BIOLOGICAL HEALTH RISKS
QUALITY OF LABORATORIES

COMMITEE OF EXPERTS

EXTERNAL QUALITY ASSESSMENT
IN VETERINARY DIAGNOSIS

DEFINITIVE GLOBAL REPORT

VETERINARY MEDICINE

CAPRIPOX (CAPX)

SURVEY 2022/5

Sciensano/PT VET CAPX/4-E

Biological health risks
Quality of laboratories
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1 INTRODUCTION

Details relevant to the proficiency test (PT) are available in the procedure SOP 2.5/01 'Management of the proficiency tests organized by the scientific directorate infectious diseases in animals'. The PT was organized according to the ISO17043 'Conformity assessment - General requirements for proficiency testing' norm.

2 AIM

The aim of this PT was to evaluate the ability of the participating laboratories to identify the absence or presence of antibodies to capripox (CAPX) viruses in serum of ruminants (Serology component of the PT: PT2022CAPXSER) and/or to assess the ability of the participating laboratories to detect CAPX virus DNA in different matrices (virology component of the PT: PT2022CAPXVIR).

3 MATERIALS AND METHODS

3.1 Performance of diagnostic tests

Within the **serology component** of the PT, participants were asked to test predefined serum samples using their primary diagnostic assay(s) for serological diagnosis. Within the **virology component** of the PT, participants were asked to test predefined 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 tests in the serology part and all parts in the virology part are individually scored. This is in contrast with previous PT's, where only the final diagnostic was scored. Only participants that submitted results for all samples in a specific part of the virological component receive a final evaluation for that part.
All participants received a supporting document containing a background of the samples (species from which the sample is taken and type of sample).

3.2 Reference samples

Twenty-nine laboratories received the PT2022CAPXSER panel containing 10 aliquots of serum and thirty-four laboratories received the PT2022CAPXVIR panel containing 10 aliquots of blood, cell culture supernatant and tissue suspension.
The samples were prepared by the European Union Reference Laboratory (EURL) for diseases caused by capripox viruses, Scientific Directorate Infectious Diseases in Animals, Sciensano. Afterwards, the PT panels were prepared separately and within each panel samples were randomized from 1 to 10, by the Quality of Laboratories, Sciensano.

3.2.1 PT2022CAPXSER PANEL: REFERENCE SERUM SAMPLES

3.2.1.1 Origin of the samples

Replicates of 10 reference serum samples, either free from detectable antibodies to capripox viruses (n=2; coded PT20222022CAPXSER_SERN1 and PT20222022CAPXSER_SERN2) or containing detectable antibodies to capripox viruses (n = 8; coded PT20222022CAPXSER_SERP1, PT20222022CAPXSER_SERP2, PT20222022CAPXSER_SERP3, PT20222022CAPXSER_SERP4, PT20222022CAPXSER_SERP5, PT20222022CAPXSER_SERP6, PT20222022CAPXSER_SERP7 and PT20222022CAPXSER_SERP8) were used.

In total, 290 aliquots were distributed to 29 participating laboratories. PTCAPXSER_SERP2 and PTCAPXSER_SERP3 were dilutions of respectively PTCAPXSER_SERP1 of ½ and 1/5. PT20222022CAPXSER_SERP6 and PT20222022CAPXSER_SERP7 were dilutions of respectively 1/2 and 1/5 of PT20222022CAPXSER_SERP5. The participants received 10 aliquots: 1 aliquot of each sample. The positions of the reference samples were randomized for each participant.

For each serum sample, the status was determined based on the background of the animals from which the samples originated and the results obtained during pre-verification, hereby using the ELISA ID Screen® Capripox Double Antigen Multi-species (ID.Vet), the immunoperoxidase monolayer assay (IPMA) (Haegeman *et al.* 2020) and the virus neutralization test with the serum titrated against a constant titer of capripox virus (VNT).

Table 1: Origin of the samples in the PTCAPX2022 panel. (GTPV = goatpox virus; LSDV = lumpy skin disease virus; SPPV = sheeppox virus; POS = positive; NEG = negative).

Sample ID	Origin	Background
PT2022CAPXSER_SERP1	Ovine	SPPV infected
PT2022CAPXSER_SERP2	Ovine	SPPV infected
PT2022CAPXSER_SERP3	Ovine	SPPV infected
PT2022CAPXSER_SERP4	Bovine	LSDV Infected
PT2022CAPXSER_SERP5	Bovine	LSDV vaccinated + infected
PT2022CAPXSER_SERP6	Bovine	LSDV vaccinated + infected
PT2022CAPXSER_SERP7	Bovine	LSDV vaccinated + infected
PT2022CAPXSER_SERP8	Bovine	Vaccinated LSDV
PT2022CAPXSER_SERN1	Ovine	Commercial serum
PT2022CAPXSER_SERN2	Bovine	Commercial serum

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 VNT. For the ELISA, the same qualitative result was obtained for all 10 aliquots of the same reference serum sample. When performing IPMA with heterologous virus by the EURL, a difference in status was found for samples PT2022CAPXSER_SERP1, PT2022CAPXSER_SERP2 and PT2022CAPXSER_SERP3 compared to the homologous virus. The IPMA status of these samples was therefore considered as positive or doubtful for these 3 samples. Sample PTCAPX2022SER_SERP8 was considered as doubtful in the VNT, because four aliquots were negative and six positive during homogeneity testing.

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 VNT.

The reference serum samples PT2022CAPXSER_SERN1 and PT2022CAPXSER_SERN2 were considered as negative samples in all tests. In the ELISA ID Screen® Capripox Double Antigen Multi-species (ID.Vet) the reference serum samples, PT2022CAPXSER_SERP1, PT2022CAPXSER_SERP2, PT2022CAPXSER_SERP3, PT2022CAPXSER_SERP4, PT2022CAPXSER_SERP5, PT2022CAPXSER_SERP6, PT2022CAPXSER_SERP7 and PT2022CAPXSER_SERP8 as positive samples. In the IPMA, the reference serum PT2022CAPXSER_SERP1, PT2022CAPXSER_SERP2 and PT2022CAPXSER_SERP3 were considered as doubtful, while all other reference serum samples had the same status as in the ELISA. For the VNT, PT2022CAPXSER_SERP8 was considered as doubtful, while all other reference serum samples have the same status as in the ELISA.

3.2.1.2 Final sample status

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

Table 2: The final status of each sample in the PT2022CAPXSER panel (POS = positive; NEG = negative; NI = doubtful).

Sample ID	ELISA	IPMA	VN
PT2022CAPXSER_SERP1	POS	NI	POS
PT2022CAPXSER_SERP2	POS	NI	POS
PT2022CAPXSER_SERP3	POS	NI	POS
PT2022CAPXSER_SERP4	POS	POS	POS
PT2022CAPXSER_SERP5	POS	POS	POS
PT2022CAPXSER_SERP6	POS	POS	POS
PT2022CAPXSER_SERP7	POS	POS	POS
PT2022CAPXSER_SERP8	POS	POS	NI
PT2022CAPXSER_SERN1	NEG	NEG	NEG
PT2022CAPXSER_SERN2	NEG	NEG	NEG

3.2.1.3 Randomization and panel composition

The samples were randomized differently for each laboratory, an overview of the randomization can be found in the preliminary report.
The PTCAPXSER2022 panel was constituted of 10 samples of 500µl.

3.2.1.4 Stability

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

3.2.2 PT2022CAPXVIR PANEL: REFERENCE CELL CULTURE SUPERNATANT, BLOOD AND TISSUE HOMOGENATE SAMPLES

3.2.2.1 Origin of the samples

Replicates of 2 reference cell culture supernatants containing detectable capripox virus DNA (n = 2; coded PT2022CAPXVIR_VP1 andPT20CAPXVIR_VP2) were used as well as replicates of 3 reference blood samples, either free from detectable capripox virus DNA (n = 1; coded PT20222022CAPXVIR_BN1) or containing detectable capripox virus DNA (n = 2; coded PT20222022CAPXVIR_BP1 and PT2022CAPXVIR_BP2). The remaining 5 reference samples were tissue homogenate samples containing detectable capripox virus DNA (n = 4; coded PT20222022CAPXVIR_TP1, PT2022CAPXVIR_TP2, PT2022CAPXVIR_TP3 and PT20222022CAPXVIR_TP4). PT20222022CAPXVIR_TP4 was represented in the panel twice.

In total, 340 aliquots were distributed to 34 participating laboratories. These participants received 10 aliquots. 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 3: Origin of the samples in the PTCAPX2022 panel. (GTPV = goatpox virus; LSDV = lumpy skin disease virus; SPPV = sheeppox virus; NEG = negative)

Sample ID	Origin	Background
PT2022CAPXVIR_TP1	Caprine tissue	GTPV Gorgon
PT2022CAPXVIR_TP2	Bovine tissue	LSDV Bulgaria
PT2022CAPXVIR_TP3	Ovine tissue	SSPV Moroccan
PT2022CAPXVIR_TP4	Bovine tissue	LSDV Israel (2x in panel)
PT2022CAPXVIR_VP1	Cell culture	LSDV Recombinant (Vietnam strain)
PT2022CAPXVIR_VP2	Cell culture	LSDV vaccine Neethling
PT2022CAPXVIR_BP1	Ovine blood	SPPV vaccine Romania
PT2022CAPXVIR_BP2	Bovine blood	LSDV Israel
PT2022CAPXVIR_BN1	Bovine blood	Negative blood

After aliquoting the different samples, a homogeneity check was performed on 10 aliquots of each sample. The homogeneity check was performed using the real-time PCR for capripox D5R (Haegeman *et al*. 2013) and DIVA tests (Agianniotaki *et al*. 2016; Haegeman *et al*. 2016; Chibssa *et al*. 2018). 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. PT2022CAPXVIR_VP1 was detected as LSDV vaccine in the DIVA assay. Since this is a recombinant strain, the sample was considered as doubtful. Moreover, 3 additional aliquots of each reference sample were tested once the PT deadline had passed using the real-time PCR for capripox D5R (Haegeman *et al*., 2013) and DIVA tests (Agianniotaki *et al*. 2016; Haegeman *et al*. 2016; Chibssa *et al*. 2018) in order to confirm the stability and status of the samples (post -PT verification).

For the **detection of capripox virus DNA**, the sample PT20222022CAPXVIR_BN1 was considered as a capripox virus negative sample and the samples PT20222022CAPXVIR_VP1, PT20222022CAPXVIR_VP2, PT20222022CAPXVIR_TP1, PT20222022CAPXVIR_TP2, PT20222022CAPXVIR_TP3, PT20222022CAPXVIR_TP4, PT20222022 CAPXVIR_BP1 and PT20222022CAPXVIR_BP2 as positive samples. For samples PT20222022CAPXVIR_VP1 andPT20222022CAPXVIR_VP2 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 sample PT2022CAPXVIR_BN1 was considered as a negative sample, the samples PT2022CAPXVIR_TP3 and PT2022CAPXVIR_BP1 as SPPV positive samples (where SPPV or SPPV/GTPV results were considered acceptable), the samples PT2022CAPXVIR_VP1, PT2022CAPXVIR_VP2, PT20222022CAPXVIR_TP2, PT20222022CAPXVIR_TP4 and PT2022CAPXVIR_BP2 as LSDV positive samples (where LSDV was considered acceptable) and sample PT2022CAPXVIR_TP1 as GTPV positive sample (where GTPV or SPPV/GTPV was considered acceptable).

Finally, for the **field or vaccine strain differentiation**, the sample PT20222022CAPXVIR_TN1 was considered as negative sample, the samples PT20222022CAPXVIR_TP2, PT20222022CAPXVIR_TP4, and PT20222022CAPXVIR_BP2 as LSDV field strains and PT20222022CAPXVIR_TP3 as SPPV field strain. PT20222022CAPXVIR_BP1 and PT20222022CAPXVIR_VP2 were considered as respectively SPPV and LSDV vaccine strain. For PT022CAPXVIR_TP1 and PT20222022CAPXVIR_VP1 which contained respectively GTPX DNA and LSDV recombinant DNA, no DIVA test is available and therefore all answers were considered in agreement with the assigned status.

3.2.2.2 Final sample status

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

Table 4: The final status of each sample in the PT2022CAPXVIR panel (GTPV = goatpox virus; LSDV = lumpy skin disease virus; SPPV = sheeppox virus; NEG = negative).

Sample ID	Final status CapX DNA detection	Final status species differentiation	Final status DIVA
PT2022CAPXVIR_TP1	POS	GTPV	GTPV
PT2022CAPXVIR_TP2	POS	LSDV	LSDV Field
PT2022CAPXVIR_TP3	POS	SPPV	SPPV Field
PT2022CAPXVIR_TP4	POS	LSDV	LSDV Field
PT2022CAPXVIR_VP1	POS	LSDV	LSDV
PT2022CAPXVIR_VP2	POS	LSDV	LSDV Vaccine
PT2022CAPXVIR_BP1	POS	SPPV	SPPV Vaccine
PT2022CAPXVIR_BP2	POS	LSDV	LSDV Field
PT2022CAPXVIR_BN1	NEG	NEG	NEG

3.2.2.3 Randomization and panel composition

The samples were randomized differently for each laboratory, an overview of the randomization can be found in the preliminary report.
The PTCAPXVIR2022 panel was constituted of 10 samples of 600 µl.

3.2.2.4 Stability

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

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

3.3.1 CLASSIFICATION OF RESULTS

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

3.3.2 LEVEL OF AGREEMENT

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

3.3.3 THRESHOLD FOR QUALIFICATION

Following the procedure, a participating laboratory is only qualified if the level of agreement for the tested aliquots of reference samples for each panel is at least 90% The threshold for qualification will be determined separately for each test. Therefore the participants will achieve a satisfactory or unsatisfactory result for each test. Only participants that submitted results for all samples in a specific part of the virological component receive a final evaluation for that part.

4 THE PARTICIPANTS

Twenty-four NRL's of European Union Member states and eleven non-EU member states participated in the capripox virus proficiency test.

Table 5: NRL's for Capripox virus from EU member states.

Country	Name of the laboratory	Participation in PT serology	Participation in PT virology
Austria	Austrian Agency for Health and Food Safety	1	1
Belgium	Sciensano; Department 'Exotic viruses and particular diseases'	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; Department Virology	0	1
Czech Republic	State Veterinary Institute Prague	1	1

Denmark	Statens Serum Institut; Department Veterinary Virology	1	1
Finland	Finnish Food Authority; Department Virology	1	1
France	LNR poxviroses des ruminants, UMR Cirad-Inra ASTRE, "Anima, santé, Territoires, Risques et Ecosystèmes"	1	1
Germany	Friedrich-Loeffler-Institut	1	1
Greece	Dep.Mol.Diagnosis,F.M.D.,Virol. Rik.&Exotic Diseases, Athens Veterinary Directorate, Ministry of Rural Development and Food	1	1
Hungary	National Food Chain Safety; Department Veterinary Diagnostic Directorate, Laboratory for Molecular Biology	0	1
Ireland	Virology Division - CVR Laboratory; Department of Agriculture	1	1
Italy	Istituto Zooprofilattico Sperimentale dell'Abruzzo e del Molise -Centro di Referenza Nazionale per lo studio e l'accertamento delle malattie esotiche degli animali (CESME)	1	1
Latvia	Institute for Food Safety, Animal Health and Environment "BIOR", Animal Disease Diagnostic Laboratory	1	1
Lithuania	National Food and Veterinary Risk Assessment Institute (NFVRAI); Department of serology	1	1
Malta	Veterinary and Phytosanitary Regulation; Department National Veterinary Laboratory	1	0
Poland	National Veterinary Research Institute; Department of Virology	1	1
Portugal	Instituto Nacional de Investigaçao Agraria e Veterinaria (INIAV), Laboratório Nacional de Referência para a Saude animal	1	1
Romania	Institute for diagnosis and animal health	1	1
Slovakia	State veterinary and food institute, Veterinary institute in Zvolen	1	1
Slovenia	University of Ljubljana, Veterinary faculty/National Veterinary Institute, Institute of Microbiology and Parasitology, Department of Virology	1	1
Spain	Laboratorio Central De Veterinaria (LCV) (ALGETE) M.A.P.A.	1	1
The Netherlands	Wageningen Bioveterinary Research	1	1

Table 6: The non-EU member states participants.

Country	Name of the laboratory	Participation in PT serology	Participation in PT virology
Albania	Food Safety and Veterinary Insitute; Department of Animal Health, Molecular Biology	0	1
Georgia	Laboratory of the Ministry of Agriculture (LMA) of Georgia	0	1
Kazakhstan	National Veterinary Reference Centre Astana	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
Republic of Moldova	Republican Veterinary Diagnostic Center	0	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
United Kingdom	Pirbright institute	1	1

5 SURVEY TIMELINE

Transfer of the samples from NRL to QL: 25/04/2022
Randomization of the samples by QL: 26/04/2022 (serology) + 28/04/2022 (virology)
Sending samples (frozen at - 20 °C) to participants: 04/05/2022
Deadline for the submitting the results: 09/06/2022
Preliminary report: 22/06/2022
Definitive global report: 26/09/2022

6 COMPLIANCE WITH THE PROCEDURE

All participating laboratories have provided a duly dated copy of the results, except the laboratories 97644, 97645 and 97646. After several attempts, it was not possible to obtain the correct custom papers requested for sending the samples. Therefore no samples were sent to these laboratories implying that no results could be reported for the PT2022.

7 RESULTS – QUALITATIVE DATA ANALYSIS

7.1 Serology

The PT2022CAPXSER panel was composed of 8 positive and 2 negative samples.

7.1.1 ANTIBODY ELISA

In total, 29 laboratories participated and used submitted their results. Twenty-eight laboratories used the same ELISA kit, namely the ID Screen Capripox Double Antigen Multispecies kit from Innovative diagnostic. This maximum score was achieved by all laboratories using the ID Screen Capripox Double Antigen Multispecies kit.
One laboratory used an ELISA from Biostone Animal Health LLC for the detection of the cattle samples and an AGID (AGAR GEL IMMUNODIFFUSION ASSAY) for the detection of the sheep samples. This laboratory did not fully succeed and obtained a score of 70%. Raw data can be found in Annex 1.

Table 7: Results of the ELISA per participating laboratory.

Lab number	P1	P2	P3	P4	P5	P6	P7	P8	N1	N2	%
	POS								NEG		
97506	POS	POS	POS	POS	POS	POS	POS	POS	NEG	NEG	100
97600	POS	POS	POS	POS	POS	POS	POS	POS	NEG	NEG	100
97602	POS	POS	POS	POS	POS	POS	POS	POS	NEG	NEG	100
97604	POS	POS	POS	POS	POS	POS	POS	POS	NEG	NEG	100
97605	POS	POS	POS	POS	POS	POS	POS	POS	NEG	NEG	100
97606	POS	POS	POS	POS	POS	POS	POS	POS	NEG	NEG	100
97607	POS	POS	POS	POS	POS	POS	POS	POS	NEG	NEG	100
97608	POS	POS	POS	POS	POS	POS	POS	POS	NEG	NEG	100
97609	POS	POS	POS	POS	POS	POS	POS	POS	NEG	NEG	100
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
97628	POS	POS	POS	POS	NEG	NEG	NEG	POS	NEG	NEG	70

97630	POS	POS	POS	POS	POS	POS	POS	POS	NEG	NEG	**100**
97631	POS	POS	POS	POS	POS	POS	POS	POS	NEG	NEG	**100**
97632	POS	POS	POS	POS	POS	POS	POS	POS	NEG	NEG	**100**
97634	POS	POS	POS	POS	POS	POS	POS	POS	NEG	NEG	**100**
97637	POS	POS	POS	POS	POS	POS	POS	POS	NEG	NEG	**100**
97642	POS	POS	POS	POS	POS	POS	POS	POS	NEG	NEG	**100**
97643	POS	POS	POS	POS	POS	POS	POS	POS	NEG	NEG	**100**

7.1.2 VIRUS NEUTRALIZATION

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

Table 8: Results of the virus neutralization test per participating laboratory.

Lab number	**P1**	**P2**	**P3**	**P4**	**P5**	**P6**	**P7**	**P8**	**N1**	**N2**	**(%**
	POS								**NEG**		
97506	POS	POS	POS	POS	POS	POS	POS	POS	NEG	NEG	**100**
97600	POS	POS	POS	POS	POS	POS	POS	POS	NEG	NEG	**100**
97612	POS	POS	POS	POS	POS	POS	POS	POS	NEG	NEG	**100**
97618	POS	POS	POS	POS	POS	POS	POS	POS	NEG	NEG	**100**
97643	POS	POS	POS	POS	POS	POS	POS	POS	NEG	NEG	**100**

7.1.3 IMMUNOPEROXYDASE MONOLAYER ASSAY (IPMA)

Only two laboratories submitted results for the immunoperoxydase monolayer assay (IPMA). These laboratories provided qualitative results that were in full agreement with the assigned status of the reference serum samples and hence reached 100% agreement. Raw data can be found in Annex 1.

Table 9: Results of the IPMA per participating laboratory.

Lab number	**P1**	**P2**	**P3**	**P4**	**P5**	**P6**	**P7**	**P8**	**N1**	**N2**	**%**
	POS								**NEG**		
97506	POS	POS	POS	POS	POS	POS	POS	POS	NEG	NEG	**100**
97618	POS	POS	POS	POS	POS	POS	POS	POS	NEG	NEG	**100**

7.2 Virology

7.2.1 CAPRIPOX DNA DETECTION

In total, 34 laboratories participated in the capripox DNA detection part of the virological component of the proficiency test. As 31 laboratories submitted 1 dataset, 2 laboratories submitted 2 datasets and 1 laboratory submitted 3 datasets, the total number of datasets registered is thus 38. Raw data can be found in Annex 1.

7.2.1.1 Results per sample

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

Sample ID PT2022CAPXVIR_	REP	Expected results	# of POS	# of NEG	# of ND	Status*
TP1	1	POS	35	3	0	Frequently detected
TP2	1	POS	36	2	0	Frequently detected
TP3	1	POS	37	1	0	Frequently detected
TP4	2	POS	74	2	0	Frequently detected
VP1	1	POS	36	2	0	Frequently detected
VP2	1	POS	37	1	0	Frequently detected
BP1	1	POS	37	1	0	Frequently detected
BP2	1	POS	37	1	0	Frequently detected
BN1	1	NEG	0	37	1	NEG

**: for positive sample a frequently detected sample is detected by more than 95% of the participants, a detected sample is detected by more than 65% of the participants and a infrequently detected sample is detected by less than 65% of the participants (www.qcmd.org).*

7.2.1.2 Results per method

Table 11: Results per method (N = number of laboratories; NR = number of results; number of correct results; FN = false negative; ND = not determined).

Kit or reference	Target gene	N	NR	NCR	FN	ND
Haegeman *et al*. 2013	D5R/E3L	3	30	30	0	0
Bowden *et al*. 2008	P32	16	160	158	2	0
Bowden *et al*. 2008; Babiuk *et al*. 2008; SOP from Pirbright	P32	1	10	10	0	0
Indical - Indispin pathogen kit	P32	1	10	10	0	0
Roche kit	P32	2	20	20	0	0
Qiagen (MO-TO 45)	P32	1	10	10	0	0
Qiagen (MO-TO 46)	RPO30	1	10	10	0	0
Lamien CE *et al*. (2010)	P32	1	10	10	0	0
ID Gene Capripox Virus Triplex (IDvet)	/	1	10	10	0	0
Babiuk *et al.* 2008	P32	1	10	10	0	0
Path_ID qPCR	P32	1	10	10	0	0
SOP G.72	P32	1	10	10	0	0
Balinsky *et al.*	PAPS	1	10	9	0	1
UD	/	2	20	20	0	0
RT-PCR Kit: PCR-OSPA-FACTOR	/	1	10	5	5	0
RT-PCR Kit: PCR-NODULAR-DERMATITE-CRS-FACTOR	/	1	10	5	5	0
RT-PCR Kit: Nodular dermatitis and smallpox of sheep and goats	/	1	10	9	1	0
Qiamp Cador pathogen Mini kit	/	1	10	10	0	0
Invitrogen/Thermo Fisher Scientific	P32	1	10	10	0	0
TOTAL		**38**	**380**	**366**	**13**	**1**

7.2.1.3 Results per laboratory

For the detection of capripox virus DNA in the PT panel: 30 out of 34 participating laboratories provided qualitative results that were in full agreement with the assigned status of the 10 reference samples (100% of agreement), whereas three labs (LAB97619, LAB97620 and LAB97631) misclassified 1 aliquot (90% of agreement). The remaining lab (LAB97628) chose to test three different methods. The first two methods achieved a low score of 50%. While the third method obtained a score of 90%.
Furthermore, two labs did a secondary PCR (LAB97605 and LAB97607) and provided results that were in full agreement with the assigned status of the reference samples.

Table 12: Results per laboratory.

Lab number	TP1	TP2	TP3	TP4 Rep 1	TP4 Rep 2	VP1	VP2	BP1	BP2	BN1	%
	POS									NEG	
97506	POS	POS	POS	POS	POS	POS	POS	POS	POS	NEG	100
97600	POS	POS	POS	POS	POS	POS	POS	POS	POS	NEG	100
97601	POS	POS	POS	POS	POS	POS	POS	POS	POS	NEG	100
97602	POS	POS	POS	POS	POS	POS	POS	POS	POS	NEG	100
97603	POS	POS	POS	POS	POS	POS	POS	POS	POS	NEG	100
97604	POS	POS	POS	POS	POS	POS	POS	POS	POS	NEG	100
97605 (1)	POS	POS	POS	POS	POS	POS	POS	POS	POS	NEG	100
97605 (2)	POS	POS	POS	POS	POS	POS	POS	POS	POS	NEG	100
97606	POS	POS	POS	POS	POS	POS	POS	POS	POS	NEG	100
97607 (1)	POS	POS	POS	POS	POS	POS	POS	POS	POS	NEG	100
97607 (2)	POS	POS	POS	POS	POS	POS	POS	POS	POS	NEG	100
97608	POS	POS	POS	POS	POS	POS	POS	POS	POS	NEG	100
97609	POS	POS	POS	POS	POS	POS	POS	POS	POS	NEG	100
97610	POS	POS	POS	POS	POS	POS	POS	POS	POS	NEG	100
97611	POS	POS	POS	POS	POS	POS	POS	POS	POS	NEG	100
97612	POS	POS	POS	POS	POS	POS	POS	POS	POS	NEG	100
97613	POS	POS	POS	POS	POS	POS	POS	POS	POS	NEG	100
97614	POS	POS	POS	POS	POS	POS	POS	POS	POS	NEG	100
97616	POS	POS	POS	POS	POS	POS	POS	POS	POS	NEG	100
97617	POS	POS	POS	POS	POS	POS	POS	POS	POS	NEG	100
97618	POS	POS	POS	POS	POS	POS	POS	POS	POS	NEG	100
97619	POS	POS	POS	POS	POS	POS	POS	POS	POS	ND	90
97620	NEG	POS	POS	POS	POS	POS	POS	POS	POS	NEG	90
97621	POS	POS	POS	POS	POS	POS	POS	POS	POS	NEG	100
97622	POS	POS	POS	POS	POS	POS	POS	POS	POS	NEG	100
97624	POS	POS	POS	POS	POS	POS	POS	POS	POS	NEG	100
97627	POS	POS	POS	POS	POS	POS	POS	POS	POS	NEG	100
97628 (1)	POS	NEG	POS	NEG	NEG	POS	NEG	POS	NEG	NEG	50

97628 (2)	NEG	NEG	NEG	POS	POS	NEG	POS	NEG	POS	NEG	**50**
97628 (3)	POS	POS	POS	POS	POS	NEG	POS	POS	POS	NEG	**90**
97630	POS	POS	POS	POS	POS	POS	POS	POS	POS	NEG	**100**
97631	NEG	POS	POS	POS	POS	POS	POS	POS	POS	NEG	**90**
97632	POS	POS	POS	POS	POS	POS	POS	POS	POS	NEG	**100**
97634	POS	POS	POS	POS	POS	POS	POS	POS	POS	NEG	**100**
97636	POS	POS	POS	POS	POS	POS	POS	POS	POS	NEG	**100**
97637	POS	POS	POS	POS	POS	POS	POS	POS	POS	NEG	**100**
97642	POS	POS	POS	POS	POS	POS	POS	POS	POS	NEG	**100**
97643	POS	POS	POS	POS	POS	POS	POS	POS	POS	NEG	**100**

7.2.2 SPECIES DIFFERENTIATION

In total, 22 laboratories participated in the species differentiation virology part of the proficiency test. As 18 laboratories submitted 1 dataset, 3 laboratories submitted 2 datasets and 1 laboratory submitted 3 datasets, the total number of datasets registered is thus 27. The datasets of the laboratories submitting multiple datasets were taken together to get a final result for this part. Raw data can be found in Annex 1.

7.2.2.1 Results per sample

Table 13: Results per sample (REP = repetition; GTPV = goatpox virus; LSDV = lumpy skin disease virus; SPPV = sheeppox virus; NEG = negative; ND = not determined).

Sample ID PT2022CAPXVIR_	REP	Expected results	# of GTPV	# of LSDV	# of SPPV	# of NEG	# of ND	%
TP1	1	GTPV	16	0	1	4	1	71
TP2	1	LSDV	0	22	0	0	0	100
TP3	1	SPPV	0	0	19	3	0	86
TP4	2	LSDV	0	44	0	0	0	100
VP1	1	LSDV	0	20	0	2	0	91
VP2	1	LSDV	0	21	0	1	0	95
BP1	1	SPPV	0	0	19	3	0	86
BP2	1	LSDV	0	22	0	0	0	100
BN1	1	NEG	0	1	0	20	1	91

7.2.2.2 Results per method

Table 14: Results per method (N = number of laboratories; NR = number of results; number of correct results; F=False; ND = not determined).

Kit or reference	N	NR	NCR	F	ND
Agianniotaki *et al*. 2016 and in-house Taqman assay and Chibssa *et al*. 2018	1	10	10	0	0
Lamien *et al*. 2011	6	60	59	1	0
Homemade - Dual hybridization probe	1	10	9	0	1
MO-TO 47 (qiagen)	1	10	7	3	0
LSDV-DIVA duplex SPPV GTPV duplex (Wolff *et al* 2021)	1	10	10	0	0
LSDV-DIVA duplex SPPV GTPV duplex (Wolff *et al* 2021) and partial sequencing (Adedeji *et al*. 2019)	1	10	10	0	0
partial GPCR (primer pair CapGPCR-OL3F/ CapGPCR-OL3R) and Lamien *et al* (2011)	1	10	10	0	0
Galaye *et al* 2017	1	10	8	1	1
IZSTE B456.1 SOP 021 and Wolff *et al*. 2021 qPCR_GTPV	1	10	7	3	0
Qiagen SOP G.72	1	10	10	0	0
Chibssa *et al*. 2018, Gelaye *et al* 2015, Lamien *et al*, 2017	1	10	10	0	0
Homemade thermofisher	1	10	10	0	0
Batra *et al*.	1	10	9	1	0
Vidanovic *et al* (unpublished)	1	10	9	1	0
Vidanovic *et al* (2016)	1	10	5	5	0
Vidanovic *et al* (2021) and vidanovic unpublished	1	10	10	0	0
NVR-SOP-46	1	10	10	0	0
TOTAL	**22**	**220**	**202**	**15**	**2**

7.2.2.3 Results per laboratory

Fourteen out of 22 participating laboratories provided qualitative results that were in full agreement with the assigned status of the 10 reference samples (100% of agreement), whereas LAB97602, LAB97619, LAB97620 and LAB97631 misclassified 1 aliquot (90% of agreement). LAB97619, LAB97620 and LAB97631 already misclassified the same aliquot in their primary PCR for capripox DNA detection. LAB97611 misclassified two aliquots reaching a level of agreement of 80%. LAB97607 and LAB97612 misclassified 3 aliquots, reaching a level of agreement of 70%. LAB97632 misclassified 5 aliquots (Level of agreement of 50%)

Table 15: Results per laboratory.

Lab number	TP1	TP2	TP3	TP4 Rep 1	TP4 Rep 2	VP1	VP2	BP1	BP2	BN1	%
	GTPV	LSDV	SPPV	LSDV	LSDV	LSDV	LSDV	SPPV	LSDV	NEG	
97506	GTPV	LSDV	SPPV	LSDV	LSDV	LSDV	LSDV	SPPV	LSDV	NEG	100
97600	GTPV	LSDV	SPPV	LSDV	LSDV	LSDV	LSDV	SPPV	LSDV	NEG	100
97602	ND	LSDV	SPPV	LSDV	LSDV	LSDV	LSDV	SPPV	LSDV	NEG	90
97603	GTPV	LSDV	SPPV	LSDV	LSDV	LSDV	LSDV	SPPV	LSDV	NEG	100
97607	NEG	LSDV	NEG	LSDV	LSDV	LSDV	LSDV	NEG	LSDV	NEG	70
97608	GTPV	LSDV	SPPV	LSDV	LSDV	LSDV	LSDV	SPPV	LSDV	NEG	100
97609	GTPV	LSDV	SPPV	LSDV	LSDV	LSDV	LSDV	SPPV	LSDV	NEG	100
97610	GTPV	LSDV	SPPV	LSDV	LSDV	LSDV	LSDV	SPPV	LSDV	NEG	100
97611	SPPV	LSDV	SPPV	LSDV	LSDV	LSDV	LSDV	SPPV	LSDV	ND	80
97612	GTPV	LSDV	NEG	LSDV	LSDV	NEG	LSDV	NEG	LSDV	NEG	60
97613	GTPV	LSDV	SPPV	LSDV	LSDV	LSDV	LSDV	SPPV	LSDV	NEG	100
97614	GTPV	LSDV	SPPV	LSDV	LSDV	LSDV	LSDV	SPPV	LSDV	NEG	100
97617	GTPV	LSDV	SPPV	LSDV	LSDV	LSDV	LSDV	SPPV	LSDV	NEG	100
97618	GTPV	LSDV	SPPV	LSDV	LSDV	LSDV	LSDV	SPPV	LSDV	NEG	100
97619	GTPV	LSDV	SPPV	LSDV	LSDV	LSDV	LSDV	SPPV	LSDV	LSDV	90
97620	NEG	LSDV	SPPV	LSDV	LSDV	LSDV	LSDV	SPPV	LSDV	NEG	90
97630	GTPV	LSDV	SPPV	LSDV	LSDV	LSDV	LSDV	SPPV	LSDV	NEG	100
97631	NEG	LSDV	SPPV	LSDV	LSDV	LSDV	LSDV	SPPV	LSDV	NEG	90
97632	NEG	LSDV	NEG	LSDV	LSDV	NEG	NEG	NEG	LSDV	NEG	50
97634	GTPV	LSDV	SPPV	LSDV	LSDV	LSDV	LSDV	SPPV	LSDV	NEG	100
97642	GTPV	LSDV	SPPV	LSDV	LSDV	LSDV	LSDV	SPPV	LSDV	NEG	100
97643	GTPV	LSDV	SPPV	LSDV	LSDV	LSDV	LSDV	SPPV	LSDV	NEG	100

7.2.3 DIVA PCR

In total, 22 laboratories participated in the DIVA PCR virology part of the proficiency test. As 18 laboratories submitted 1 dataset, 3 laboratories submitted 2 datasets and 1 laboratory submitted 3 datasets, the total number of datasets registered was thus 27. The datasets of the laboratories submitting multiple datasets were taken together to get a final result for this part. Raw data can be found in Annex 1. For samples TP1 and VP1, all answers were considered correct, since no DIVA test is already available for these samples.

7.2.3.1 Results per sample

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

Sample ID	REP	Expected results	# of GTPV	# of LSDV Wild	# of LSDV Vaccine	# of SPPV Wild	# of SPPV Vaccine	# of NEG	# of ND	%
TP1	1	GTPV	6	0	0	0	0	10	6	27
TP2	1	LSDV Wild	0	21	0	0	0	1	0	96
TP3	1	SPPV Wild	0	0	0	10	0	7	5	45
TP4	2	LSDV Wild	0	42	0	0	0	2	0	96
VP1	1	LSDV	0	2	11	0	0	3	6	59
VP2	1	LSDV Vaccine	0	0	21	0	0	0	1	96
BP1	1	SPPV Vaccine	0	0	0	0	10	7	5	45
BP2	1	LSDV Wild	0	21	0	0	0	1	0	96
BN1	1	NEG	0	0	0	0	0	19	3	86

7.2.3.2 Results per method

Table 17: Results per method (N = number of laboratories; NR = number of results; number of correct results; F = false ; ND = not determined).

Kit or reference	N	NR	NCR	F	ND
Agianniotaki *et al*. 2016 and in-house Taqman assay and Chibssa *et al*. 2018 and unpublished recombinant DIVA	1	10	10	0	0
Kit from FLI Riems	1	10	10	0	0
Wolff *et al*. 2021 and Chibbsa *et al*. 2018	1	10	10	0	0
Wolff *et al*. 2021	1	10	8	0	2
Agianniotaki *et al*. 2017 and Haegeman. *et al* 2015	3	30	30	0	0
Vidanovic *et al*. 2016	2	20	16	4	0
Agianniotaki *et al* 2017	2	20	15	2	3
ID Gene LSD DIVA Triplex - IDvet	1	10	8	0	2
Qiagen SOP G.72	1	10	10	0	0
Menasherow *et al*. 2016 (LSDV); Chibssa *et al*. 2018 (SPPV); Gelaye *et al*. 2015 (GTPV)	1	10	10	0	0
Agianniotaki *et al* 2017, Menasherow *et al* 2016, Chibssa *et al* 2018	1	10	10	0	0

ID Gene LSD DIVA Triplex – Idvet and Chibssa *et al* 2018	1	10	10	0	0
Other- in house	1	10	6	0	4
Wolff *et al*. 2021 and Möller *et al*. 2019	1	10	8	2	0
Moller *et al* 2019	1	10	4	6	0
Vidanovic *et al* 2021	1	10	8	2	0
Vidanovic *et al* 2016, Agianniotaki *et al* 2017	1	10	8	2	0
Other - Thermofisher	1	10	7	0	3
TOTAL	**22**	**220**	**188**	**18**	**14**

7.2.3.3 Results per laboratory

Ten out of 22 laboratories provided results that were in full agreement with the assigned status of the reference samples (100% agreement). Eight laboratories (LAB97608, LAB97610; LAB97612, LAB97613, LAB97630, LAB97631, LAB97634 and LAB97637) misclassified 2 aliquots (80% agreement). All of these laboratories misclassified PT2022CAPXVIR_TP3 and PT2022CAPXVIR_BP1 as NEG or ND instead of respectively SPPV wild and SPPV vaccine. Two laboratories misclassified (LAB97611 and LAB97643) misclassified 3 aliquots, reaching a level of agreement of 70%. They also misclassified PT2022CAPXVIR_TP3 and PT2022CAPXVIR_BP1 as ND instead of respectively SPPV wild and SPPV vaccine. In addition PT2022CAPXVIR_BN1 was also misclassified as ND instead of NEG. The two remaining laboratories misclassified 4 aliquots (LAB97622) and 6 aliquots (LAB97632) reaching a level of agreement of respectively 60 and 40%

Table 18: Results per laboratory.

Lab number	TP1	TP2	TP3	TP4 Rep 1	TP4 Rep 2	VP1	VP2	BP1	BP2	BN1	%
	GTPV	LSDV Wild	SPPV Wild	LSDV Wild	LSDV Wild	LSDV	LSDV Vac	SPPV Vac	LSDV wild	NEG	
97506	GTPV	LSDV Wild	SPPV Wild	LSDV Wild	LSDV Wild	LSDV Wild	LSDV Vac	SPPV Vac	LSDV Wild	NEG	**100**
97600	ND	LSDV Wild	SPPV Wild	LSDV Wild	LSDV Wild	LSDV Vac	LSDV Vac	SPPV Vac	LSDV Wild	NEG	**100**
97602	GTPV	LSDV Wild	SPPV Wild	LSDV Wild	LSDV Wild	LSDV Vac	LSDV Vac	SPPV Vac	LSDV Wild	NEG	**100**
97608	ND	LSDV Wild	ND	LSDV Wild	LSDV Wild	ND	LSDV Vac	ND	LSDV Wild	NEG	**80**
97609	NEG	LSDV Wild	SPPV Wild	LSDV Wild	LSDV Wild	LSDV Vac	LSDV Vac	SPPV Vac	LSDV Wild	NEG	**100**
97610	NEG	LSDV Wild	NEG	LSDV Wild	LSDV Wild	LSDV Vac	LSDV Vac	NEG	LSDV Wild	NEG	**80**

97611	ND	LSDV Wild	ND	LSDV Wild	LSDV Wild	LSDV Vac	LSDV Vac	ND	LSDV Wild	ND	**70**
97612	NEG	LSDV Wild	NEG	LSDV Wild	LSDV Wild	NEG	LSDV Vac	NEG	LSDV Wild	NEG	**80**
97613	NEG	LSDV Wild	NEG	LSDV Wild	LSDV Wild	LSDV Vac	LSDV Vac	NEG	LSDV Wild	NEG	**80**
97614	GTPV	LSDV Wild	SPPV Wild	LSDV Wild	LSDV Wild	LSDV Vac	LSDV Vac	SPPV Vac	LSDV Wild	NEG	**100**
97617	GTPV	LSDV Wild	SPPV Wild	LSDV Wild	LSDV Wild	LSDV Vac	LSDV Vac	SPPV Vac	LSDV Wild	NEG	**100**
97618	GTPV	LSDV Wild	SPPV Wild	LSDV Wild	LSDV Wild	LSDV Vac	LSDV Vac	SPPV Vac	LSDV Wild	NEG	**100**
97620	ND	LSDV Wild	SPPV Wild	LSDV Wild	LSDV Wild	LSDV Wild	LSDV Vac	SPPV Vac	LSDV Wild	NEG	**100**
97621	NEG	LSDV Wild	SPPV Wild	LSDV Wild	LSDV Wild	LSDV Vac	LSDV Vac	SPPV Vac	LSDV Wild	NEG	**100**
97622	ND	LSDV Wild	ND	LSDV Wild	LSDV Wild	ND	ND	ND	LSDV Wild	ND	**60**
97630	NEG	LSDV Wild	NEG	LSDV Wild	LSDV Wild	NEG	LSDV Vac	NEG	LSDV Wild	NEG	**80**
97631	NEG	LSDV Wild	ND	LSDV Wild	LSDV Wild	LSDV Vac	LSDV Vac	ND	LSDV Wild	NEG	**80**
97632	NEG	NEG	NEG	NEG	NEG	NEG	LSDV Vac	NEG	NEG	NEG	**40**
97634	NEG	LSDV Wild	NEG	LSDV Wild	LSDV Wild	LSDV Vac	LSDV Vac	NEG	LSDV Wild	NEG	**80**
97637	NEG	LSDV Wild	NEG	LSDV Wild	LSDV Wild	LSDV Vac	LSDV Vac	NEG	LSDV Wild	NEG	**80**
97642	GTPV	LSDV Wild	SPPV Wild	LSDV Wild	LSDV Wild	LSDV Vac	LSDV Vac	SPPV Vac	LSDV Wild	NEG	**100**
97643	ND	LSDV Wild	ND	LSDV Wild	LSDV Wild	ND	LSDV Vac	ND	LSDV Wild	ND	**70**

8 DISCUSSION

The purpose of this PT was to assess the performance 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.

8.1 Serology component of the PT

For the detection of specific antibodies to capripox virus in reference serum samples, using ELISA and in some cases VNT or IPMA, 28 out of 29 laboratories provided qualitative results that were in full agreement with the assigned status of the reference serum samples. The remaining laboratory performed an AGID (AGAR GEL IMMUNODIFFUSION ASSAY) and misclassified 3 aliquots, resulting in a level of agreement of 70% and did therefore not obtained a satisfactory result

8.2 Virology component of the PT

For the detection of capripox virus DNA by real-time PCR (RT-PCR) in the PT panel : 30 out of 34 participating laboratories provided qualitative results that were in full agreement with the assigned status of the 10 reference samples (100% of agreement), whereas LAB97619, LAB 97620 and LAB97631 misclassified 1 sample (90% of agreement). LAB 97619 misclassified the PT2022CAPXVIR_BN1 as ND instead of NEG. The two other laboratories both misclassified PT2022CAPXVIR_TP1, being the GTPV sample, as NEG instead of POS.
The remaining laboratory (LAB97628) performed three different PCRs. In the first two they misclassified 5 aliquots. PTC2022CAPXVIR_TP2 was misclassified as NEG instead of POS in both tests. The other 8 samples were correctly classified in one PCR and misclassified in the other PCR. In the third PCR, only one aliquot was misclassified (PT2022CAPXVIR_VP1). This was the recombinant LSDV strain. LAB97628 thus reached a level of agreement of 50% in the two first PCRs and a level of agreement of 90% in the third PCR.

For the differentiation of capripox virus species, fourteen out of 22 participating laboratories provided qualitative results that were in full agreement with the assigned status of the 10 reference samples (100% of agreement), whereas LAB97602, LAB97619, LAB97620 and LAB97631 misclassified 1 aliquot (90% of agreement). Three of these (LAB97602, LAB97620 and LAB97631) laboratories misclassified PT2022CAPXVIR_TP1 respectively as ND, NEG and NEG instead of GTPV. LAB97619 misclassified PT2022CAPXVIR_BN1 as LSDV instead of NEG. LAB97619, LAB97620 and LAB97631 misclassified the same aliquot in their pan capripox PCR as well. LAB97611 misclassified 2 aliquots (PT2022CAPXVIR_TP1 and PT2022CAXVIR_BN1) respectively as SPPV and ND instead of GTPV and NEG. The assay they used should normally be able to classify the GTPV sample correctly. LAB97607 and LAB97612 misclassified 3 aliquots. For LAB97607 these were PT2022CAPXVIR_TP1, PT2022CAPXVIR_TP3 and PT2022CAPXVIR_BP1. All of these were misclassified as NEG instead of respectively GTPV, SPPV and SPPV. This laboratory used a LSDV specific PCR and was thus unable to classify the GTPV/SPPV samples. The LSDV samples were correctly classified. LAB97612 misclassified PT2022CAPXVIR_TP3, PT2022CAPXVIR_VP1 and PT2022CAPXVIR_BP1. These were respectively the two SPPV samples and the recombinant LSDV strain that were misclassified as NEG. The assays that were used were able to detect GTPV and LSDV. LAB97632 misclassified aliquots PT2022CAPXVIR_TP1, PT2022CAPXVIR_TP3, PT2022CAPXVIR_VP1, PT2022CAPXVIR_VP2 and PT2022CAPXVIR_BP1 as NEG instead of respectively GTPV, SPPV, LSDV, LSDV and SPPV. This laboratory used an assay to detect LSDV field strains and the LSDV field aliquots were correctly classified.

For the differentiation between capripox virus field and vaccine strains, two aliquots were given the status doubtful (PT2022CAPXVIR_TP1 and PT2022CAPXVIR_VP1), because no DIVA assay is currently available for GTPV (TP1) and LSDV recombinants (VP1). Therefore, NEG and ND were accepted for these aliquots in addition to respectively GTPV and LSDV.

Ten out of 22 participating laboratories provided qualitative results that were in full agreement with the assigned status of the samples they analyzed (100% of agreement). Eight laboratories (LAB97608, LAB97610; LAB97612, LAB97613, LAB97630, LAB97631, LAB97634 and LAB97637) misclassified 2 aliquots. All of these laboratories misclassified PT2022CAPXVIR_TP3 and PT2022CAPXVIR_BP1 as NEG or ND instead of respectively SPPV wild and SPPV vaccine. All of these laboratories used only an assay for the differentiation of LSDV species and could therefore not make the differentiation of the SPPV strains. The LSDV samples were correctly classified. Two more laboratories (LAB97611 and LAB97643) misclassified the same aliquots and also misclassified the negative sample (PT2022CAPXVIR_BN1) as ND instead of NEG. LAB97611 also used an assay that was only capable of differentiating LSDV strains, while LAB97643 used an unpublished assay. Both of these labs used an assay that could not differentiate the SPPV strains and they did not test the negative sample. LAB97622 misclassified 4 aliquots. Three of them being the same as the LAB97611 and LAB97643. In addition they also misclassified PT2022CAPXVIR_VP2, which contained the LSDV vaccine strain. The lab classified all these aliquots as ND. They used an in-house assay, that only detect LSDV wild strains. All LSDV wild strains in the panel were correctly classified. LAB97632 misclassified 6 aliquots. PT2022CAPXVIR_TP2, PT2022CAPXVIR_TP3, PT2022CAPXVIR_TP4 (2x), PT2022CAPXVIR_BP1 and PT2022CAPXVIR_BP2 were all misclassified as NEG. They used an assay capable of differentiating LSDV strains. However, only PT2022CAPxVIR_VP2 was correctly classified.

9 CONCLUSIONS

According to the procedure currently in force, the performance of a participating laboratory is satisfactory if at least 90% of the results provided by this laboratory is in agreement with the status of the reference samples assigned by the European Union Reference Laboratory for disease caused by capripox viruses of the Scientific Directorate Infectious Diseases in Animals of Sciensano. Each part of the PT is separately evaluated.
Only laboratories that provided a complete dataset are rated and will be able to get a satisfactory performance. The remaining laboratories will be evaluated on the samples that could be analyzed with the used assay. However, a satisfactory performance cannot be awarded, since not all aliquots were analyzed for a specific part.

For the detection of specific antibodies to capripox viruses in bovine and ovine sera, all laboratories except LAB97628, achieved a satisfactory results on all of the tests they performed (ELISA, VNT and/or IPMA).
LAB97268 performed an ELISA in combination with an AGID (AGAR GEL IMMUNODIFFUSION ASSAY) and misclassified 3 aliquots as NEG instead of POS, reaching a level of agreement of 70%. This laboratory therefore achieved an unsatisfactory result.

For the detection of capripox virus nucleic acid in the cell culture supernatant, blood or tissue homogenate samples, all 34 laboratories achieved a satisfactory performance for all the datasets they submitted.
LAB97628 that submitted 3 datasets based on different assays did not achieve satisfactory results using their first and second PCR (level of agreement of 50%) test while the third PCR provided a satisfactory performance.

For the differentiation of capripox virus species, LAB97506, LAB97600, LAB97602, LAB97603, LAB97608, LAB97609, LAB97610, LAB97613, LAB97614, LAB97617, LAB97618, LAB97619, LAB97620, LAB97630, LAB97631, LAB97634, LAB97642 and LAB97643 submitted a complete dataset and achieved a satisfactory performance.

LAB97611 used an assay that should be able to detect all capripox species. The lab did not retest the negative sample and misclassified the GTPV sample as SPPV. Therefore they cannot be awarded a satisfactory performance.
LAB97607 used an assay that was only able to differentiate LSDV strains and did therefore not submit a complete dataset and a satisfactory result cannot be awarded. However, all LSDV aliquots were correctly classified.
LAB97612 used an assay to detect GTPV and LSDV and thus did not submit a complete dataset. The aliquot containing the LSDV recombinant strain was also classified NEG, so they achieved an unsatisfactory performance for this virological part of the proficiency test.
LAB97632 misclassified 5 aliquots (GTPV, SPPV and LSDV samples). The performance on this part was unsatisfactory for this lab.
The laboratories that did not obtained a satisfactory performance for the species differentiation part of the proficiency test are advised to implement additional diagnostic tests for species differentiation and/or to optimize the already implemented tests that led to incorrect results.

For the differentiation between capripox virus field and vaccine strains, LAB97506, LAB97600, LAB97602, LAB97609, LAB97614, LAB97617, LAB97618, LAB97620, LAB97621 and LAB97642 submitted a complete dataset and achieved a satisfactory result for this part of the virology component of the PT.
LAB97608, LAB97610; LAB97612, LAB97613, LAB97630, LAB97631, LAB97634 and LAB97637 used an assay that can only differentiate between vaccine and wild type of LSDV. All of these laboratories classified all LSDV samples correctly. A satisfactory performance to this part of the proficiency could however not be granted since the SPPV samples were not classified and thus no complete dataset was submitted. LAB97611 and LAB97632 also used an assay that can only differentiate between vaccine and wild type of LSDV. Therefore, they were also unable to submit a complete dataset. In addition they also did not test the negative samples, so a satisfactory performance was not achieved. However, both laboratories classified all LSDV samples correctly.
LAB97622 used an assay that only detects LSDV wild strains. The aliquots containing an LSDV vaccine strain, SPPV field strain and vaccine strain could not be classified, resulting in an incomplete dataset. Therefore a satisfactory performance cannot be awarded. The LSDV field strains were however correctly classified.
LAB97632 misclassified aliquots containing both LSDV and SPPV strains and use an assay that is able to differentiate between wild and vaccine LSDV strains. In total, they misclassified 6 aliquots. Therefore, the performance was unsatisfactory.
The laboratories that did not obtained a satisfactory performance for the DIVA part of the proficiency test are advised to implement additional diagnostic tests and/or to optimize the already implemented tests that led to incorrect results.

In conclusion, almost all laboratories participating in the virology part of the PT were able to correctly classify the aliquots for which they have tests available. However, it is recommended to implement additional test(s) to also differentiate SPPV and GTPV and to differentiate between wild and vaccine strains of SPPV and LSDV.
Furthermore, the recombinant strain (PT2022CAPXVIR_VP1) was detected as vaccine type by 11 out of the 22 participants. It will therefore be recommendable to implement an additional DIVA test that is capable to classify the recombinants strains as wild type field strains once such tests become available.

10 ANNEXES (NOT UNDER ACCREDITATION)

10.1 Annex 1: Raw data

10.1.1 SERO: ANTIBODY ELISA

Value	Sample	Laboratory number									
		97506	97600	97602	97604	97605	97606	97607	97608	97609	97611
Optical density (OD)	Positive control (mean)	101,0	0,7	0,7	0,7	0,7	0,8	0,9	0,1	0,9	0,8
	Negative control (mean)	3,1	0,1	0,05	0,1	0,05	0,1	0,04	1,0	0,05	0,1
	PT2022CAPXSER_SERP1	1,3274	1,197	1,054	1,144	1,359	1,324	1,4145	1,48	1,182	1,3595
	PT2022CAPXSER_SERP2	1,1562	1,127	1,121	1,084	1,245	1,287	1,4175	1,48	1,183	1,4305
	PT2022CAPXSER_SERP3	1,0751	1,016	0,978	1,006	1,191	1,1	1,315	1,33	1,069	1,188
	PT2022CAPXSER_SERP4	0,8056	0,61	0,689	0,627	0,75	0,836	0,885	1	0,768	0,824
	PT2022CAPXSER_SERP5	2,6298	2,077	2,151	2,076	2,134	2,415	2,4845	2,66	2,154	2,456
	PT2022CAPXSER_SERP6	2,6604	2,115	1,959	1,975	2,174	1,922	2,261	2,58	1,826	2,4195
	PT2022CAPXSER_SERP7	2,4142	1,7	1,716	0,467	1,973	1,94	2,077	2,36	1,856	2,1615
	PT2022CAPXSER_SERP8	0,6829	0,344	0,498	0,538	0,561	0,667	0,5915	0,69	0,536	0,5415
	PT2022CAPXSER_SERN1	0,0492	0,07	0,049	0,063	0,05	0,124	0,0445	0,06	0,054	0,0585
	PT2022CAPXSER_SERN2	0,0506	0,09	0,056	0,071	0,049	0,144	0,039	0,06	0,046	0,057
Normalized data	PT2022CAPXSER_SERP1	170,618	179	161,7	163,06	195,67	171,2	165	156,5	138	177,83
	PT2022CAPXSER_SERP2	148,606	168	172,5	153,904	178,64	165,8	165	156,6	138,12	187,54
	PT2022CAPXSER_SERP3	136,378	151	149,5	142,035	170,645	139,1	153	140,3	124,24	154,48
	PT2022CAPXSER_SERP4	99,803	87	103,1	84,09	104,705	101,1	101	104	87,58	104,915
	PT2022CAPXSER_SERP5	347,371	316	338,1	305,298	311,43	327,8	293	287,9	256,39	327,37
	PT2022CAPXSER_SERP6	351,523	322	307,2	290,013	317,4	257,1	266	278,1	216,38	322,585
	PT2022CAPXSER_SERP7	318,111	258	268,2	59,73	287,38	259,6	244	254,4	220,1	287,125
	PT2022CAPXSER_SERP8	83,151	46	72,3	70,445	76,55	76,83	66	69,7	59,26	66,4575
	PT2022CAPXSER_SERN1	-2,85	3	0,2	-2,051	0,225	-1,156	1	-0,6	0,55	0,69324
	PT2022CAPXSER_SERN2	-2,66	6	1,3	-0,868	0	1,77	0	-0,3	-0,43	0,46803
Interpretation	PT2022CAPXSER_SERP1	POS	POS	POS	POS	POS	POS	POS	POS	POS	POS
	PT2022CAPXSER_SERP2	POS	POS	POS	POS	POS	POS	POS	POS	POS	POS
	PT2022CAPXSER_SERP3	POS	POS	POS	POS	POS	POS	POS	POS	POS	POS
	PT2022CAPXSER_SERP4	POS	POS	POS	POS	POS	POS	POS	POS	POS	POS
	PT2022CAPXSER_SERP5	POS	POS	POS	POS	POS	POS	POS	POS	POS	POS
	PT2022CAPXSER_SERP6	POS	POS	POS	POS	POS	POS	POS	POS	POS	POS
	PT2022CAPXSER_SERP7	POS	POS	POS	POS	POS	POS	POS	POS	POS	POS
	PT2022CAPXSER_SERP8	POS	POS	POS	POS	POS	POS	POS	POS	POS	POS
	PT2022CAPXSER_SERN1	NEG	NEG	NEG	NEG	NEG	NEG	NEG	NEG	NEG	NEG
	PT2022CAPXSER_SERN2	NEG	NEG	NEG	NEG	NEG	NEG	NEG	NEG	NEG	NEG
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
	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
	Short or long incubation protocol (if applicable)	/	Short	Short	Short	Short	/	/	/	Short	/
	Formula to calculate the normalized data	S/P%= (OD(sample)-OD(NC))/(OD(PC)-OD(NC))*100	/	/	/	ELISA Indirect	/	S/P%= ((DOéchantillon-DO CN)/(DOCP-DO CN))*100	/	S/P%= (ODsample-ODnc)x100/(ODpc-ODnc)	OD Sample-OD Neg / OD Pos- OD Neg *100
	Used cut-off	30	30	30	30	30	30	30	30	30	30
	Remark(s)	/	/	/	/	/	/	/	/	/	/

Value	Sample	Laboratory number									
		97612	97613	97614	97615	97616	97617	97618	97619	97620	97621
Optical density (OD)	Positive control (mean)	0,8	0,7	1,0	0,9	0,7	0,9	0,5	0,9	0,7	0,6
	Negative control (mean)	0,1	0,1	0,05	0,1	0,1	0,1	0,1	0,1	0,04	0,1
	PT2022CAPXSER_SERP1	1,161	1,2842	1,525	1,3095	1,265	1,409	214,3	1,514	1,058	1,28
	PT2022CAPXSER_SERP2	1,173	1,3896	1,549	1,323	1,268	1,493	204,15	1,547	0,987	1,27
	PT2022CAPXSER_SERP3	1,083	1,1227	1,371	1,233	1,074	1,38	186,72	1,285	0,894	1,09
	PT2022CAPXSER_SERP4	0,772	0,8491	0,848	0,7265	0,718	1,003	107,67	0,996	0,682	0,68
	PT2022CAPXSER_SERP5	2,252	2,3482	2,453	2,35	2,186	2,317	324,48	2,465	2,267	2,3
	PT2022CAPXSER_SERP6	2,105	2,3009	2,29	2,224	2,151	2,325	347,3	2,318	2,092	1,98
	PT2022CAPXSER_SERP7	1,976	2,1199	2,209	1,954	1,938	2,141	278	2,257	1,953	1,86
	PT2022CAPXSER_SERP8	0,61	0,4856	0,663	0,501	0,467	0,586	65,76	0,69	0,583	0,4
	PT2022CAPXSER_SERN1	0,048	0,0532	0,055	0,0595	0,053	0,055	0,41	0,049	0,042	0,06
	PT2022CAPXSER_SERN2	0,051	0,0489	0,043	0,0605	0,06	0,059	0	0,05	0,043	0,05
Normalized data	PT2022CAPXSER_SERP1	145	178,44	161,694	157,062	195	154,3	/	169,6	/	223
	PT2022CAPXSER_SERP2	147	193,71	164,317	158,757	196	164	/	173,5	/	221
	PT2022CAPXSER_SERP3	135	155,04	144,863	147,458	164	151	/	142,9	/	187
	PT2022CAPXSER_SERP4	94	115,41	87,705	83,867	107	107,9	/	109,3	/	113
	PT2022CAPXSER_SERP5	288	332,57	263,115	287,696	343	258,2	/	280,3	/	408
	PT2022CAPXSER_SERP6	269	325,72	245,301	271,877	338	259,1	/	263,2	/	349
	PT2022CAPXSER_SERP7	252	299,5	236,448	237,979	303	238,1	/	256,1	/	328
	PT2022CAPXSER_SERP8	73	62,75	67,486	55,556	67	60,2	/	73,7	/	63
	PT2022CAPXSER_SERN1	0	0,11	1,038	0,126	0	-0,6	/	0,93	/	0
	PT2022CAPXSER_SERN2	0	-0,51	-0,273	0,251	1	-0,1	/	0,81	/	0
Interpretation	PT2022CAPXSER_SERP1	POS	POS	POS	POS	POS	POS	POS	POS	POS	POS
	PT2022CAPXSER_SERP2	POS	POS	POS	POS	POS	POS	POS	POS	POS	POS
	PT2022CAPXSER_SERP3	POS	POS	POS	POS	POS	POS	POS	POS	POS	POS
	PT2022CAPXSER_SERP4	POS	POS	POS	POS	POS	POS	POS	POS	POS	POS
	PT2022CAPXSER_SERP5	POS	POS	POS	POS	POS	POS	POS	POS	POS	POS
	PT2022CAPXSER_SERP6	POS	POS	POS	POS	POS	POS	POS	POS	POS	POS
	PT2022CAPXSER_SERP7	POS	POS	POS	POS	POS	POS	POS	POS	POS	POS
	PT2022CAPXSER_SERP8	POS	POS	POS	POS	POS	POS	POS	POS	POS	POS
	PT2022CAPXSER_SERN1	NEG	NEG	NEG	NEG	NEG	NEG	NEG	NEG	NEG	NEG
	PT2022CAPXSER_SERN2	NEG	NEG	NEG	NEG	NEG	NEG	NEG	NEG	NEG	NEG
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
	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
	Short or long incubation protocol (if applicable)	/	Short	Short	Short	Short	/	Short	Short	Short	/
	Formula to calculate the normalized data	S/P % = [(ODsample - ODnc) / (ODpc - ODnc)] * 100	SP%=((sample OD - mean NC OD)/(mean PC OD - mean NC OD))*100	S/P% = (OD sample - OD NC) / (OD PC - OD NC) x 100	As per Insert CPVDA version 0117GB	/	S/P%=(ODsample-OD negative)/ (OD positive-OD negative)x100	ELISA Indirect	/	S/P%=(ODsample-ODnc)/(ODpc-ODnc)*100	((OD sample - OD NC)/(OD PC - OD NC)) *100
	Used cut-off	30	30	30	30	30	30	30	30	30	30
	Remark(s)	/	/	/	/	Values for Antibody ELISA column are S/P in %	/	/	/	/	/

Value	Sample	Laboratory number								
		97622	97628	97630	97631	97632	97634	97637	97642	97643
Optical density (OD)	Positive control (mean)	0,9	1,5	0,6	1,0	0,8	0,8	0,7	0,7	0,9
	Negative control (mean)	0,1	0,04	0,1	0,1	0,1	0,1	0,1	0,1	0,04
	PT2022CAPXSER_SERP1	1,458	/	1,0353	1,509	1,158	1,256	1,46	1,081	1,49545
	PT2022CAPXSER_SERP2	1,455	/	1,0559	1,425	1,154	1,383	1,508	1,083	1,3476
	PT2022CAPXSER_SERP3	1,273	/	0,994	1,265	1,058	1,19	1,288	0,936	1,23175
	PT2022CAPXSER_SERP4	0,861	147,948	0,6637	0,865	0,836	0,801	0,88	0,655	0,8514
	PT2022CAPXSER_SERP5	2,602	21,2142	2,0347	2,869	2,276	2,334	2,665	1,864	1,9982
	PT2022CAPXSER_SERP6	2,358	18,5926	1,81135	2,666	2,069	2,411	2,356	1,794	2,0662
	PT2022CAPXSER_SERP7	2,22	14,2463	1,6191	2,254	1,931	2,091	2,16	1,64	2,0182
	PT2022CAPXSER_SERP8	0,613	73,5081	0,4989	0,734	0,563	0,615	0,604	0,455	0,4948
	PT2022CAPXSER_SERN1	0,052	/	0,0617	0,054	0,044	0,053	0,073	0,054	0,04505
	PT2022CAPXSER_SERN2	0,051	13,5564	0,05495	0,058	0,046	0,046	0,065	0,055	0,0504
Normalized data	PT2022CAPXSER_SERP1	158	/	165,12	155,79	/	167,6	215,74	169,2	174,1
	PT2022CAPXSER_SERP2	158	/	168,6	146,78	/	185,3	223,15	169,6	156,4
	PT2022CAPXSER_SERP3	137	/	158,16	129,58	/	158,4	189,20	145,3	142,5
	PT2022CAPXSER_SERP4	91	/	102,53	86,71	/	104,18	126,23	98,8	96,98
	PT2022CAPXSER_SERP5	287	/	333,46	301,55	/	317,84	401,70	298,8	234,3
	PT2022CAPXSER_SERP6	259	/	295,83	279,74	/	328,57	354,01	287,3	242,45
	PT2022CAPXSER_SERP7	244	/	263,46	235,64	/	283,97	323,77	261,8	236,7
	PT2022CAPXSER_SERP8	63	/	74,77	72,72	/	78,26	83,64	65,8	54,285
	PT2022CAPXSER_SERN1	0	/	1,13	-0,16	/	-0,07	1,70	-0,7	0,4251
	PT2022CAPXSER_SERN2	0	/	-0,01	0,27	/	-1,05	0,46	-0,5	1,0656
Interpretation	PT2022CAPXSER_SERP1	POS	POS	POS	POS	POS	POS	POS	POS	POS
	PT2022CAPXSER_SERP2	POS	POS	POS	POS	POS	POS	POS	POS	POS
	PT2022CAPXSER_SERP3	POS	POS	POS	POS	POS	POS	POS	POS	POS
	PT2022CAPXSER_SERP4	POS	POS	POS	POS	POS	POS	POS	POS	POS
	PT2022CAPXSER_SERP5	POS	POS	POS	POS	POS	POS	POS	POS	POS
	PT2022CAPXSER_SERP6	POS	POS	POS	POS	POS	POS	POS	POS	POS
	PT2022CAPXSER_SERP7	POS	NEG	POS	POS	POS	POS	POS	POS	POS
	PT2022CAPXSER_SERP8	POS	POS	POS	POS	POS	POS	POS	POS	POS
	PT2022CAPXSER_SERN1	NEG	NEG	NEG	NEG	NEG	NEG	NEG	NEG	NEG
	PT2022CAPXSER_SERN2	NEG	NEG	NEG	NEG	NEG	NEG	NEG	NEG	NEG
/Information	Name ELISA kit producer	ID.VET	Detection Ab's specific to the Capx virus in the serum/plasma of goats, sheep, cattle and other susceptible species. Manufacturer the BioStone Animal Health. Detection Ab's to sheep and goat pox: Test kit for the diagnosis of sheep pox by method AGID. Manufacturer the Research institute for biological safety problems	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
	Short or long incubation protocol (if applicable)	/	Short	Short	Short	Short	Short	/	/	/
	Formula to calculate the normalized data	S/P in %	PP=OD450test sample-NC/PC-NC*100%	S/P%=100*((Odsample-Odnc)/(Odpc-Odnc))	/	/	S/P%=(OD sample-OD NC)/(OD PC-OD NC)*100	/	[OD(sample)-OD(NC)]/[OD(PC)-OD(NC)]*100	(ODsample-ODnc)/(ODpc-ODnc)*100
	Used cut-off	30	40	30	30	30	30	30	30	30
	Remark(s)	/	/	/	/	/	/	/	/	/

10.1.2 SERO: VIRUS NEUTRALIZATION

Value	Sample	Laboratory number				
		97506	97600	97612	97618	97643
Raw data (Ct/Cp value)	Positive control (mean)	400	480	80	20	20
	Negative control (mean)	2	5	10	5	10
	PT2022CAPXSER_SERP1	3750	160	40	640	30
	PT2022CAPXSER_SERP2	250	120	40	400	15
	PT2022CAPXSER_SERP3	750	60	40	200	10
	PT2022CAPXSER_SERP4	1250	320	10	160	120
	PT2022CAPXSER_SERP5	3750	320	160	640	30
	PT2022CAPXSER_SERP6	750	240	160	640	30
	PT2022CAPXSER_SERP7	750	120	160	640	15
	PT2022CAPXSER_SERP8	250	40	10	50	20
	PT2022CAPXSER_SERN1	2	5	5	5	10
	PT2022CAPXSER_SERN2	6	5	5	0	10
Interpretation	PT2022CAPXSER_SERP1	POS	POS	POS	POS	POS
	PT2022CAPXSER_SERP2	POS	POS	POS	POS	POS
	PT2022CAPXSER_SERP3	POS	POS	POS	POS	POS
	PT2022CAPXSER_SERP4	POS	POS	POS	POS	POS
	PT2022CAPXSER_SERP5	POS	POS	POS	POS	POS
	PT2022CAPXSER_SERP6	POS	POS	POS	POS	POS
	PT2022CAPXSER_SERP7	POS	POS	POS	POS	POS
	PT2022CAPXSER_SERP8	POS	POS	POS	POS	POS
	PT2022CAPXSER_SERN1	NEG	NEG	NEG	NEG	NEG
	PT2022CAPXSER_SERN2	NEG	NEG	NEG	NEG	NEG
Information	Used protocol/SOP	In house	After OIE Manual Chapter 3.4.12	In house	Pirbright	NVR-SOP-42
	Name (+ reference) cell type used	OA3.Ts	ESH	OA3.Ts	OA3.Ts	MDBK
	Used cut-off	1/50	5	Pos > 1:5	5	<1/10
	Remark(s)	/	/	/	/	/

10.1.3 SERO: IPMA

Value	Sample	Laboratory number	
		97506	97618
Raw data (Ct/Cp value)	Positive control (mean)	50	300
	Negative control (mean)	1	0
	PT2022CAPXSER_SERP1	300	300
	PT2022CAPXSER_SERP2	50	300
	PT2022CAPXSER_SERP3	300	300
	PT2022CAPXSER_SERP4	300	300
	PT2022CAPXSER_SERP5	300	300
	PT2022CAPXSER_SERP6	300	300
	PT2022CAPXSER_SERP7	300	300
	PT2022CAPXSER_SERP8	50	300
	PT2022CAPXSER_SERN1	1	0
	PT2022CAPXSER_SERN2	1	0
Interpretation	PT2022CAPXSER_SERP1	POS	POS
	PT2022CAPXSER_SERP2	POS	POS
	PT2022CAPXSER_SERP3	POS	POS
	PT2022CAPXSER_SERP4	POS	POS
	PT2022CAPXSER_SERP5	POS	POS
	PT2022CAPXSER_SERP6	POS	POS
	PT2022CAPXSER_SERP7	POS	POS
	PT2022CAPXSER_SERP8	POS	POS
	PT2022CAPXSER_SERN1	NEG	NEG
	PT2022CAPXSER_SERN2	NEG	NEG
Information	Used protocol/SOP	In house	EURL Capripoxviruses Protocol
	Name (+ reference) cell type used	OA3.Ts	OA3.Ts
	Used cut-off	0,02	50
	Remark(s)	/	The samples have been tested at the 1/50-1/300 dilutions

10.1.4 VIRO: RT-QPCR

Value	Sample	Laboratory number									
		97506	97600	97601	97602	97603	97604	97605 (1)	97605 (2)	97606	97607 (1)
Raw data (Ct/Cp value)	Positive control (mean)	29,7	32,1	19,0	22,4	24,5	24,5	29,6	30,2	35,6	30,3
	Negative control (mean)	50	45	0	40	0	45	45	45	45	45
	PT2022CAPXVIR_TP1	32,21	32,87	32,39	32,06	29,31	28,6	31,52	32,08	30,96	30,26
	PT2022CAPXVIR_TP2	36,15	34,45	36,3	30,85	33,05	30,5	34,05	35,05	32,76	33,22
	PT2022CAPXVIR_TP3	30,17	26,88	30,95	24,29	26,67	25,5	27,83	28,66	27,5	28,67
	PT2022CAPXVIR_TP4 (1)	36,57	32,14	37,65	27,58	31,56	28,5	34,23	34,09	33,08	34,03
	PT2022CAPXVIR_TP4 (2)	32,85	32,02	35,88	28,41	31,14	28,8	32,01	32,33	31,7	31,49
	PT2022CAPXVIR_VP1	37,74	33,75	39,43	31,45	33,94	30,6	32,93	33,54	36,06	36,35
	PT2022CAPXVIR_VP2	30,66	28,91	30,96	25,82	32,16	24,6	28,87	29,26	29,03	29,01
	PT2022CAPXVIR_BP1	33,31	30,99	32,8	29,86	30,53	26,9	31,16	32,06	30,79	30,82
	PT2022CAPXVIR_BP2	32,48	29,82	33,31	27,9	29,41	27	30,79	31,14	29,37	30,47
	PT2022CAPXVIR_BN1	50	45	0	40	0	45	45	45	0	45
Interpretation	PT2022CAPXVIR_TP1	POS	POS	POS	POS	POS	POS	POS	POS	POS	POS
	PT2022CAPXVIR_TP2	POS	POS	POS	POS	POS	POS	POS	POS	POS	POS
	PT2022CAPXVIR_TP3	POS	POS	POS	POS	POS	POS	POS	POS	POS	POS
	PT2022CAPXVIR_TP4 (1)	POS	POS	POS	POS	POS	POS	POS	POS	POS	POS
	PT2022CAPXVIR_TP4 (2)	POS	POS	POS	POS	POS	POS	POS	POS	POS	POS
	PT2022CAPXVIR_VP1	POS	POS	POS	POS	POS	POS	POS	POS	POS	POS
	PT2022CAPXVIR_VP2	POS	POS	POS	POS	POS	POS	POS	POS	POS	POS
	PT2022CAPXVIR_BP1	POS	POS	POS	POS	POS	POS	POS	POS	POS	POS
	PT2022CAPXVIR_BP2	POS	POS	POS	POS	POS	POS	POS	POS	POS	POS
	PT2022CAPXVIR_BN1	NEG	NEG	NEG	NEG	NEG	NEG	NEG	NEG	NEG	NEG
Information	Protocol/SOP used	Haegeman *et al*. (2013)	Bowden *et al*. 2008 - modified by FLI	Indispin pathogen kit	Bowden *et al*. 2008	Bowden *et al*. 2008;Babiuk *et al*. 2008; SOP from Pirbright	6.3.51	Bowden *et al*. 2008	Haegeman *et al*. 2013	DNA extraction and real time PCR (Bowden *et al*. 2008)	MO-TO 45
	Producer extraction protocol/kit	Macherey Nagel	Biosellal	Indical Bioscience	Invitrogen/Thermo Fisher Scientific	Roche	Roche	Roche	Roche	Qiagen	Qiagen
	Name extraction protocol/kit	/	Biosellal Superball	Indispin pathogen kit	MagMAX Core Nucleic Acid Purification Kit	MagNa Pure 96 DNA	MagNa Pure 96 DNA	Roche-MP 96/ Viral NA SV	Roche-MP 96/ Viral NA SV	QIAamp 96 Virus QIAcube HT Kit	Qiagen DNAesay Blood and tissue
	RT-qPCR protocol/kit	Fast start DNA polymerase	Qiagen - QuantiTect Multiplex PCR Kit NoRox	Virotype Mix + IC (JOE) - DNA	Home Made	Path-ID qPCR Master Mix (Thermo Fisher Scientific)	Qiagen - QuantiTect Probe	/	/	QuantiNova Probe PCR Kit	SsoAdvanced Universal Probes Supermix
	Target of the RT primer	D5R	ORF074 (P32)	ORF074 (P32)	ORF074 (P32)	ORF074 (P32)	ORF074 (P32)	ORF074 (P32)	E3L	ORF074 (P32)	ORF074 (P32)
	Used cut-off	42	/	40	/	35	38	/	/	/	/
	Remark(s)		/	/	/	/	/	/	/	/	/

Value	Sample	Laboratory number									
		97607 (2)	97608	97609	97610	97611	97612	97613	97614	97616	97617
Raw data (Ct/Cp value)	Positive control (mean)	31,4	25,5	27,4	26,7	26,5	28,7	24,3	24	35	21,2
	Negative control (mean)	45	45	45	38	38	39	40	40	35	40
	PT2022CAPXVIR_TP1	30,89	28,2	29,12	32,3	28,9	27,4	27,7	32,2	28,84	28,31
	PT2022CAPXVIR_TP2	34,15	30,9	32,23	33,9	28,98	31,2	31,72	32,3	31,86	30,77
	PT2022CAPXVIR_TP3	28,8	27,4	25,7	27,63	23,1	25,2	25,77	25,4	25,61	23,04
	PT2022CAPXVIR_TP4 (1)	38,33	31,3	31,45	32,93	27,2	28,3	31,4	30,9	31,07	28,06
	PT2022CAPXVIR_TP4 (2)	31,5	30,9	31,67	33,17	27,6	28,7	30,39	30,4	29,86	27,93
	PT2022CAPXVIR_VP1	35,4	31,7	33,93	33,63	28,8	31,4	30,64	35,9	30,94	28,83
	PT2022CAPXVIR_VP2	29,7	26,4	26,28	28,32	24,4	25,9	26,43	26,7	26,41	24
	PT2022CAPXVIR_BP1	31,68	28,9	27,96	31,82	27,9	28,9	27,81	30,7	28,42	26,51
	PT2022CAPXVIR_BP2	31,31	27	27,51	29,37	25,2	27,4	26,6	29,8	27,6	24,76
	PT2022CAPXVIR_BN1	45	45	0	38	38	39	40	40	0	40
Interpretation	PT2022CAPXVIR_TP1	POS	POS	POS	POS	POS	POS	POS	POS	POS	POS
	PT2022CAPXVIR_TP2	POS	POS	POS	POS	POS	POS	POS	POS	POS	POS
	PT2022CAPXVIR_TP3	POS	POS	POS	POS	POS	POS	POS	POS	POS	POS
	PT2022CAPXVIR_TP4 (1)	POS	POS	POS	POS	POS	POS	POS	POS	POS	POS
	PT2022CAPXVIR_TP4 (2)	POS	POS	POS	POS	POS	POS	POS	POS	POS	POS
	PT2022CAPXVIR_VP1	POS	POS	POS	POS	POS	POS	POS	POS	POS	POS
	PT2022CAPXVIR_VP2	POS	POS	POS	POS	POS	POS	POS	POS	POS	POS
	PT2022CAPXVIR_BP1	POS	POS	POS	POS	POS	POS	POS	POS	POS	POS
	PT2022CAPXVIR_BP2	POS	POS	POS	POS	POS	POS	POS	POS	POS	POS
	PT2022CAPXVIR_BN1	NEG	NEG	NEG	NEG	NEG	NEG	NEG	NEG	NEG	NEG
Information	Protocol/SOP used	MO-TO 46	Pan-Capripox-p32-Taq (Wolff *et al* 2021)	Bowden *et al* Generic Capripox virus detection Real Time PCR	Lamien CE et. al. (2010)	Haegeman *et al* 2013	ID Gene Capripox Virus Triplex (IDvet)	Babiuk *et al*. 2008; Transbound Emerg Dis 55(7) pp299-307	SOP G.72	Path-ID qPCR	Bowden *et al*. 2008
	Producer extraction protocol/kit	Qiagen	/	Indical Bioscience	Indical Bioscience	Qiagen	Roche	Indical Bioscience	Qiagen	Qiagen	Indical Bioscience
	Name extraction protocol/kit	DNAesay Blood and tissue	/	Indispin pathogen kit	IndiMag Pathogen Kit (384)	QIAamp Viral RNA Mini Kit	High Viral Nucleic Acid kit	Indispin pathogen kit	QIAamp Viral RNA Mini Kit	QIAmp DNA mini kit	/
	RT-qPCR protocol/kit	SsoAdvanced Universal Probes Supermix	/	QuantiFast Pathogen+ IC PCR Kit	/	IndiMag Pathogen Kit (384)	ID.VET - ID GENE® CAPRIPOX VIRUS TRIPLEX	Home Made	/	Path-ID qPCR Master Mix	/
	Target of the RT primer	/	/	ORF074 (P32)	ORF074 (P32)	E3L	/	ORF074 (P32)	ORF074 (P32)	ORF074 (P32)	ORF074 (P32)
	Used cut-off	/	/	37	38	38	/	40	37	35	40
	Remark(s)	/	/	/	/	/	/	/	/	We did not perform species differentiation and DIVA PCR due to financial restrictions.	/

Value	Sample	Laboratory number									
		97618	97619	97620	97621	97622	97624	97627	97628 (1)	97628 (2)	97628 (3)
Raw data (Ct/Cp value)	Positive control (mean)	21,3	33	24,0	35	27	24,4	30,7	/	/	/
	Negative control (mean)	0	0	45	40	0	0	0	/	/	/
	PT2022CAPXVIR_TP1	28,43	30	0	27,5	33,49	34,9	30,65	/	/	/
	PT2022CAPXVIR_TP2	33,85	33	34,73	30,5	35,76	39,3	34,54	/	/	/
	PT2022CAPXVIR_TP3	27,37	27	27,12	26,9	30,51	34,6	28,48	/	/	/
	PT2022CAPXVIR_TP4 (1)	30,32	33	30,97	30,5	37,06	37,2	33,98	/	/	/
	PT2022CAPXVIR_TP4 (2)	30,53	33	31,98	31,9	40	38,9	34,76	/	/	/
	PT2022CAPXVIR_VP1	32,53	35	35,08	32,4	36	37,6	35,33	/	/	/
	PT2022CAPXVIR_VP2	25,09	29	29,33	26,4	29,08	34,1	29,85	/	/	/
	PT2022CAPXVIR_BP1	28,57	34	32,7	26,7	31,2	34,3	29,11	/	/	/
	PT2022CAPXVIR_BP2	27,96	34	31,8	25,3	32,05	36,2	26,26	/	/	/
	PT2022CAPXVIR_BN1	0	37	0	40	0	0	0	/	/	/
Interpretation	PT2022CAPXVIR_TP1	POS	POS	NEG	POS	POS	POS	POS	POS	NEG	POS
	PT2022CAPXVIR_TP2	POS	POS	POS	POS	POS	POS	POS	NEG	NEG	POS
	PT2022CAPXVIR_TP3	POS	POS	POS	POS	POS	POS	POS	POS	NEG	POS
	PT2022CAPXVIR_TP4 (1)	POS	POS	POS	POS	POS	POS	POS	NEG	POS	POS
	PT2022CAPXVIR_TP4 (2)	POS	POS	POS	POS	POS	POS	POS	NEG	POS	POS
	PT2022CAPXVIR_VP1	POS	POS	POS	POS	POS	POS	POS	POS	NEG	NEG
	PT2022CAPXVIR_VP2	POS	POS	POS	POS	POS	POS	POS	NEG	POS	POS
	PT2022CAPXVIR_BP1	POS	POS	POS	POS	POS	POS	POS	POS	NEG	POS
	PT2022CAPXVIR_BP2	POS	POS	POS	POS	POS	POS	POS	NEG	POS	POS
	PT2022CAPXVIR_BN1	NEG	ND	NEG	NEG	NEG	NEG	NEG	NEG	NEG	NEG
Information	Protocol/SOP used	Bowden *et al*. 2008	Balinsky *et al*.	Bowden TR Babiuk SL Parkyn GR Copps JS Boyle DB (2008)	Bowden *et al*. 2008	In house	IAEA Bowden et.al	Cat. No. 69506	OIE manual	OIE manual	OIE manual
	Producer extraction protocol/kit	Invitrogen/Thermo Fisher Scientific	/	Invitrogen/Thermo Fisher Scientific	Qiagen	Roche	Qiagen	Qiagen	/	/	/
	Name extraction protocol/kit	Pure Link Genomic DNA Mini Kit (Invitrogen)	/	MagMAX Core Nucleic Acid Purification Kit	MagAttract 96 cador Pathogen Kit	High Viral Nucleic Acid kit	QIAamp Viral RNA Mini Kit	DNAesay Blood and tissue	DNA/RNA-?-FACTOR	DNA/RNA-?-FACTOR	DNA/RNA-?-FACTOR
	RT-qPCR protocol/kit	Invitrogen by life technologies/Platinum Quantitative PCR SuperMix-UDG with ROX	/	QuantiTect Virus Kit	/	Roche kit	/	ID.VET - ID GENE® CAPRIPOX VIRUS TRIPLEX	PCR-OSPA-FACTOR (cat. No. D12016-VET) VET FACTOR	PCR-NODULAR-DERMATITE-CRS-FACTOR (cat. No. D12416-VET) VET FACTOR	Nodular dermatitis and smallpox of sheep and goats (Capripoxvirus Viruses) Fractal Bio
	Target of the RT primer	ORF074 (P32)	ORF068 (PAPS)	ORF074 (P32)	ORF074 (P32)	ORF074 (P32)	ORF074 (P32)	/	/	/	/
	Used cut-off	/	37	45	35	/	/	/	/	/	/
	Remark(s)	/	PT2022CAPXVIR_BN1 → under LOD / weak pos / doubt	/	/	/	/	/	/	/	/

10.1.5 VIRO: SPECIES DIFFERENTIATION

Value	Sample	Laboratory number									
		97506	97600	97602	97603	97607	97608	97609	97610	97611	97612
Raw data (Ct/Cp value)	Positive control (mean)	22,48	14,53	20,47	172	32,86	25,4	/	25,43	75,73	25,6
	Negative control (mean)	50	45	40	0	45	45	/	38	37	39
	PT2022CAPXVIR_TP1	50	24,92	40	172	45	30,5	/	32,51	75,4	39
	PT2022CAPXVIR_TP2	35,27	25,19	33,26	172	34,23	31,7	/	35,76	76,4	31,1
	PT2022CAPXVIR_TP3	50	23,85	25,81	151	45	26,4	/	34,1	75,4	39
	PT2022CAPXVIR_TP4 (1)	36,28	21,09	29,39	172	35,4	31,2	/	33,74	76,4	28,9
	PT2022CAPXVIR_TP4 (2)	32,86	21,23	30,28	172	32,7	31,3	/	33,63	76,6	29,7
	PT2022CAPXVIR_VP1	35,27	22,59	33,78	172	37,6	45	/	33,32	76,4	39
	PT2022CAPXVIR_VP2	30,18	17,56	26,84	172	29,9	26,2	/	27,57	76,2	25,9
	PT2022CAPXVIR_BP1	50	28,39	30,79	151	45	29	/	33,52	75,2	39
	PT2022CAPXVIR_BP2	30,84	18,41	28,88	172	32,2	26,9	/	29,68	76,2	27,6
	PT2022CAPXVIR_BN1	50	45	40	0	45	45	/	/	/	39
Interpretation	PT2022CAPXVIR_TP1	GTPV	GTPV	ND	GTPV	NEG	GTPV	GTPV	GTPV	SPPV	GTPV
	PT2022CAPXVIR_TP2	LSDV	LSDV	LSDV	LSDV	LSDV	LSDV	LSDV	LSDV	LSDV	LSDV
	PT2022CAPXVIR_TP3	SPPV	SPPV	SPPV	SPPV	NEG	SPPV	SPPV	SPPV	SPPV	NEG
	PT2022CAPXVIR_TP4 (1)	LSDV	LSDV	LSDV	LSDV	LSDV	LSDV	LSDV	LSDV	LSDV	LSDV
	PT2022CAPXVIR_TP4 (2)	LSDV	LSDV	LSDV	LSDV	LSDV	LSDV	LSDV	LSDV	LSDV	LSDV
	PT2022CAPXVIR_VP1	LSDV	LSDV	LSDV	LSDV	LSDV	LSDV	LSDV	LSDV	LSDV	NEG
	PT2022CAPXVIR_VP2	LSDV	LSDV	LSDV	LSDV	LSDV	LSDV	LSDV	LSDV	LSDV	LSDV
	PT2022CAPXVIR_BP1	SPPV	SPPV	SPPV	SPPV	NEG	SPPV	SPPV	SPPV	SPPV	NEG
	PT2022CAPXVIR_BP2	LSDV	LSDV	LSDV	LSDV	LSDV	LSDV	LSDV	LSDV	LSDV	LSDV
	PT2022CAPXVIR_BN1	NEG	NEG	NEG	NEG	NEG	NEG	NEG	NEG	ND	NEG
Information	Protocol/SOP used	Agianniotaki *et al*. 2016 and in-house Taqman assay and Chibssa *et al*. 2018	Lamien *et al*. 2011	Dual hybridization probe	Lamien *et al* 2011	MO-TO 47	LSDV-DIVA duplex, SPPV GTPV duplex (Wolff *et al* 2021)	Lamien *et al* 2011 conventional PCR, partial GPCR	Lamien CE et. al. (2010)	Galaye *et al* 2017	IZSTE B456.1 SOP 021 + Wolff *et al*. 2021 qPCR_GTPV
	Producer extraction protocol/kit	Fast start DNA polymerase	Biosellal	ThermoFisher Scientific	Roche	Qiagen	/	/	Indical	Qiagen	Biosellal
	Name extraction protocol/kit	/	Biosellal Superball	/	Magna Pure Compact Nucleic Acid Isolation Kit I	/	/	IndiMag Pathogen	IndiMag Pathogen Kit (384)	QiaAmp Viral RNA mini kit kit	/
	RT-qPCR protocol/kit	Fast start DNA polymerase, Addition of EC to buffer B3	Lamien *et al*. 2011	Home made	Taq DNA Polymerase kit (Qiagen) (conventional PCR)	Home made	SsoAdvanced Universal Probes Supermix	HotStart Taq Polymerase Qiagen	Home made	Quantinova SYBR Green RT-PCR master mix 172018438	Bio-T kit Lumpy Skin Disease
	Used cut-off	42	45	/	151bp for SPPV 172bp for GTPV and LSDV	/	/	/	38	CT 37/ MT 65 to 85C	39
	Remark(s)	/	/	/	/	A neg result may represent either a truly neg sample (neg) or a GTPV/ SPPV pos sample	/	Poscontrol GTPV: LSDV/GTPV band (172 bp), Poscontrol SPPV: SPPV band (151 bp)	/	Galaye *et al* 2017 adapted in-house for Quantitect SYBR Green Master mix.	/

Value	Sample	Laboratory number									
		97613	97614	97617	97618	97619	97620	97630	97631	97632	97634
Raw data (Ct/Cp value)	Positive control (mean)	24,35	24	/	/	33	172	33,03	32	31,5	30,34
	Negative control (mean)	0	40	/	/	0	0	45	38	0	45
	PT2022CAPXVIR_TP1	30,96	34	/	/	30	0	30,327	/	0	45
	PT2022CAPXVIR_TP2	33,78	34	/	/	33	172	35,09	30,2	28,5	33,78
	PT2022CAPXVIR_TP3	37,1	29	/	/	27	151	26,959	33,83	0	45
	PT2022CAPXVIR_TP4 (1)	36	33	/	/	33	172	33,991	28,76	31,9	33,82
	PT2022CAPXVIR_TP4 (2)	32,84	33	/	/	33	172	32,9	29,76	31,3	31,34
	PT2022CAPXVIR_VP1	34,97	36	/	/	35	/	45	26,35	0	33,84
	PT2022CAPXVIR_VP2	27,96	30	/	/	29	172	29,114	20,88	0	27,1
	PT2022CAPXVIR_BP1	40,52	33	/	/	34	151	28,839	32,68	0	45
	PT2022CAPXVIR_BP2	29,72	32	/	/	34	172	28,712	27,59	31,6	28,07
	PT2022CAPXVIR_BN1	/	40	/	/	37	0	45	/	0	45
Interpretation	PT2022CAPXVIR_TP1	GTPV	GTPV	GTPV	GTPV	GTPV	NEG	GTPV	NEG	NEG	GTPV
	PT2022CAPXVIR_TP2	LSDV	LSDV	LSDV	LSDV	LSDV	LSDV	LSDV	LSDV	LSDV	LSDV
	PT2022CAPXVIR_TP3	SPPV	SPPV	SPPV/GTPV	SPPV	SPPV	SPPV	SPPV	SPPV	NEG	SPPV
	PT2022CAPXVIR_TP4 (1)	LSDV	LSDV	LSDV	LSDV	LSDV	LSDV	LSDV	LSDV	LSDV	LSDV
	PT2022CAPXVIR_TP4 (2)	LSDV	LSDV	LSDV	LSDV	LSDV	LSDV	LSDV	LSDV	LSDV	LSDV
	PT2022CAPXVIR_VP1	LSDV	LSDV	LSDV	LSDV	LSDV	/	NEG	LSDV	NEG	LSDV
	PT2022CAPXVIR_VP2	LSDV	LSDV	LSDV	LSDV	LSDV	LSDV	LSDV	LSDV	NEG	LSDV
	PT2022CAPXVIR_BP1	SPPV	SPPV	SPPV	SPPV	SPPV	SPPV	SPPV	SPPV	NEG	SPPV
	PT2022CAPXVIR_BP2	LSDV	LSDV	LSDV	LSDV	LSDV	LSDV	LSDV	LSDV	LSDV	LSDV
	PT2022CAPXVIR_BN1	NEG	NEG	NEG	NEG	LSDV	NEG	NEG	NEG	NEG	NEG
Information	Protocol/SOP used	Lamien *et al* 2011	SOP G.72	Chibssa *et al*. 2018, Gelaye *et al*. 2015, Lamien *et al*. 2011	In house	PCR followed by direct sequencing and Blast search - PCR based on publication Batra *et al*.	Lamien *et al* 2011	Wolff *et al*. 2021	Vidanovic *et al*. unpublished	Vidanovic *et al* Detection of field LSDV strains	Vidanovic *et al*. 2021, GtPV - Vidanovic unpublished, SpPV Vidanovic unpublished
	Producer extraction protocol/kit	Indical	Qiagen	Indical	ThermoFisher Scientific	/	ThermoFisher Scientific	Indical	/	/	/
	Name extraction protocol/kit	/	/	IndiMag Pathogen Kit	/	/	/	/	/	/	/
	RT-qPCR protocol/kit	Home made	/	/	Qiagen/Taq DNA Polymerase	Chemagic Viral DNA/RNA 300 Kit	Platinum PCR Supermix	QuantiTect Multiplex PCR NoROX Kit	Home Made	/	/
	Used cut-off	/	37	/	/	/	/	/	40	38	40
	Remark(s)	/	/	Species identification was performed by sequencing of the amplified fragment.	/	Please upgrade your results table possibilities for conventional PCR followed by direct Sanger sequencing	PCR product lenghts are 172bp LSDV/GTPV and 151bp SPPV.	/	/	/	/

Value	Sample	Laboratory number	
		97642	97643
Raw data (Ct/Cp value)	Positive control (mean)	17,12	45
	Negative control (mean)	40	45
	PT2022CAPXVIR_TP1	37,23	/
	PT2022CAPXVIR_TP2	36,57	/
	PT2022CAPXVIR_TP3	30,47	/
	PT2022CAPXVIR_TP4 (1)	35,94	/
	PT2022CAPXVIR_TP4 (2)	35,57	/
	PT2022CAPXVIR_VP1	36,65	/
	PT2022CAPXVIR_VP2	26,9	/
	PT2022CAPXVIR_BP1	32,58	/
	PT2022CAPXVIR_BP2	27,41	/
	PT2022CAPXVIR_BN1	0	/
Interpretation	PT2022CAPXVIR_TP1	GTPV	GTPV
	PT2022CAPXVIR_TP2	LSDV	LSDV
	PT2022CAPXVIR_TP3	SPPV	SPPV
	PT2022CAPXVIR_TP4 (1)	LSDV	LSDV
	PT2022CAPXVIR_TP4 (2)	LSDV	LSDV
	PT2022CAPXVIR_VP1	LSDV	LSDV
	PT2022CAPXVIR_VP2	LSDV	LSDV
	PT2022CAPXVIR_BP1	SPPV	SPPV
	PT2022CAPXVIR_BP2	LSDV	LSDV
	PT2022CAPXVIR_BN1	NEG	NEG
Information	Protocol/SOP used	Lamien *et al* 2011	Lamien *et al*. 2011, NVR-SOP-46
	Producer extraction protocol/kit	Maxwell RSC viral Total Nucleic Acid Purification Kit and Maxwell RSC whole Blood DNA kit	ThermoFisher Scientific
	Name extraction protocol/kit	/	/
	RT-qPCR protocol/kit	Home made	/
	Used cut-off	40	/
	Remark(s)	/	/

10.1.6 VIRO: DIVA PCR

Value	Sample	Laboratory number									
		97506	97600	97602	97608	97609 (1)	97609 (2)	97609 (3)	97610	97611	97612
Raw data (Ct/Cp value)	Positive control (mean)	22,48	25,26	25,76	25,5	30,2	27,68	/	26,2	32,3	28,4
	Negative control (mean)	50	45	40	45	45	45	/	38	38	39
	PT2022CAPXVIR_TP1	50	/	40	/	0	0	/	/	/	39
	PT2022CAPXVIR_TP2	35,27	33,71	35,03	31,7	35,97	0	/	33,12	30,7	28,5
	PT2022CAPXVIR_TP3	50	26,67	28,55	/	0	30,29	/	/	/	39
	PT2022CAPXVIR_TP4 (1)	36,28	31,3	30,97	32,2	35,16	0	/	31,35	28,6	26,1
	PT2022CAPXVIR_TP4 (2)	32,86	32,04	31,58	31,3	35,59	0	/	31,48	28,9	26,9
	PT2022CAPXVIR_VP1	36,27	37,5	40	45	36,63	0	/	31,69	30,1	39
	PT2022CAPXVIR_VP2	30,18	29,99	26,86	26,2	27,42	0	/	26,33	25,8	24,9
	PT2022CAPXVIR_BP1	50	30,82	33,74	/	0	0	/	/	/	39
	PT2022CAPXVIR_BP2	30,84	28,5	32,44	26,9	30,49	0	/	27,78	26,5	25,6
	PT2022CAPXVIR_BN1	50	45	40	/	0	0	/	/	/	39
Interpretation	PT2022CAPXVIR_TP1	GTPV	ND	GTPV wild	ND	NEG	NEG	NEG	NEG	ND	NEG
	PT2022CAPXVIR_TP2	LSDV wild	ND	LSDV wild	LSDV wild	LSDV wild	NEG	NEG	LSDV wild	LSDV wild	LSDV wild
	PT2022CAPXVIR_TP3	SPPV wild	LSDV wild	SPPV wild	ND	NEG	SPPV wild	SPPV wild	NEG	ND	NEG
	PT2022CAPXVIR_TP4 (1)	LSDV wild	SPPV wild	LSDV wild	LSDV wild	LSDV wild	NEG	NEG	LSDV wild	LSDV wild	LSDV wild
	PT2022CAPXVIR_TP4 (2)	LSDV wild	LSDV wild	LSDV wild	LSDV wild	LSDV wild	NEG	NEG	LSDV wild	LSDV wild	LSDV wild
	PT2022CAPXVIR_VP1	LSDV wild	LSDV wild	LSDV Vaccin	ND	LSDV Vaccin	NEG	NEG	LSDV Vaccin	LSDV Vaccin	NEG
	PT2022CAPXVIR_VP2	LSDV Vaccin	LSDV Vaccin	LSDV Vaccin	LSDV Vaccin	LSDV Vaccin	NEG	NEG	LSDV Vaccin	LSDV Vaccin	LSDV Vaccin
	PT2022CAPXVIR_BP1	SPPV Vaccin	SPPV Vaccin	SPPV Vaccin	ND	NEG	NEG	SPPV Vaccin	NEG	ND	NEG
	PT2022CAPXVIR_BP2	LSDV wild	LSDV wild	LSDV wild	LSDV wild	LSDV wild	NEG	NEG	LSDV wild	LSDV wild	LSDV wild
	PT2022CAPXVIR_BN1	NEG	NEG	NEG	NEG	NEG	NEG	NEG	NEG	ND	ND
Information	Protocol/SOP used	Agianniotaki *et al*., in-house Taqman ass ay, Chibssa *et al*, unpublished recombinant DIVA	Confidential (from FLI Riems)	Wolf *et al*. 2021 /Chibbsa *et al*. 2018	LSDV-DIVA duplex (Wolff *et al* 2021)	DIVA LSDV Real Time PCR Agianniotaki *et al* 2017	SPPV WT Sybr Green Real Time PCR / Haegeman *et al* 2015	SPPV DIVA conventional PCR Haegeman *et al* 2015	Vidanovic *et al* (2016)	Agianniotaki *et al* 2017	ID Gene LSD DIVA Triplex - IDvet
	Producer extraction protocol/kit	/	Biosellal	/	/	Indical	Indical	Indical	Indical	Qiagen	ID.VET
	Name extraction protocol/kit	/	Biosellal Superball	/	/	Indispin Pathogen kit (manual)	IndiMag Pathogen kit	IndiMag Pathogen kit	IndiMag Pathogen Kit (384)	Qiagen QiaAmp Viral RNA mini kit kit lot:56901711	/
	RT-qPCR protocol/kit	Fast start DNA polymerase	Quantitec multiplex PCR kit (Qiagen)	Home made	Home made	Pt Taq DNA Polymerase Invitrogen	/	HotStart Taq DNA Polymerase kit Qiagen	Home made	Thermofisher TaqMan Fast Virus 1-step Master Mix	ID GENE® LSD DIVA TRIPLEX
	Used cut-off	42	/	/	/	38	38	/	38	38	/
	Remark(s)	Recombinant strain based on the Recombinant DIVA	/	/	Differentiating between field and vaccine strains of GTPV and SPPV is meaningless because the genetic markers are not clearly and robustly defined (Biswas *et al*. 2020). + recently described evidence from recombinant LSDV also shows that the genetic markers for classification into field and vaccine viruses are not absolutely fixed here either.	/	Haegeman etal 2015 SPPV WT adapted to Sybr Real Time PCR kit	/	/	/	Our lab only performs LSD DIVA

Value	Sample	Laboratory number									
		97613	97614	97617	97618	97619	97620 (1)	97620 (2)	97621 (1)	97621 (2)	97622
Raw data (Ct/Cp value)	Positive control (mean)	30,33	24	/	35,71	/	24,55	218	35	/	35,73
	Negative control (mean)	0	40	/	0	/	40	0	40	/	/
	PT2022CAPXVIR_TP1	0	34	/	40	/	0	0	40	/	/
	PT2022CAPXVIR_TP2	33,32	34	/	37,83	/	33,62	336	33,2	/	34,43
	PT2022CAPXVIR_TP3	0	34	/	0	/	0	302	40	/	/
	PT2022CAPXVIR_TP4 (1)	33,82	34	/	36,41	/	29,34	336	31,5	/	35,45
	PT2022CAPXVIR_TP4 (2)	34,22	34	/	36,24	/	30,86	336	33,3	/	36,52
	PT2022CAPXVIR_VP1	32,8	34	/	37,71	/	0	336	34	/	/
	PT2022CAPXVIR_VP2	28,27	34	/	29,46	/	29,89	336	26	/	/
	PT2022CAPXVIR_BP1	0	34	/	0	/	0	218	40	/	/
	PT2022CAPXVIR_BP2	28,82	34	/	33,73	/	29,37	336	25,9	/	31,01
	PT2022CAPXVIR_BN1	0	40	/	0	/	0	0	40	/	/
Interpretation	PT2022CAPXVIR_TP1	NEG	GTPV wild	GTPV wild	GTPV Vaccin	ND	NEG	ND	NEG	NEG	NI
	PT2022CAPXVIR_TP2	LSDV wild	LSDV wild	LSDV wild	LSDV wild	ND	LSDV wild	LSDV wild	LSDV wild	NEG	LSDV wild
	PT2022CAPXVIR_TP3	NEG	SPPV Vaccin	SPPV wild	SPPV Vaccin	ND	NEG	SPPV wild	NEG	SPPV wild	NI
	PT2022CAPXVIR_TP4 (1)	LSDV wild	LSDV wild	LSDV wild	LSDV wild	ND	LSDV wild	LSDV wild	LSDV wild	NEG	LSDV wild
	PT2022CAPXVIR_TP4 (2)	LSDV wild	LSDV wild	LSDV wild	LSDV wild	ND	LSDV wild	LSDV wild	LSDV wild	NEG	LSDV wild
	PT2022CAPXVIR_VP1	LSDV Vaccin	LSDV Vaccin	LSDV Vaccin	LSDV Vaccin	ND	NEG	LSDV wild	LSDV Vaccin	NEG	NI
	PT2022CAPXVIR_VP2	LSDV Vaccin	LSDV Vaccin	LSDV Vaccin	LSDV Vaccin	ND	LSDV Vaccin	LSDV wild	LSDV Vaccin	NEG	NI
	PT2022CAPXVIR_BP1	NEG	SPPV wild	SPPV Vaccin	SPPV Vaccin	ND	NEG	SPPV Vaccin	NEG	SPPV Vaccin	NI
	PT2022CAPXVIR_BP2	LSDV wild	LSDV wild	LSDV wild	LSDV wild	ND	LSDV wild	LSDV wild	LSDV wild	NEG	LSDV wild
	PT2022CAPXVIR_BN1	NEG	NEG	NEG	NEG	ND	NEG	NEG	NEG	NEG	NI
Information	Protocol/SOP used	Agianniotaki *et al*. (2017)	Gelaye et. al. 2015, Le Goff et. al. 2009, SOP G.72	Menasherow *et al*. 2016 (LSDV); Chibssa *et al*. 2018 (SPPV); Gelaye *et al*. 2015 (GTPV)	DIVA GREECE Agianniotaki *et al* 2017	The DIVA GREECE Agianniotaki *et al* 2017 protocol is dedicated mainly for LSD	ID Gene LSD DIVA Triplex Real time PCR	Chibssa T.R. Grabherr R. Loitsch A. *et al*. A gel-based PCR method to differentiate sheeppox virus field isolates from vaccine strains. (2018)	Agianniotaki *et al*. (2017)	Haegeman *et al*. 2015	In house
	Producer extraction protocol/kit	Indical	Qiagen	Indical	ThermoFisher Scientific	/	ThermoFisher Scientific	ThermoFisher Scientific	Qiagen	Qiagen	/
	Name extraction protocol/kit	/	/	IndiMag Pathogen Kit	Thermo Fisher Scientific	/	/	/	/	/	/
	RT-qPCR protocol/kit	Home made	Home made	/	Invitrogen by Thermo Fisher Scientific/Platinum Taq DNA Polymerase	/	ID.VET - ID GENE® LSD DIVA TRIPLEX	/	/	/	/
	Used cut-off	/	37	/	/	/	40	/	35	/	/
	Remark(s)	/	/	/	/	/	/	/	No difference could be made between LSDV wild or vaccine	No difference could be made between LSDV wild or vaccine	/

Value	Sample	Laboratory number							
		97630	97631	97632	97634	97637 (1)	97637 (2)	97642	97643
Raw data (Ct/Cp value)	Positive control (mean)	28,71	32,3	31,1	30,34	17,75	17,74	17,49	/
	Negative control (mean)	45	40	0	45	/	/	40	45
	PT2022CAPXVIR_TP1	45	/	0	45	/	/	0	/
	PT2022CAPXVIR_TP2	35,09	30,2	0	33,78	34,52	34,44	34,48	/
	PT2022CAPXVIR_TP3	45	/	0	45	/	37,85	0	/
	PT2022CAPXVIR_TP4 (1)	33,991	28,76	0	33,82	33,55	34,24	31,59	/
	PT2022CAPXVIR_TP4 (2)	32,9	29,76	0	31,34	32,61	33,11	31,12	/
	PT2022CAPXVIR_VP1	45	26,3	0	33,84	31,67	36,29	34,57	/
	PT2022CAPXVIR_VP2	29,114	20,88	26,1	27,1	25,58	30,29	27,17	/
	PT2022CAPXVIR_BP1	45	/	0	45	/	/	0	/
	PT2022CAPXVIR_BP2	28,644	27,59	0	28,07	27,46	28,46	28,94	/
	PT2022CAPXVIR_BN1	45	/	0	45	/	/	0	/
Interpretation	PT2022CAPXVIR_TP1	NEG	NEG	NEG	NEG	NEG	NEG	GTPV wild	ND
	PT2022CAPXVIR_TP2	LSDV wild	LSDV wild	NEG	LSDV wild	LSDV wild	LSDV wild	LSDV wild	LSDV wild
	PT2022CAPXVIR_TP3	NEG	ND	NEG	NEG	NEG	LSDV wild	SPPV wild	ND
	PT2022CAPXVIR_TP4 (1)	LSDV wild	LSDV wild	NEG	LSDV wild	LSDV wild	LSDV wild	LSDV wild	LSDV wild
	PT2022CAPXVIR_TP4 (2)	LSDV wild	LSDV wild	NEG	LSDV wild	LSDV wild	LSDV wild	LSDV wild	LSDV wild
	PT2022CAPXVIR_VP1	NEG	LSDV Vaccin	NEG	LSDV Vaccin	LSDV Vaccin	LSDV Vaccin	LSDV Vaccin	ND
	PT2022CAPXVIR_VP2	LSDV Vaccin	LSDV Vaccin	LSDV Vaccin	LSDV Vaccin	LSDV Vaccin	LSDV Vaccin	LSDV Vaccin	LSDV Vaccin
	PT2022CAPXVIR_BP1	NEG	ND	NEG	NEG	NEG	NEG	SPPV Vaccin	ND
	PT2022CAPXVIR_BP2	LSDV wild	LSDV wild	NEG	LSDV wild	LSDV wild	LSDV wild	LSDV wild	LSDV wild
	PT2022CAPXVIR_BN1	NEG	NEG	NEG	NEG	NEG	NEG	NEG	NEG
Information	Protocol/SOP used	Wolff *et al*. 2021 (Möller *et al*. 2019)	Vidanovic *et al* (2021)	Moller *et al* 2019-Experimental LSD infection of cattle	Vidanovic *et al* (2021)	Vidanovic *et al* (2021)	Agianniotaki *et al*. (2017)	Agianniotaki *et al*. (2017)	Unpublished assay
	Producer extraction protocol/kit	Indical	/	/	/	/	/	/	ThermoFisher Scientific
	Name extraction protocol/kit	/	/	/	/	/		/	/
	RT-qPCR protocol/kit	QuantiTect Multiplex PCR NoROX Kit	Home made	/	Vidanovic *et al* (2021)	Vidanovic *et al* (2021)	Vidanovic *et al* (2021)	Home made	Unpublished assay
	Used cut-off	/	/	/	40	38	38	40	/
	Remark(s)	Negative results in the interpretation of this assay refer only to the LSDV wild and vaccine strains. Results of the samples for GTPV and SPPV are included in the species differentiation assay.	/	/	Only LSDV DIVA was performed	/	/	Wild type LSDV according to Eirini *et al*l (2017)	Sample TP4: Its negative according to Vidanovic *et al*. (2016). Suspected sample?

10.2 Annex 2: Boxplots

Besides qualitative data analysis (positive, negative or doubtful result), also quantitative data analysis was performed. The quantitative data analysis in this report was not used to evaluate the participants in this PT, but should only be considered as educational information for the participants in order to evaluate their performance and/or to standardize their different diagnostic tests.

For the virology RT-qPCR, box plots of the Ct-values per reference sample and per participating laboratory are shown in Figures I and II. Because there was one repetition, only the sample PT2022CAPXVIR_TP4 was included in the figures down below.

The boxplots, shown down below, were created by using the following software programme: shiny.chemgrid.org/boxplotr/.

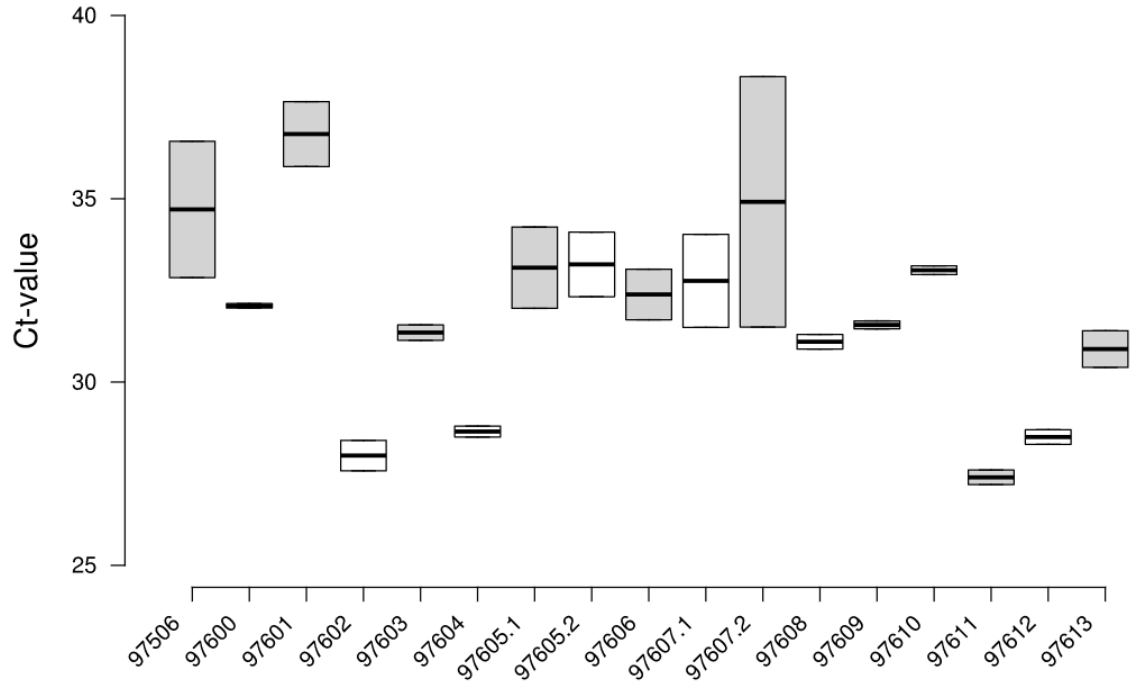


Figure I: Distribution of the Ct-values data (boxplots) for the PT2022CAPXVIR_TP4 samples (LAB97506-LAB97613).

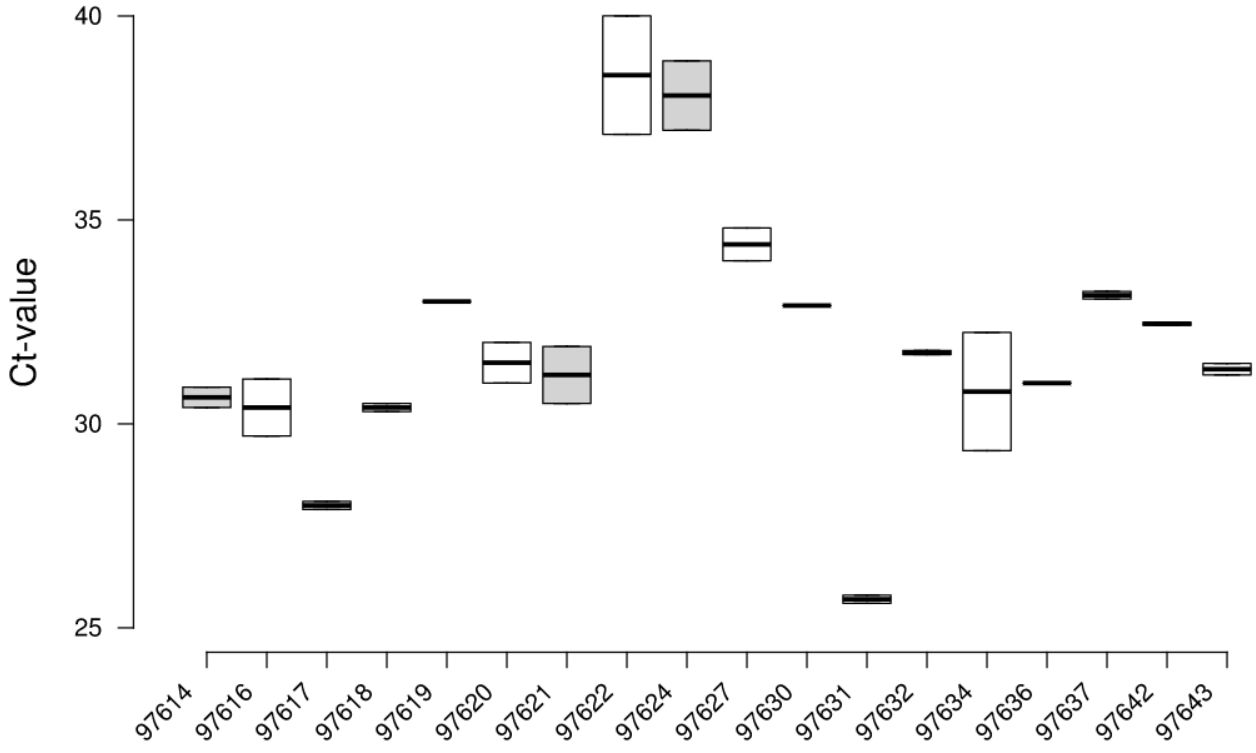


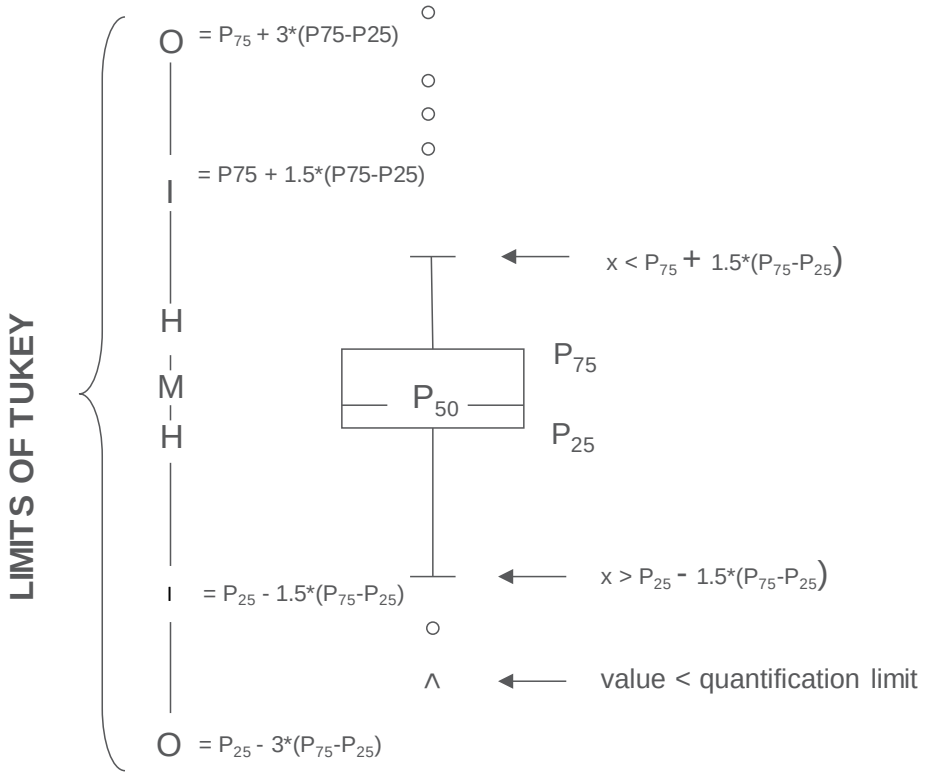
Figure II: Distribution of the Ct-values data (boxplots) for the PT2022CAPXVIR_TP4 samples (LAB97614-LAB97643).

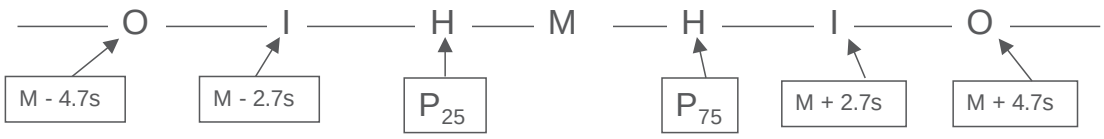
10.3 Annex 3: Additional information

Graphical representation

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

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





Corresponding limits in case of normal distribution

The preliminary report of this survey is available on our website via the following link:
https://www.wiv-isp.be/QML/activities/PT%20VET/fr/originaux/rapports_annee.htm

The calendar for Proficiency Testing in Veterinary diagnosis is available on our website:
https://www.wiv-isp.be/QML/activities/external_quality/calendar/kalender.htm

END