	0,9	0,795	0,816
	PT2021CAPXSER_SERP6	2,059	1,755	2,129	0,895	1,83	1,998	219,27	1,897	2,2	1,631	1,829
PT2021CAPXSER_SERP7	1,942	1,728	2,13	1,895	1,726	1,911	201,55	1,79	2,039	1,625	1,591	
PT2021CAPXSER_SERP8	1,795	1,499	1,717	1,548	1,417	1,789	173,05	1,477	1,665	1,366	1,521	
	PT2021CAPXSER_SERN1	0,000	-3,117	0,171	0,718	0,478	1,724	109,132	0,481	0,837	-1,708	-0,471
	PT2021CAPXSER_SERN2	0,111	-3,865	1,423	0,000	0,239	0,928	134,703	0,241	0,451	-1,553	0,235
	PT2021CAPXSER_SERP1	169,633	150,748	192,544	206,034	181,720	198,939	16389,498	168,833	182,550	196,894	164,235
	PT2021CAPXSER_SERP2	98,665	100,499	109,676	120,977	93,070	136,737	7932,877	108,183	132,968	112,578	89,529
	PT2021CAPXSER_SERP3	60,178	59,601	79,397	69,109	55,317	75,199	4788,584	59,928	90,599	56,988	52,941
	PT2021CAPXSER_SERP4	210,011	196,509	221,571	246,121	204,779	244,695	17573,059	205,295	242,692	223,758	190,824
	PT2021CAPXSER_SERP5	90,879	88,404	104,781	110,632	83,632	130,106	7991,324	103,971	109,659	113,82	86,941
	PT2021CAPXSER_SERP6	224,138	208,728	236,938	120,977	212,545	256,631	20017,808	222,142	277,077	243,634	206,118
	PT2021CAPXSER_SERP7	211,123	205,362	237,052	264,655	200,119	245,093	18399,543	209,266	256,343	242,702	178,118
PT2021CAPXSER_SERP8	194,772	176,808	190,040	214,799	163,202	228,912	15796,804	171,600	208,178	202,484	169,882	
	PT2021CAPXSER_SERN1	NEG	NEG	NEG	NEG	NEG	NEG	NEG	NEG	NEG	NEG	NEG
	PT2021CAPXSER_SERN2	NEG	NEG	NEG	NEG	NEG	NEG	NEG	NEG	NEG	NEG	NEG
	PT2021CAPXSER_SERP1	POS	POS	POS	POS	POS	POS	POS	POS	POS	POS	POS
	PT2021CAPXSER_SERP2	POS	POS	POS	POS	POS	POS	POS	POS	POS	POS	POS
	PT2021CAPXSER_SERP3	POS	POS	POS	POS	POS	POS	POS	POS	POS	POS	POS
	PT2021CAPXSER_SERP4	POS	POS	POS	POS	POS	POS	POS	POS	POS	POS	POS
	PT2021CAPXSER_SERP5	POS	POS	POS	POS	POS	POS	POS	POS	POS	POS	POS
	PT2021CAPXSER_SERP6	POS	POS	POS	POS	POS	POS	POS	POS	POS	POS	POS
	PT2021CAPXSER_SERP7	POS	POS	POS	POS	POS	POS	POS	POS	POS	POS	POS
PT2021CAPXSER_SERP8	POS	POS	POS	POS	POS	POS	POS	POS	POS	POS	POS	
Information	Name ELISA kit producer:	ID.VET	ID.VET	ID.VET	ID.VET	ID.VET	ID.VET	ID.VET	ID.VET	ID.VET	ID.VET	ID.VET
	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	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	ID SCREEN® CAPRIPox DOUBLE ANTIGEN MULTI-SPECIES	ID SCREEN® CAPRIPox DOUBLE ANTIGEN MULTI-SPECIES	ID SCREEN® CAPRIPox DOUBLE ANTIGEN MULTI-SPECIES
	Short or long incubation protocol (if applicable):	/	short	short	/	short	/	long	/	/	short	/
	Formula to calculate the normalized data:	ELISA Indirect	ELISA Indirect	ELISA Indirect	ELISA Indirect	ELISA Indirect	ELISA Indirect	ELISA Indirect	ELISA Indirect	ELISA Indirect	ELISA Indirect	ELISA Indirect
	Used Cut-off :	30,00	30,00	30,00	30,00	30,00	30,00	30,00	30,00	30,00	30,00	30,00
	Batch ELISA kit:	G66	H35	G66	E83	H35	E83	H35	E83	E83	H35	H35
Remark(s)	N/A	N/A	N/A	N/A	N/A	Batch expired. Extra controls were added to validate the assay	N/A	N/A	N/A	N/A	N/A	

value	sample	97630	97631	97632	Labnr 97633	97634	97638	97642
OD	<b>positive kit control (mean)</b>	0,69	0,926	1,013	0,941	1,109	0,992	0,869
	<b>negative kit control (mean)</b>	0,05	0,042	0,041	0,064	0,152	0,048	0,057
	PT2021CAPXSER_SERN1	0,05	0,044	0,041	0,064	0,127	0,049	0,051
	PT2021CAPXSER_SERN2	0,05	0,0495	0,044	0,066	0,101	0,05	0,042
	PT2021CAPXSER_SERP1	1,25	1,6365	1,617	1,693	1,568	1,699	1,802
	PT2021CAPXSER_SERP2	0,73	1,087	1,064	1,36	1,169	1,108	1,059
	PT2021CAPXSER_SERP3	0,33	0,9105	0,65	0,758	0,8095	0,739	0,563
	PT2021CAPXSER_SERP4	1,26	1,9545	1,828	2,153	2,0295	1,9	1,866
	PT2021CAPXSER_SERP5	0,7	0,9225	0,97	1,168	1,0825	1,126	0,865
	PT2021CAPXSER_SERP6	1,49	2,2055	2,025	2,454	2,1545	2,332	2,236
	PT2021CAPXSER_SERP7	1,42	2,088	1,929	1,689	2,1725	1,949	1,99
	PT2021CAPXSER_SERP8	1,21	1,939	1,787	1,952	1,885	1,864	1,749
	PT2021CAPXSER_SERN1	0	0,226	0	0	-2,612	0,106	-0,739
	PT2021CAPXSER_SERN2	0	0,848	0,309	0,228	-5,329	0,212	-1,847
	PT2021CAPXSER_SERP1	187,5	180,373	162,14	185,747	147,962	174,894	214,901
	PT2021CAPXSER_SERP2	106,25	118,213	105,247	147,777	106,27	112,288	123,399
	PT2021CAPXSER_SERP3	43,75	98,247	62,654	79,133	68,704	73,199	62,315
	PT2021CAPXSER_SERP4	189,063	216,346	183,848	238,198	196,186	196,186	222,783
	PT2021CAPXSER_SERP5	101,563	99,604	95,576	125,884	97,231	114,195	99,507
	PT2021CAPXSER_SERP6	225	244,74	204,115	272,52	209,248	241,949	268,350
	PT2021CAPXSER_SERP7	214,063	231,448	194,239	185,291	211,139	201,377	238,054
	PT2021CAPXSER_SERP8	181,25	214,593	179,63	215,279	181,452	192,373	208,374
	PT2021CAPXSER_SERN1	NEG	NEG	NEG	NEG	NEG	NEG	NEG
	PT2021CAPXSER_SERN2	NEG	NEG	NEG	NEG	NEG	NEG	NEG
	PT2021CAPXSER_SERP1	POS	POS	POS	POS	POS	POS	POS
	PT2021CAPXSER_SERP2	POS	POS	POS	POS	POS	POS	POS
	PT2021CAPXSER_SERP3	POS	POS	POS	POS	POS	POS	POS
	PT2021CAPXSER_SERP4	POS	POS	POS	POS	POS	POS	POS
	PT2021CAPXSER_SERP5	POS	POS	POS	POS	POS	POS	POS
	PT2021CAPXSER_SERP6	POS	POS	POS	POS	POS	POS	POS
	PT2021CAPXSER_SERP7	POS	POS	POS	POS	POS	POS	POS
	PT2021CAPXSER_SERP8	POS	POS	POS	POS	POS	POS	POS
Information	<b>Name ELISA kit producer:</b>	ID.VET	ID.VET	ID.VET	ID.VET	ID.VET	ID.VET	ID.VET
	<b>Name ELISA kit:</b>	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	ID SCREEN® CAPRIPOX DOUBLE ANTIGEN MULTI- SPECIES	ID SCREEN® CAPRIPOX DOUBLE ANTIGEN MULTI- SPECIES	ID SCREEN® CAPRIPOX DOUBLE ANTIGEN MULTI- SPECIES
	<b>Short or long incubation protocol (if applicable):</b>	/	/	short	/	short	short	/
	<b>Formula to calculate the normalized data:</b>	ELISA Indirect	ELISA Indirect	ELISA Indirect	ELISA Indirect	ELISA Indirect	ELISA Indirect	ELISA Indirect
	<b>Used Cut-off :</b>	30,00	30,00	30,00	30,00	30-40	30,00	30,00
	<b>Batch ELISA kit:</b>	H35	G66	G66	H35	G66	G66	H35
	<b>Remark(s)</b>	N/A	N/A	N/A	N/A	N/A	N/A	N/A

Raw data VN

value		Labnr				
sample		97506	97600	97612	97618	97633
Antibody titer	positive kit control (mean)	0,025	160	20	20	128
	negative kit control (mean)	2	5	5	5	8
	PT2021CAPXSER_SERN1	0,1	5	5	5	8
	PT2021CAPXSER_SERN2	0,1	5	5	5	8
	PT2021CAPXSER_SERP1	0,0008	160	80	320	32
	PT2021CAPXSER_SERP2	0,0008	320	20	1280	64
	PT2021CAPXSER_SERP3	0,0013	240	10	640	16
	PT2021CAPXSER_SERP4	0,004	80	160	160	32
	PT2021CAPXSER_SERP5	0,0013	480	20	320	128
	PT2021CAPXSER_SERP6	0,00016	320	320	1280	128
PT2021CAPXSER_SERP7	0,004	320	160	640	32	
PT2021CAPXSER_SERP8	0,0013	160	80	80	32	
Information	PT2021CAPXSER_SERN1	NEG	NEG	NEG	NEG	NEG
	PT2021CAPXSER_SERN2	NEG	NEG	NEG	NEG	NEG
	PT2021CAPXSER_SERP1	POS	POS	POS	POS	POS
	PT2021CAPXSER_SERP2	POS	POS	POS	POS	POS
	PT2021CAPXSER_SERP3	POS	POS	POS	POS	POS
	PT2021CAPXSER_SERP4	POS	POS	POS	POS	POS
	PT2021CAPXSER_SERP5	POS	POS	POS	POS	POS
	PT2021CAPXSER_SERP6	POS	POS	POS	POS	POS
	PT2021CAPXSER_SERP7	POS	POS	POS	POS	POS
PT2021CAPXSER_SERP8	POS	POS	POS	POS	POS	
Information	Protocol/SOP used	In house	OIE Manual 3.4.12	In house	Pirbright	In house
	Name (+Reference) cell type used	OA3.Ts	ESH	OA3.Ts	OA3.Ts	UD
	Starting dilution of PT serum samples tested:	100,00	5	0,2	0,2	2
	Virus dose used in test (TCID50): e.g. 100 TCID50	100 TCID50	84 TCID50	100 TCID50	100 TCID50	100 TCID50
	Positive control serum used:	R6F 45dpi (LSDV) and S1F 15 dpi (SPPV)	Sheep 1341: home made	In house	Antiserum Pirbright	experimental bovine serum.32 day p.i.
	Cut-off for positive:	0,02	5	5	0,2	8
	Name (+ reference) virus strain used:	LSDV Neethling or B1/10	LSDV Neethling	LSDV Neethling	LSDV Neethling	LSDV Dagestan
	Dilutions of PT serum samples tested:	0,5	2	0,2-0,0016	0,2-0,0008	2
	Expected antibody titer in positive control serum:	0,025	120	20	20	128



# Raw data IPMA

value	sample	Labnr	
		97506	97618
Antibody titer	<b>positive kit control (mean)</b>	50	300
	<b>negative kit control (mean)</b>	0	0
	PT2021CAPXSER_SERN1	0	0
	PT2021CAPXSER_SERN2	0	0
	PT2021CAPXSER_SERP1	50	300
	PT2021CAPXSER_SERP2	50	300
	PT2021CAPXSER_SERP3	50	300
	PT2021CAPXSER_SERP4	50	300
	PT2021CAPXSER_SERP5	50	300
	PT2021CAPXSER_SERP6	50	300
PT2021CAPXSER_SERP7	50	300	
PT2021CAPXSER_SERP8	50	300	
	PT2021CAPXSER_SERN1	NEG	NEG
	PT2021CAPXSER_SERN2	NEG	NEG
	PT2021CAPXSER_SERP1	POS	POS
	PT2021CAPXSER_SERP2	POS	POS
	PT2021CAPXSER_SERP3	POS	POS
	PT2021CAPXSER_SERP4	POS	POS
	PT2021CAPXSER_SERP5	POS	POS
	PT2021CAPXSER_SERP6	POS	POS
	PT2021CAPXSER_SERP7	POS	POS
PT2021CAPXSER_SERP8	POS	POS	
Information	<b>Protocol/SOP used</b>	in house	EURL Capripoxviruses Protocol
	<b>Name (+ reference) cell type used:</b>	OA3.Ts	OA3.Ts
	<b>Starting dilution of PT serum samples tested:</b>	0,02	0,02
	<b>Virus dose used in test (TCID50): e.g. 100 TCID50</b>	100 TCID50	100 TCID50
	<b>Positive control serum used:</b>	R6F 45dpi (LSDV) and S6F (SPPV)	Antiserum Pirbright
	<b>Secondary antibody (+reference) used for staining:</b>	Anti bovine IgG (g) whole molecule -peroxidase produced in rabbit (Sigma A5295)	Anti-Bovine Ig G Peroxidase antibody produced in rabbit A 5295 SIGMA
	<b>Cut-off for positive:</b>	50	50
	<b>Name (+ reference) virus strain used:</b>	LSDV Neethling	LSDV Neethling
	<b>Dilutions of PT serum samples tested:</b>	1/50	1/50-1/300
	<b>Expected antibody titer in positive control serum:</b>	0,0025	50
<b>Dilution of secondary antibody used:</b>	0,0001	0,001	

### VIII. Annex 2 Boxplots (not under accreditation)

Besides qualitative data analysis (positive, negative or non-interpretable result), also quantitative data analysis was performed.

The quantitative data analysis in this report was not used to evaluate the participants in this PT, but should only be considered as educational information for the participants in order to evaluate their performance and/or to standardize their different diagnostic tests.

For the Pan-capripox PCR, box plots of the ct values per reference sample and per participating laboratory are shown in Figures 1a, 1b, 2a and 2b. Only the samples PT2021CAPXVIR\_TP1 and PT2021CAPXVIR\_TP2 are included in the figure because there was only one aliquot in the panel for the other samples.

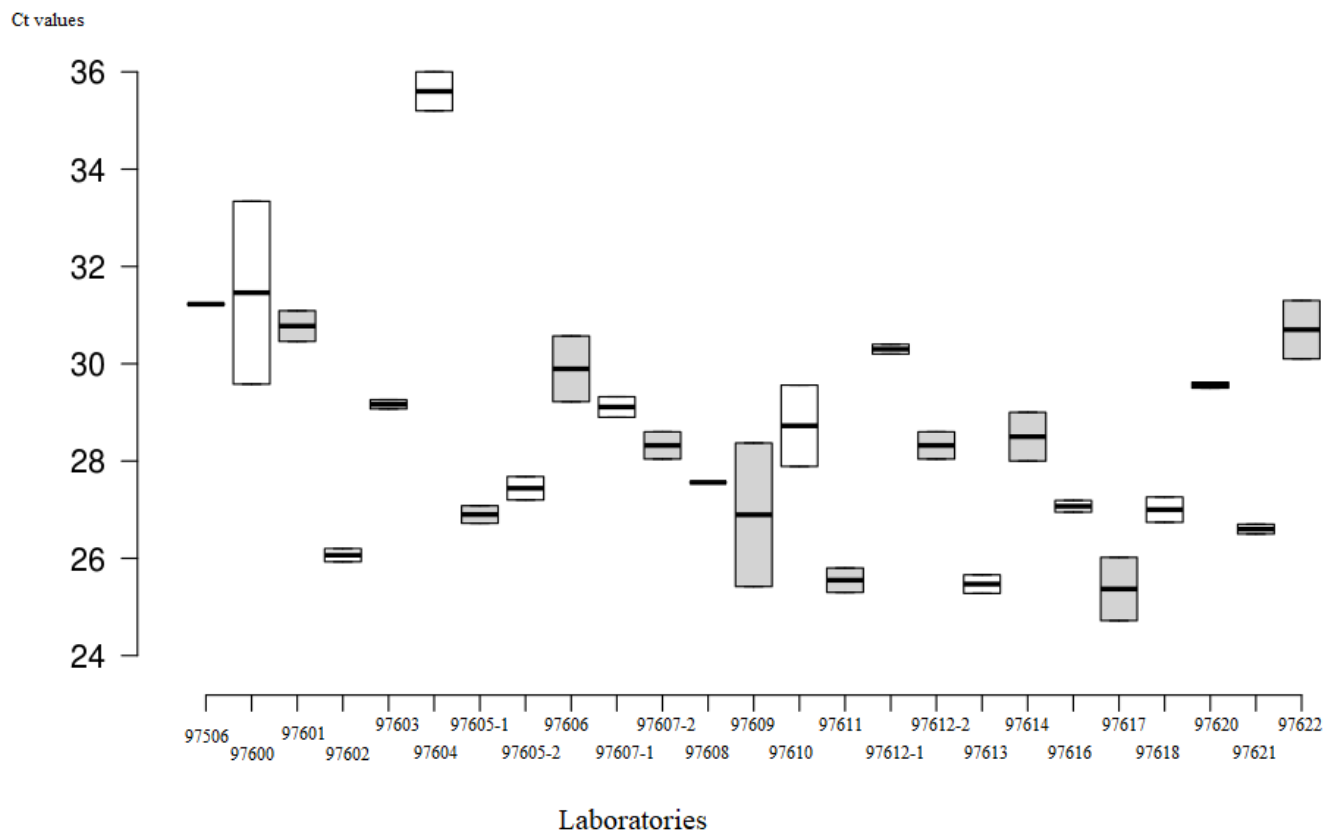


Figure 1a : Distribution of the Ct values data (boxplots) for the PTCAPXVIR\_TP1 samples (LAB97506-LAB97622). Data are represented for both the primary and secondary PCR (if performed).

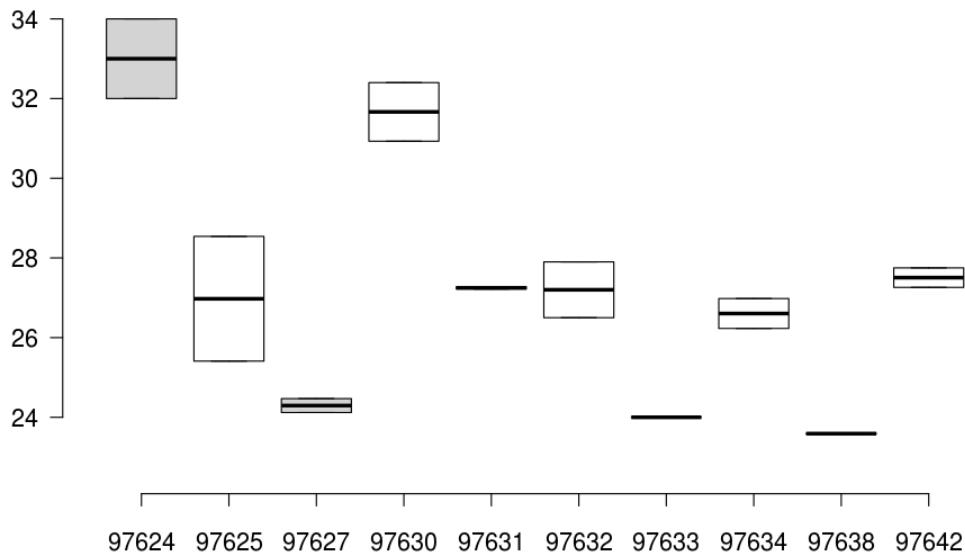


Figure 1b : Distribution of the Ct values data (boxplots) for the PTCAPXVIR\_TP1 samples (LAB97624-LAB97642).

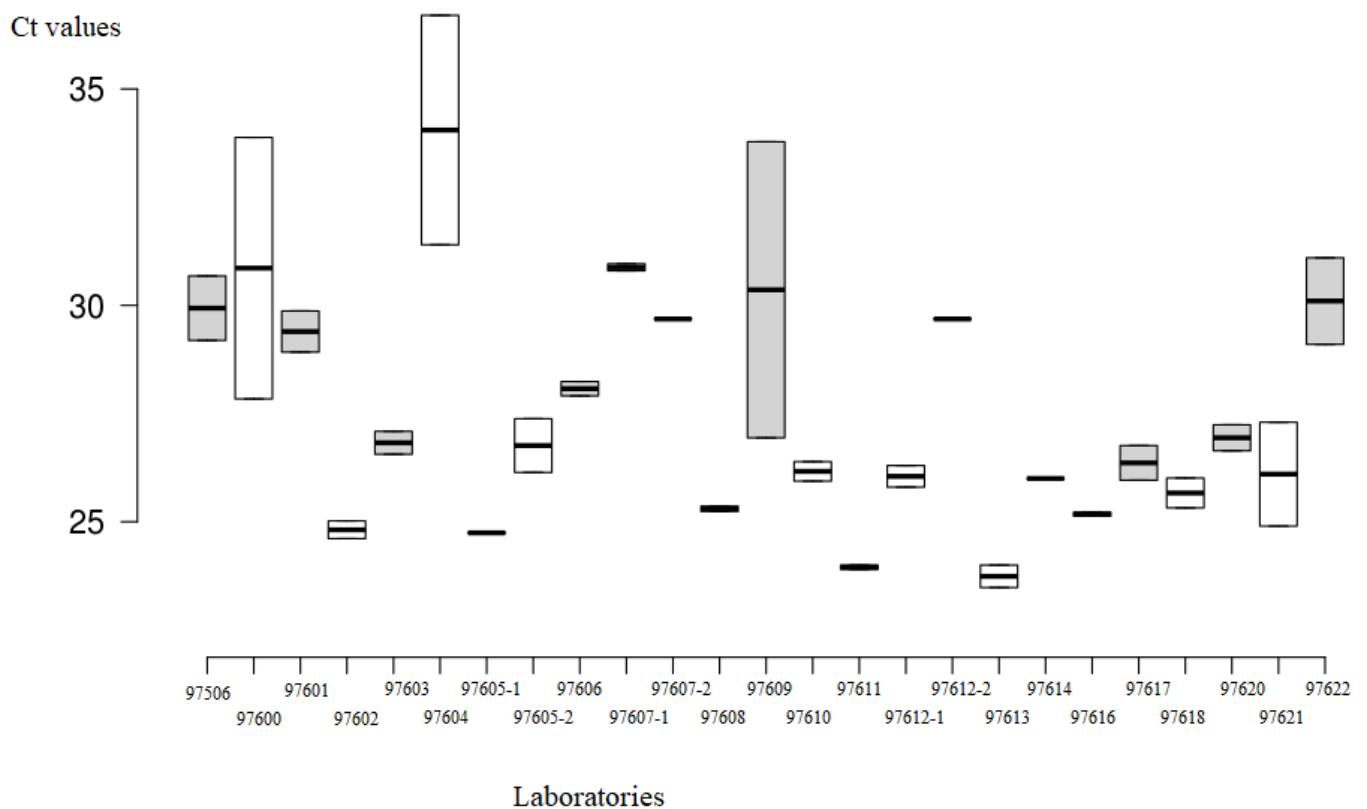


Figure 2a : Distribution of the Ct values data (boxplots) for the PTCAPXVIR\_TP2 samples (LAB97506-LAB97622). Data are represented for both the primary and secondary PCR (if performed).

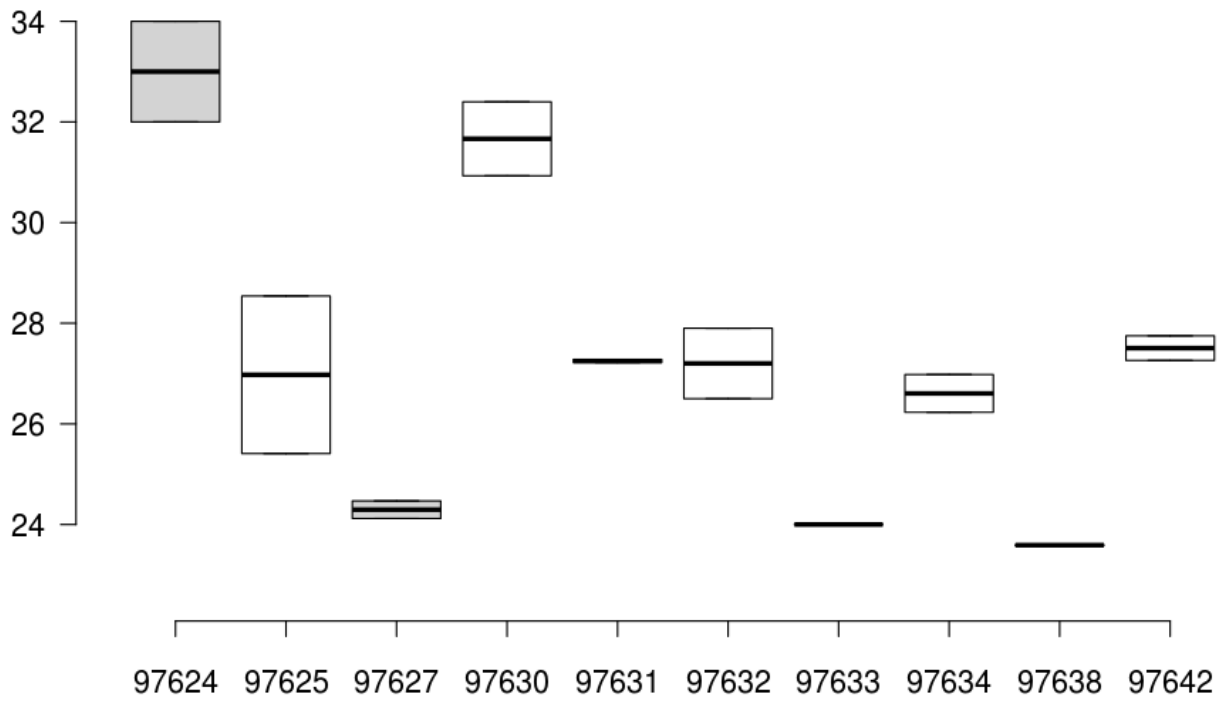
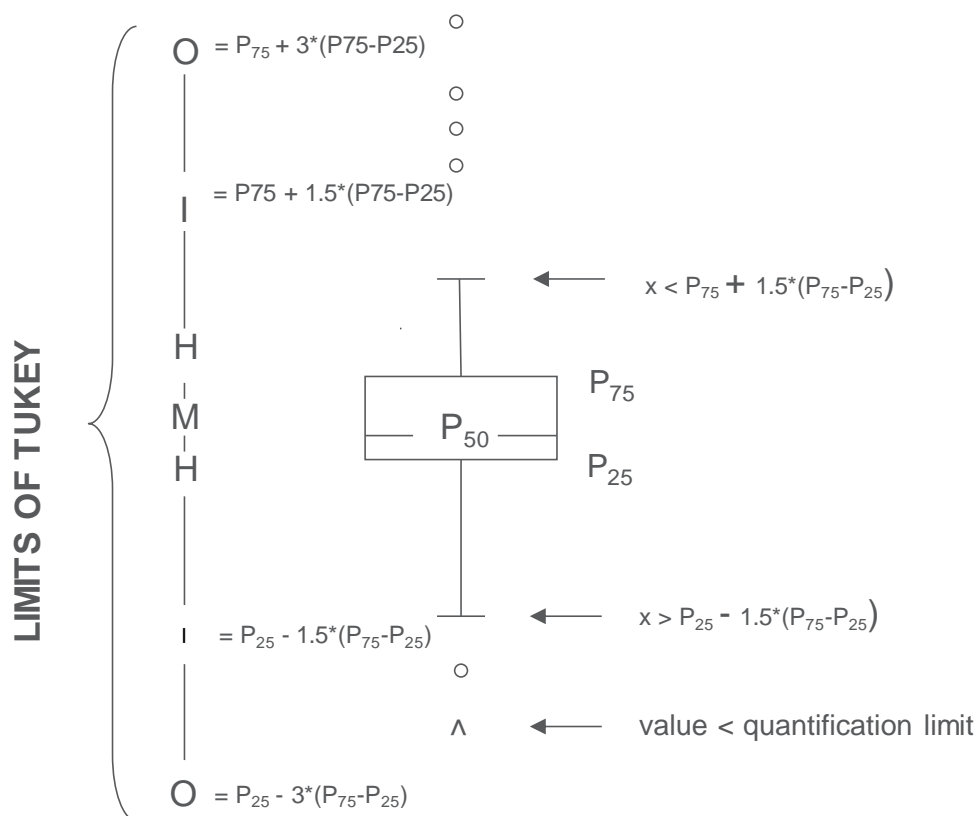


Figure 2b : Distribution of the Ct values data (boxplots) for the PTAPXVIR\_TP2 samples (LAB97624-LAB97642).

## 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

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