VALIDATION REPORT

Product: IDCPV

ID Gene™ Capripox Virus Triplex

Method	Real time PCR – Triplex - Qualitative
Species	Bovine, Caprine, Ovine
Matrices	Ruminant whole blood, nasal and oral swabs, tissue (skin lesions)
Reference	IDCPV

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1 INTRODUCTION

Lumpy skin disease (LSD) is a highly infectious disease of cattle caused by a virus belonging to the genus *Capripox* of the *Poxviridae* family.

This disease is characterized by growth nodule with often necrosis stage, anorexia, hyperthermia and usually death for the most serious cases. Ovine and caprine species can be affected by other Capripox viruses: sheeppox and goatpox viruses.

Innovative Diagnostics has developed a molecular biology tool to detect all Capripox viruses. This kit is a real time triplex PCR assay. It simultaneously amplifies a target gene sequence, an endogenous internal control and an exogenous internal control.

The internal control is an ubiquitous endogenous DNA sequence that is constitutively present in cells. It allows for detection of DNA from sample cells.

The exogenous internal control (NTPC-CPV) is a different DNA virus from Capripox virus that is not present in the domain of activity used. It is hereafter called the "Mimic". This Mimic validates all the analytical steps of the system (extraction, purification, amplification).

2 KIT OVERVIEW

The **ID Gene™ Capripox virus Triplex**, reference: **IDCPV**, is developed, manufactured, controlled and conditioned at the headquarters of Innovative Diagnostics : 310 rue Louis Pasteur 34790 Grabels.

2.1 Kit description

The IDCPV kit allows for detection of Capripox virus DNA by real time PCR.

Target gene "CPV": target gene sequence conserved in all Capripox viruses.

Endogenous "Non Target Positive Control": Gene constitutively present in the sample.

Exogenous "Non Target Positive Control": Gene from a non-pathogenic DNA virus.

Type of PCR: Triplex Real-time PCR, allowing a qualitative analysis.

Validated species: cattle; sheep and goat.

Matrices: Ruminant whole blood, oral and nasal swabs, tissues or organs (including skin lesions).

Kit format: 50 or 100 reactions.

2.2 Kit composition, storage and stability

All Innovative Diagnostics contain reagents and biological matrices internally lyophilized.

The IDCPV kit is composed of the reagents listed in the table below.

Reference	*Component*	*Volume*	*Description*
TPC-CPV	*Target Positive Control*	*550 µl 1 vial*	*Whole negative blood spiked with LSD inactivated vaccine strain freeze dried and calibrated between 10 and 100 times the method detection limit (MDL). Freezed-dried pellet to be reconstituted in 550 µl distilled or nuclease-free water.*
NTPC-CPV	*Non Target Positive Control*	*2200 µl 1 vial*	*Non-pathogenic inactivated virus strain. Freezed-dried pellet to be reconstituted in 2200 µl distilled or nuclease-free water.*
ARM-CPV	*Amplification Reaction Mix*	*400 µl 1 or 2 tubes (white caps)*	*Ready-to-use reaction mixture containing Taq polymerase and oligonucleotides for amplification and detection of Capripox virus, the endogenous and exogenous non-target positive controls.*

All components should be stored at ≤ -16°C. It is recommended to prepare aliquots (minimum 100 µl) in order to avoid multiple freeze/thaw cycles (> 3 not recommended).

The kit is stable at ≤ -16°C from the manufactured date.

2.3 Precautions

The material used contains less than 0.1% hazardous or carcinogenic materials, MSDS sheets are thus not required. However it is recommended to always take appropriate precautions with all biochemicals such as wearing appropriate protective clothing. The material used should be of quality suitable for molecular biology.

3 PRESENTATION OF VALIDATION DATA

3.1 Validation protocol

The **ID Gene™ Capripox virus Triplex** kit was evaluated and validated by the CODA-CERVA, Center for Veterinary and Agrochemical Studies and Research, Uccle, Bruxelles, BELGIUM.

The protocols of validated methods are described in Appendices 1 and 2.

3.2 PCR Characterization

3.2.1 Analytic specificity

3.2.1.1 *In silico* specificity

The *in silico* specificity of the IDCPV PCR kit was evaluated by DNA sequence alignment the target PCR (primers and probes) with the databases available on the NCBI (National Center for Biotechnology Information).
After alignment, 100% of specificity *in silico* was obtained for the target gene. The alignments do not show high sequence homology with pathogens in the same ecological niche.

3.2.1.2 Experimental specificity

3.2.1.2.1 Inclusivity study

The inclusivity study was conducted on a collection of 18 Capripox Virus reference isolates supplied by **Friedrich-Loeffler** Institute (FLI).

Identification	Strain	Virus	Dilution factor	Result
LSDV 1	LSDV vaccine Neethling	LSDV	10-1	Detected
LSDV 2	LSDV vaccine Neethling	LSDV	10-2	Detected
LSDV 3	LSDV vaccine Neethling	LSDV	10-3	Detected
LSDV 4	LSDV vaccine Neethling	LSDV	10-4	Detected
LSDV 5	LSDV vaccine Neethling	LSDV	10-5	Detected
LSDV 6	LSDV vaccine Neethling	LSDV	10-6	Detected
Capripox-Ringtest 2016 DNA01	Sheeppox Russia (2003)	Sheeppox	10-1	Detected
Capripox-Ringtest 2016 DNA02	LSDV Neethling vaccine	LSDV	10-1	Detected
Capripox-Ringtest 2016 DNA03	LSDV Neethling 2490	LSDV	10-1	Detected
Capripox-Ringtest 2016 DNA04	Goatpox FZ (India)	Goatpox	10-1	Detected
Capripox-Ringtest 2016 DNA05	LSDV Macedonia 2016	LSDV	10-1	Detected
Capripox-Ringtest 2016 DNA06	LSDV Vaccine SIS-Type	LSDV	10-1	Detected
Capripox-Ringtest 2016 DNA07	Negative Rinder DNA EDTA Blood	-	neg	Not Detected
Capripox-Ringtest 2016 DNA08	LSDV Neethling vaccine	LSDV	10-3	Detected
Capripox-Ringtest 2016 DNA09	LSDV Neethling 2490	LSDV	10-3	Detected
Capripox-Ringtest 2016 DNA10	Negative Rinder DNA EDTA Blood	-	neg	Not Detected
Capripox-Ringtest 2016 DNA11	LSDV Macedonia 2016	LSDV	10-3	Detected
Capripox-Ringtest 2016 DNA12	LSDV Vaccine SIS Type	LSDV	10-3	Detected

Conclusion:
The IDCPV kit successfully detected all the Capripox virus isolates in this panel.

3.2.1.2.2 Exclusivity study

The exclusivity study was conducted on a panel of strains which may have genetic similarities with the target sequence of interest and / or are found within the same ecological niche.

Sample type	Origin	IDCPV results
Bacteria		
Anaplasma Phagocytophilum	France	Not detected
Chlamydophila abortus	France	Not detected
Mycoplasma bovis	France	Not detected
Mycobacterium avium Phlei	France	Not detected
Viruses		
BTV 1	France	Not detected
BTV 8	France	Not detected
H7N1	France	Not detected
Bovine Respiratory Syncitial Virus	France	Not detected
Bovine Viral Diarrhea Virus	France	Not detected
Bovine Leucose Virus	France	Not detected
SDRP US	France	Not detected
H1N1	France	Not detected
IBV	France	Not detected
NDV	France	Not detected
H5N2	France	Not detected
SBV	France	Not detected
Parasites		
Leishmania infantum	France	Not detected
Neospora caninum	France	Not detected

Conclusion:
The IDCPV kit is amplifies and detect specifically its target only (Capripox virus).

3.2.1.2.3 Detectability study

Inclusivity study is complete with a detectability assay on 1 LSD field strain virus (Macedonia 2016) and 3 vaccine strains against LSDV (Neethling, Herbivac®LS, BOVIVAX LSD).
A serial dilution of each strain is carried out in nuclease free water and extracted before amplification using the IDCPV.

Identification	Dilution	Results (Cq values)
		CPV target (FAM)
LSDV-Macedonia 2016	pure	12.53
	10^{-1}	15.82
	10^{-2}	19.21
	10^{-3}	22.13
	10^{-4}	25.56
	10^{-5}	28.25
	10^{-6}	33.63
	10^{-7}	38.92
LSDV-Neethling Vaccine	pure	13.40
	10^{-1}	16.83
	10^{-2}	20.16
	10^{-3}	23.32
	10^{-4}	26.31
	10^{-5}	29.06
	10^{-6}	32.75
	10^{-7}	Not detected
LSDV Herbivac®LS	pure	20.37
	10^{-1}	26.65

	10^{-2}	38.57
	10^{-3}	Not detected
BOVIVAX LSD-N (Maroc)	pure	16.70
	10^{-1}	23.51
	10^{-2}	29.23
	10^{-3}	Not detected

Conclusion:
The different dilution levels are detected correctly.

3.2.2 Analytic sensitivity

Experimental estimate of the LD_{PCR}

The limit of detection of the PCR (LD_{PCR}) is the smallest number of copies of nucleic acid target per unit volume that can be detected in 95% of cases. To determine the LD_{PCR} different amounts of nucleic acid were used flanking the supposed LD_{PCR} value in repeatable conditions with intra-assay replicates and inter-assay (independent experiment) replicates.
The LD_{PCR} is determined using a PLS-CPV plasmid quantified with a 'QuantiFluor® dsDNA System' and a 'Quantus™ Fluorometer' from Promega. The quantification on the Quantus™ Fluorometer is performed 10 times. The plasmid quantification is determined by calculating the average of the 10 analysis.
The LD_{PCR} was determined with a PCR program called "rapid" (45 min ± 10 min depending on the thermocycler used). Another program called "classic" can be used.
The program is the following:

	Rapid amplification program
Step 1: Single time	**Activation of the polymerase:** 2 min at 95°C
Step 2: Repeat 40 times	**DNA denaturation:** 10 seconds at 95°C **Annealing:** 30 seconds at 60°C

Experimental design for the estimation of the LD_{PCR} (95%):

Number of dilutions	Number of replicates / dilution	Number of independent experimental runs
6	4	1

Rapid program results:

Number of copies/PCR	Positives in run 1	Total number of runs	Detection frequency
50	4	4	100.00%
25	4	4	100.00%
5	4	4	100.00%
2.5	3	4	75.00%
1	3	4	75.00%
0.5	2	4	50.00%

Conclusion:
The estimated 95% limit of detection of the IDCPV PCR with the rapid amplification program is around **5 copies / PCR**.

3.2.3 Determination of the linearity range and the efficiency of the PCR

The linearity of a quantitative assay is its ability to generate results that are proportional to the concentration of targets present in a given range and that can be modeled by a linear equation. A linear regression exists between the instrumental response (example: Cq) and the logarithm of the target quantity (copy number of target per PCR). The establishment of the parameters of the regression line (type y = x '+ b) and the validation of the linear model requires compliance with an evaluation protocol and monitoring of data analysis that has several purposes:

— Define the equation of the line
— Determine efficiency
— Determine and validate the performance of the linear regression

Parameters for straight line calibration and determination of PCR efficiency

The efficiency (E) evaluates the performance of the reaction of each real-time PCR.
For each run, we calculate the efficiency E according to the equation:

$$E = (10^{-1/a} - 1) \times 100$$

For your information, the slope (a) is generally between - 4.115 and - 2.839, which corresponds to an E between 75% and 125%.

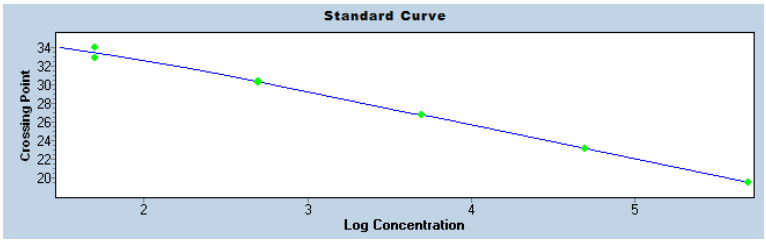
Experimental determination of the efficiency (E) and R^2

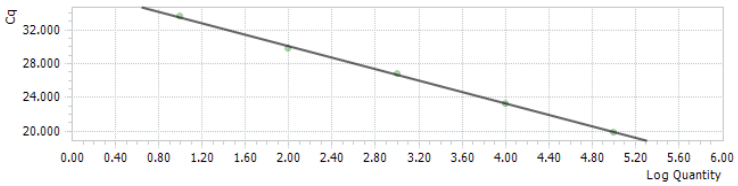
A range of 4 points of nucleic acid dilution made from the previously calibrated CPV plasmid is prepared to implement the following experimental design on the Light Cycler 480 and 96 device.

Minimum number of runs	Minimum number of operators	Minimum number of independent ranges	Minimum number of dilutions tested per range	Number of replicates per dilution tested
2	1	1	5	1

Rapid program results

	Slope (a)	Y-intercept (b)	Efficiency
Run 1	-3.6	40.08	89%
Run 2	-3.4	36.82	97%
Average	-3.5	38.4	93%





Conclusion with the rapid amplification program:

The average efficiency is 93%

3.3 Characterization of the complete method

Without any access to field samples naturally infected and calibrated by a reference laboratory, Innovative Diagnostics decided to perform the approach of the method detection limit by spiking a quantified vaccine strain CPV in a negative sheep blood sample.

3.3.1 Methodology

A negative sheep blood sample is spiked with LSD virus neethling vaccinale strain from CODA CERVA calibrated at $12.5.10^4$ copies / µl. It is important to notify that this method does not take into account the influence of target inclusion in the analysed matrix.

3.3.2 Experimental modality to approach the method detection limit

The LD_{METHOD} is estimated as the last dilution level where 4 repetitions are positive. The limit estimated is thus not the absolute detection limit of the method which is at a lower concentration of target sequences.

Experimental design for the experimental estimation of the LD_{METHOD}:

Number of run	Minimum number of operators	Minimum number of replicates per dilution
1	1	4

Validated methods are:

- SPIN50/SPIN250: manual extraction on silica columns.
- MAGFAST384: automated extraction with magnetic beads in 20 minutes.

Each method has been validated with the "rapid" amplification programs with MAGFAST384 and SPIN50/SPIN250 results on sheep whole blood.

	Copies/PCR	50	25	12.5	6.25	3.125	1.56
MAGFAST384	Replicate 1	31.27	32.41	33.84	35.99	37.54	-
	Replicate 2	31.21	32.35	33.02	35.45	37.74	-
	Replicate 3	31.77	32.73	33.92	35.15	-	37.7
	Replicate 4	31.82	33	33.71	35.66	36.49	38.3
	Average Cq	31.52	32.62	33.62	35.6	36.83	38
	% of detection	100%	100%	100%	100%	75%	50%
SPIN50/SPIN250	Replicate 1	33.92	35.11	35.66	36.42	37.42	-
	Replicate 2	34.44	34.87	35.72	36.56	38.56	-
	Replicate 3	34.35	34.78	35.89	36.78	38.68	-
	Replicate 4	34.62	35.36	35.64	37.45	-	38.9
	Average Cq	34.33	35.03	35.72	36.30	38.22	38.9
	% of detection	100%	100%	100%	100%	75%	25%

Conclusion:

The limit of detection of the method on whole blood with SPIN50/SPIN250 and the rapid amplification program is **6.25 copies/PCR or $1.25.10^3$ copies/ml**

The limit of detection of the method on whole blood with MAGFAST384 and the rapid amplification program is **6.25 copies/PCR or $1.25.10^3$ copies/ml**

3.4 Diagnostic Specificity and Sensitivity

3.4.1 Experimental modalities

The method must be evaluated on a representative panel of samples from the field or from an infection experiment. Samples should have known positive and negative status (subject to availability).

This evaluation study was conducted by the OIE reference laboratory CODA CERVA and by Institute Friedriech Loeffler (FLI).
The status of these samples has been previously validated by a reference method (in-house reference labs methods).

If possible the panel should include samples with three levels of viral load (*e.g.* slightly positive samples. averagely positive samples and strongly positive samples). The panel should also include negative samples.

Results will be presented as a percentage of positives found among the expected positives for the diagnostic sensitivity (Se) and as the percentage of negatives found among the expected negatives for the diagnostic specificity (Sp).

Se = TP / (TP + FN) ; TP = True Positive ; FN = False Negative

Sp = TN / (TN + FP) ; TN = True Negative ; FP = False Positive

3.4.2 MAGFAST384 results obtained on whole blood

The results were obtained with Method 1 (automated magnetic bead extraction - Protocol in Appendix 1) with the rapid amplification program.

- 56 blood samples from an experimental infection study achieved by the OIE reference laboratory CODA CERVA are used to evaluate the diagnostic sensitivity and specificity of the IDCPV PCR kit.
- 24 blood samples from Institute Friedriech Loeffler
- 25 positive swab (oral and nasal) samples from Institute Friedriech Loeffler
- 25 negative swab (oral and nasal) samples from Innovative Diagnostics
- 16 tissues or organs from Institute Friedriech Loeffler
- 7 negative organs from Innovative Diagnostics

The status of each sample is predetermined using a reference method (in-house validated PCR for the detection of all capripox viruses).

3.4.2.1 Results of whole blood samples panel with MAGFAST384:

		PCR status with a validated method		
		Positive	**Negative**	**Total**
IDCPV method results	**Positive**	*57*	*0*	**57**
	Negative	*3**	*20*	**23**
	Total	*60*	*20*	**80**
		Se = 95%	**Sp = 100%**	

Raw data of the study are detailed in the Appendix 6

*3 samples are detected with D5R PCR from CODA-CERVA but not detected with LSDIVA PCR. The 2 PCRs not being carried out at the same time and the Cq values were around 35; the posititivity of the samples is therefore very low, at the detection limit of the method.

Conclusions:

The sensitivity and specificity diagnostic results obtained with MAGFAST384 and IDCPV kits are the following:
Se = 95% et Sp = 100%

The number of samples (n) used to evaluate the diagnostic specificity and sensitivity has an impact on the error of the estimation.
The determination of the error (e), at 95% of confidence of a diagnostic sensitivity or specificity value (ɵ), estimated according to the number (n) of samples analysed, is used as described by Greiner and Gardner

$$e = 1{,}96\sqrt{\frac{\theta(1-\theta)}{n}}$$

For a diagnostic sensitivity (Se) of 88% determided by analyzing 60 samples known as positive, the error (e), at the 95% confidence, is estimated at 5.5% (corresponding to a confidence interval between 94.5% and 100%).

3.4.2.2 Results of whole blood samples panel with SPIN50/250 :

		PCR status with a validated method		
		Positive	Negative	Total
IDCPV method results	Positive	33	0	33
	Negative	3*	20	23
	Total	36	20	56
		Se = 92%*	Sp = 100%	

Raw data of the study are detailed in the Appendix 5.5.1 and 5.5.2

*3 samples are detected with D5R PCR from CODA-CERVA but not detected with LSDIVA PCR. The 2 PCRs not being carried out at the same time and the Cq values were around 35; the posititivity of the samples is therefore very low, at the detection limit of the method

Conclusions:
The sensitivity and specificity diagnostic results obtained with SPIN50/250 and IDCPV kits are the following:
Se = 92%* et Sp = 100%

For a diagnostic sensitivity (Se) of 92% determinated by analysing 36 samples known to be positive, error (e), at the 95% confidence, is estimated at 8.8% (corresponding to a range of Se confidence interval between 83.2% and 100%).

3.4.2.3 Results of oral and nasal swabs panel:

		PCR status with a validated method		
		Positive	Negative	Total
IDCPV method results	Positive	25	0	25
	Negative	0	25	25
	Total	25	25	50
		Se = 100%	Sp = 100%	

Raw data of the study are detailed in the Appendix 6

Conclusions:
The sensitivity and specificity diagnostic results obtained with MAGFAST384 and IDCPV kits are the following:
Se = 100% et Sp = 100%

3.4.2.4 Results of tissues / organs panel:

		PCR status with a validated method		
		Positive	Negative	Total
IDCPV method results	Positive	16	0	16
	Negative	0	7	7
	Total	16	7	23
		Se = 100%	Sp = 100%	

Raw data of the study are detailed in the Appendix 6

Conclusions:
The sensitivity and specificity diagnostic results obtained with MAGFAST384 and IDCPV kits
Se = 100% et Sp = 100%

3.5 Robustness

Robustness is evaluated on DNA from negative bovine blood sample spiked with LSDV vaccine strain calibrated at 10x LD_{METHOD} and on the PLS-CPV diluted at 3x LD_{PCR}. The samples are performed in 8 replicates.

3.5.1 Temperature Variations

Three PCR amplifications were performed on a single Roche LightCycler®96 thermocycler with the rapid amplification program (*i.e.* the most critical conditions):

	Setpoint temperatures -1°C	Setpoint temperatures	Setpoint temperatures + 1°C
Step 1: Single time	**Activation of the polymerase:** 2 mins at 94°C	**Activation of the polymerase:** 2 mins at 95°C	**Activation of the polymerase:** 2 mins at 96°C
Step 2: Repeated 40 times	**DNA denaturation:** 10 seconds at 94°C **Annealing:** 30 seconds at 59°C	**DNA denaturation:** 10 seconds at 95°C **Annealing:** 30 seconds at 60°C	**DNA denaturation:** 10 seconds at 96°C **Annealing:** 30 seconds at 61°C

	Average of 8 Cq values for each temperature			
	-1°C	Setpoint temperatures	+1°C	CV %
10x LD_{METHOD}	30.7	30.9	30.8	**0.3%**
3x LD_{PCR}	32.9	33.5	33.2	**0.7%**

Conclusion:

For amplification temperature variations of ± 1°C. the IDCPV kit has variation coefficients below 1%.

3.5.2 Variations in DNA volume

Three PCR amplifications were performed on a single Roche LightCycler®96 thermocycler with DNA volumes ±10% with the rapid amplification program (*i.e.* the most critical conditions).

Set volume -10%	Set volume	Set volume +10%
4.5 µl	5µl	5.5 µl

	Average of 8 Cq values for each DNA volume tested			
	-10%	Set volume	+10%	CV
10x LD_{METHOD}	31.3	30.9	30.6	**0.9%**
3x LD_{PCR}	33.42	33.15	32.46	**1.2%**

Conclusion:

For changes of DNA volume ± 10%. the IDCPV kit has variation coefficients below 1.5%.

3.5.3 Repeatability

Caprin negative blood was spiked with viral inactivated strains provided by the CODA-CERVA at medium (Cq = 28/29) and low (Cq = 32/33) concentrations. These samples were extracted 5 times for the strongest sample and 10 times for the weakest sample with the MAGFAST384 kit. Then amplified using the IDCPV kit and the rapid amplification program.

Stonghly positive blood (Cq values)				
28.35	29.52	28.88	28.84	29.12

CV = 1.3%

Weakly positive blood (Cq values)									
32.66	32.31	31.48	31.97	31.42	32.62	31.68	31.92	32.34	32.27

CV = 1.31%

Conclusion:

The IDCPV kit provides good repeatability regardless of the level of virus used.

3.5.4 Reproducibility

Experimental plan for reproducibility testing:

Load levels	Number of replicates / load level	Number of independent sessions
2	3	3

Negative blood was spiked with LSD viral and vaccine strains with 2 different load levels. These samples were then extracted 3 times with the MAGFAST384 kit. The extracts were amplified with the IDCPV kit and the rapid amplification program.

	ROCHE LC®96		LIFE ABI 7500	
Sample	**Run 1**	**Run 2**	**Run 3**	**CV**
Viral target level 1	25.2	25.8	25.5	**1.5%**
Viral target level 1	25.4	25.6	24.8	
Viral target level 1	24.9	25.8	24.9	
Viral target level 2	27.9	28.2	28.6	**1.1%**
Viral target level 2	28.6	27.9	28.2	
Viral target level 2	28.9	28.1	28.2	
Vaccin target level 1	27.5	27.5	27.8	**1.1%**
Vaccin target level 1	28.1	27.6	27.9	
Vaccin target level 1	27.8	28.2	28.5	
Vaccin target level 2	32.5	32.8	31.8	**1.2%**
Vaccin target level 2	32.1	32.9	31.9	
Vaccin target level 2	31.9	32.4	32.1	

Conclusion:

The IDCPV kit ensures good reproducibility regardless of the level of virus and vaccine and the thermocycler instrument used.

3.6 Cross-contamination tests

3.6.1 MAGFAST384 with IDEAL™

The following study was performed to ensure no cross-contamination upon extraction on magnetic beads with the IDEAL™ robot.

A range of LSD virus strains was spiked into negative caprin whole blood and deposited alternately with unspiked negative whole blood according to the diagram below:

POS1	NEG	NEG	NEG	POS5	NEG	NEG	NEG	POS5	NEG	NEG	POS1
POS2	NEG	POS1	NEG	NEG	NEG	POS1	NEG	NEG	NEG	POS1	POS2
POS3	NEG	NEG	NEG	POS6	NEG	NEG	NEG	POS6	NEG	NEG	POS3
POS4	NEG	POS2	NEG	NEG	NEG	POS2	NEG	NEG	NEG	POS2	POS4
POS5	NEG	NEG	NEG	POS7	NEG	NEG	NEG	POS7	NEG	NEG	POS5
POS6	NEG	POS3	NEG	NEG	NEG	POS3	NEG	NEG	NEG	POS3	POS6
POS7	NEG	NEG	NEG	POS8	NEG	NEG	NEG	POS8	NEG	NEG	POS7
POS8	NEG	POS4	NEG	NEG	NEG	POS4	NEG	NEG	NEG	POS4	POS8

3.6.1.1 Results of MAGFAST384 on the IDEAL™

28.5	NEG	NEG	NEG	36.8	NEG	NEG	NEG	36.3	NEG	NEG	28.2
30.2	NEG	28.2	NEG	NEG	NEG	27.9	NEG	NEG	NEG	28.2	30.5
33.6	NEG	NEG	NEG	-	NEG	NEG	NEG	37.5	NEG	NEG	33.9
34.8	NEG	30.8	NEG	NEG	NEG	29.8	NEG	NEG	NEG	30.6	34.8
37.1	NEG	NEG	NEG	-	NEG	NEG	NEG	-	NEG	NEG	35.9
-	NEG	33.9	NEG	NEG	NEG	33.5	NEG	NEG	NEG	33.8	37.5
-	NEG	NEG	NEG	-	NEG	NEG	NEG	-	NEG	NEG	-
-	NEG	35.2	NEG	NEG	NEG	35.2	NEG	NEG	NEG	34.7	-

Conclusion :

All the negative samples remain negative. The MAGFAST384 magnetic bead extraction method with the IDEAL™ does not display cross-contamination.

3.6.2 MAGFAST384 with KingFisher™ mL

The following study was performed to ensure no cross-contamination upon extraction on magnetic beads with LSD virus strains using the KingFisher mL robot:

A negative caprin whole blood sample was spiked with a LSD virus strains and was loaded alternately with the negative caprin whole blood sample according to the table below:

POS	NEG	NEG	NEG	POS
NEG	NEG	POS	NEG	NEG
POS	NEG	NEG	NEG	POS

3.6.2.1 MAGFAST384 results on the KingFisher mL

24.2	NEG	NEG	NEG	23.9
NEG	NEG	24.1	NEG	NEG
23.6	NEG	NEG	NEG	23.9

Conclusion :

All the negative samples remain negative. The MAGFAST384 magnetic bead extraction method with the KingFisher mL does not display cross-contamination.

3.7 PCR Stability

An accelerated stability study for 4 weeks at 22°C and for 14 weeks at 4°C already allows us to predict a stability of 12 months at ≤ -16°C.

3.7.1 Accelerated Stability Data

To make this prediction the following formula derived from the Arrhenius equation is commonly used; Predicted Stability = Accelerated Stability x $2^{\Delta T/10}$.

Here Δ T is the difference in temperature between the expected storage temperature and the temperature tested.

Note that the greater the Δ T is, less reliable prediction will be. It is therefore appropriate to apply a correction factor (reducing) to the predicted data based on data provided by our suppliers on the components of the assay.

The calculated predictions are shown in the table below:

Study starting from the manufacture of the IDCPV test (in weeks)	Prediction for an accelerated stability at +4°C (in months)	Prediction for an accelerated stability at +22°C (in months)
2	1.5	5
3	2.5	8
4	3	12
5	4	
6	5	
10	8	
14	12	

3.7.1.1 Data analysed for stability evaluation

The real and accelerated stabilities are derived in the limiting conditions of the classic amplification kit, namely:

- 3 times the LD_{PCR} or 15 copies / PCR
- 10 times the LD_{METHOD} or 62.5 copies / PCR (MAGFAST384).

The analysis of the target positive control (TPC–CPV) will allow the evaluation of a possible drift.

3.7.1.2 Preliminary data for the actual stability study

The actual stability study is ongoing.

The first results of stability at a storage temperature ≤ -16°C for 12 months are indiacted on the next page.

Stability at -20°C during 4 months.

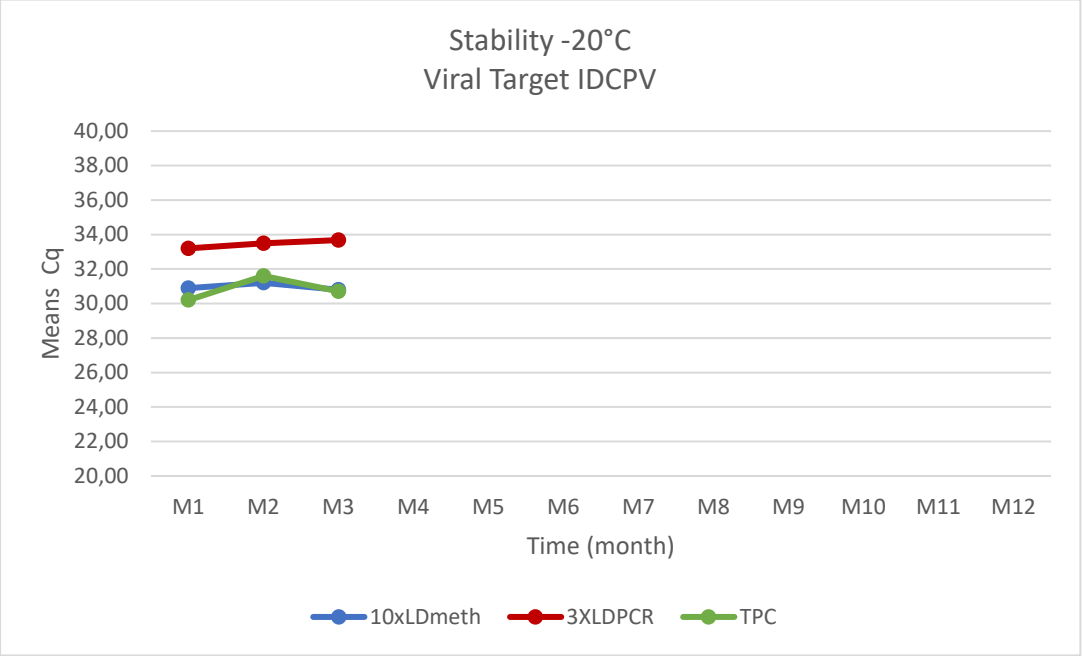


Figure 1: Monitoring the value of the viral target Cq signal versus time at a -20°C conservation temperature

Conclusion:

The real stability study of IDCPV kit is currently evaluated for 3 months at a storage temperature ≤ -16 °C. However the accelerated stability data enable to predict the stability of the kit for at least 12 months at a storage temperature ≤ -16 ° C.

4 OVERALL CONCLUSION

According to the EURL reference laboratory CODA CERVA, the **ID Gene™ Capripox virus triplex** kit is validated to perform a confident detection of Capripox virus.

HISTORY OF REVISIONS

Version	Edit date	Reference	Type of revision	Revision made
1217	07/2025	doc0215	**Update**	- Correction of the version number to match the version of the associated instructions for use (version 1217) - Innovative Diagnostics now mentionned as the kit's manufacturer
0518	05/2018	doc1121	**Update**	Update of the chapter Diagnostic sensitivity

Appendix n°1: Instructions for the MAGFAST extraction system

INSTRUCTIONS FOR USE

ID GENE™ MAG FAST EXTRACTION KIT

FAST EXTRACTION OF NUCLEIC ACIDS WITH MAGNETICS BEADS

PATHOGENS	All types of pathogens
NUCLEIC ACID TYPE	DNA and RNA
SPECIES	Multiple species
SAMPLE TYPES	All types of samples
ADDITIONAL REFERENCES	• Positive Extraction Control (product code: PEC) • or Target Positive Control (product code: TPC)
ASSOCIATED PCR PRODUCTS	• ID Gene™ range • ID Gene Lyo™ range
PRODUCT CODE	MAGFAST

In vitro use

December 2024

Changes were made to the IFU, please refer to the history of revisions for details.

MAGFAST version 1224 EN, rev. December 2024

With you at every step

TABLE OF CONTENTS

RISKS AND PRECAUTIONS OF USE

Read the instructions carefully before use. A general guide to the use of molecular biology techniques and Material Safety Data Sheets (MSDS) are available upon request from info@innovative-diagnostics.com.

⚠ Caution: Some components contain hazardous chemicals. Wear appropriate Personal Protective Equipment. Follow Good Laboratory Practice (GLP) and safety guidelines. Dispose of reagents and biological waste (notably those with a potential infectious risk) in accordance with applicable regulations.

⚠ Some buffers contain chaotropic salts which might form highly reactive compounds if mixed with bleach or acids. Therefore, bleach or acids should not be added directly to sample preparation waste.

GENERAL INFORMATION

Characteristics

The ID Gene™ Mag Fast Extraction Kit – 384 extractions format (product-code: MAGFAST-384) is a system for fast nucleic acid extraction and purification using magnetic beads, applicable to any matrices and all pathogens.

Kit composition and storage conditions

The ID Gene™ Mag Fast Extraction Kit, format 384 reactions, contains the following reagents:

REFERENCE	DESCRIPTION	VOLUME	
		On receipt	After reconstitution
LYS-FAST	Lysis buffer	60 mL	*Not necessary*
MAGBEADS	Magnetic beads suspension	4,5 mL	*Not necessary*
BB-FAST	FAST Binding buffer	90 mL	180 mL
WASH1	Wash Buffer 1	81 mL	125 mL
WASH2	Wash buffer 2	81 mL	125 mL
ELU	Elution buffer	60 mL	*Not necessary*
-	General user manual		-

The kit should be stored at 21°C (± 5°C) until the date indicated on the box and on the Quality Control sheet.

Equipment, materials, reagents and consumables required to be supplied by the user

Equipment and materials:

- For optimal results, the use of the following automatic magnetic rod extraction robots, consumables and programmes is recommended:

ROBOT	CONSUMABLES		PROGRAM TO USE**
	PRODUCT CODE	DESCRIPTION	
IDEAL™ 32*	IDTIP-32A	Plastic tip combs-32	IDGeneMAGFAST
	IDWELL-32A	Deepwell plates -32	
IDEAL™ 96*	IDTIP-96	Plastic tip combs-96	IDGeneFAST or IDGeneFastv3.
	IDWELL-96	Deepwell plates -96	
	IDELU-96	Elution plates -96	
KingFisher™ 96 /Flex	Supplier : Thermo Fisher Scientific		ID Gene MAGFAST_96

A presentation of the IDEAL™ is accessible online by flashing the QR code above or by following the link www.innovative-diagnostics.com/espece/instruments/

*Availability varies between countries. ** If necessary, software scripts can be provided upon request. Please contact info@innovative-diagnostics.com for more information.*

Note : When using other robots, it is important to contact Innovative Diagnostics BEFORE running the tests in order to verify software script compatibility. Assistance can be provided to choose compatible programs.

- Precision pipettes capable of delivering volumes from 1 µL to 1 000 µL
- Personal Protective Equipment (single-use gloves and lab coat, possibly with safety glasses, mask...)
- ⚠ Where there is a risk of the presence of highly airborne pathogens and/or zoonotic pathogens, it is recommended that the samples be processed under a biosafety cabinet until the end of the lysis step.

Reagents:

- Absolute ethanol, of molecular biology grade, if possible
- Distilled water for WASH3 buffer preparation, eventually
- Nuclease (DNAse and RNAse)-free water, of molecular biology grade

Consumables :

- Nuclease-free filter tips
- For the robots, the above-mentioned plastic consumables

PREPARATION OF REAGENTS

Magnetic beads suspension MAGBEADS-BB-FAST

Before the first use of BB-FAST binding buffer, reconstitute by adding 90 mL of absolute ethanol to the vial. Tick the dedicated box on the label to indicate that ethanol has been added.

Each extraction reaction requires 310 µL of MAGBEADS-BB-FAST suspension to be made up with 10 µL of MAGBEADS beads and 300 µL of BB-FAST binding buffer.

- Before each extraction, prepare the necessary MAGBEADS-BB-FAST suspension by multiplying the indicated quantities for one single reaction by the number of samples and controls to be analysed and adding a dead volume of 10%.
  Example: For 10 reactions, mix 110 µL of MAGBEADS with 3300 µL of BB-FAST binding buffer.
- Mix the suspension thoroughly by inversion, or by successive pipetting, just before use.
- Distribute 310 µL of this mixture in the Deepwell plate, according to the positions indicated, and in as many wells as there are samples and controls to extract.

WASH buffer 1 (WASH1) and WASH buffer 2 (WASH2)

Before the first use of these buffers, reconstitute by adding 44 mL of absolute ethanol to the vial. Tick the dedicated boxes on the labels to indicate that ethanol has been added.

WASH buffer 3 (WASH3)

WASH Buffer 3 consists of an 80% ethanol solution, to be prepared.

- Ensure that the final volume prepared covers at least the requirements of the analyses to be carried out, adding a dead volume of 10%. The excess can be stored at room temperature.
- Mix the required proportions of absolute ethanol and distilled or Nuclease-free water.
- Mix thoroughly before use.

PRE-TREATMENT OF SAMPLES AND CONTROLS

Preparation of samples

⚠ For each type of matrix, and depending on the pathogens to be detected, Innovative Diagnostics offers a specific pre-treatment protocol.

A list of currently available protocols can be accessed online by flashing the following QR code or using the link below:

www.innovative-diagnostics.com/produit/id-gene-mag-fast-extraction-kit

These protocols are available upon request at info@innovative-diagnostics.com.

Note: Additional equipment, materials, reagents and consumables, to be supplied by the user, may be required.

Preparation of extraction controls

⚠**They must be processed in the same way as samples, starting from the pre-treatment when necessary and nucleic acid extraction.** They are used to validate the efficiency of the nucleic acid extraction and (RT-) qPCR amplification processes.

- The Positive Extraction Control must contain the target pathogen(s).
  Innovative Diagnostics can provide Positive Extraction Control (product code: PEC) or Target Positive Control (product code: TPC) that contains the target pathogen(s) diluted in a matrix.
  For more information, please refer to the package insert for reconstitution and storage conditions. These products can be used as a sentinel to monitor variations in analytical sensitivity. In that case, users must first determine the dilution adapted to their conditions of use.
- The Negative Extraction Control (NEC) must not contain the target pathogen. It consists of :
  - either a negative matrix: NEC-matrix.
  - or Nuclease-free water (RNase /DNase free), or any other buffer used during the pre-treatment step: NEC-process.

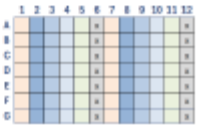
⚠ The step at which these controls are to be included, and the test volume, may vary depending on the matrices and pathogens to be analysed. Please refer to the specific pre-treatment protocol for the matrix/pathogen in question.

With the IDEAL™ 32 robot

1. PREPARE CONSUMABLES

- Prepare 1 or 2 Deepwell IDWELL-32A plates depending on the number of samples and controls to be extracted. One plate is sufficient for up to 16 samples.
- A half-row (covering columns 1-6 or 7-12) is required for the extraction and purification of a sample or control. Label each half-row with the name of the sample or control to be processed.

2. DISTRIBUTE a) ALL BUFFERS b) SAMPLES/CONTROLS AND c) THE BEAD SUSPENSION ACCORDING TO THE FOLLOWING PLAN:

STEP		MATRIX OR REAGENT	VOLUME	POSITION PLATE	POSITION COLUMN
LYSIS	a)	LYS-FAST *	125 µL	Deepwell IDWELL-32A #1 and/ or #2  	Column 1 and/or 7
	b)	• Sample # • PEC or TPC • NEC-matrix or NEC-process	Volume mentioned in the specific pre-treatment protocol # or 125 µL max		
	c)	MAGBEADS-BB-FAST**	310 µL		
FIRST WASH		WASH 1	300 µL		2 and/or 8
SECOND WASH		WASH 2	300 µL		3 and/or 9
THIRD WASH		WASH 3 (80% EtOH)	300 µL		4 and/or 10
ELUTION		ELU	80 µL		5 and/or 11
-		-	Empty		6 and/or 12

3. LOAD THE ROBOT AND LAUNCH THE EXTRACTION/PURIFICATION

- Protect the robot magnetic rods with the 8-tip plastic combs IDTIP-32A.
- Load the prepared Deepwell plate(s) on the robot tray.
- Select the *IDGeneMAGFAST* program.
- Press *Run* to start the extraction.

4. RECOVER THE NUCLEIC ACID ELUATES

- At the end of the extraction program, transfer the eluates from the columns 5 and/or 11 of the Deepwell plate(s) into:

  - identified microtubes

  - or into a 96-well microplate and cover with an adhesive film for storage.

 or 

- Discard the plastic consumables.

5. STORE THE ELUATES

The eluted nucleic acids can be stored:

- at 5°C (± 3°C) if the qPCR is performed within the same day as extraction.
- at 5°C (± 3°C) if RT-qPCR is performed within 2 hours after extraction.
- below -16°C for longer-term storage or, if possible, -70°C for better RNA preservation.

** For assays where an Exogenous Non-Target Positive Control (NTPC) is supplied with the ID Gene™ or ID Gene Lyo™ (RT) qPCR amplification Kit, follow the LYS-FAST+NTPC reagent preparation procedure outlined in the dedicated pre-treatment protocol.*

*** Follow the MAGBEADS-BB-FAST reagent preparation procedure. ⚠ Mix the suspension thoroughly by inversion, or by successive pipetting, just before use.*

With the IDEAL™ 96 and KingFisher™ 96 /Flex robots

1. PREPARE CONSUMABLES

- Prepare and identify 4 x IDWELL-96 Deepwell plates or equivalent: LYSIS (#1), WASH1 (#2), WASH2 (#3) and WASH3 (#4).
- Prepare and identify one elution IDELU-96 microplate or equivalent, counting one well per sample or control to be processed.

2. DISTRIBUTE a) ALL BUFFERS b) SAMPLES/CONTROLS AND c) THE BEAD SUSPENSION ACCORDING TO THE FOLLOWING PLAN:

STEP		MATRIX OR REAGENT	VOLUME		POSITION
LYSIS	a)	LYS-FAST *	125 µL		Deepwell IDWELL-96 #1
	b)	• Sample # • PEC or TPC • NEC-matrix or NEC-process	Volume mentioned in the specific pre-treatment protocol	# or 125 µL max	
	c)	MAGBEADS-BB-FAST**	310 µL		
FIRST WASH		WASH 1	300 µL		Deepwell IDWELL-96 #2
SECOND WASH		WASH 2	300 µL		Deepwell IDWELL-96 #3
THIRD WASH		WASH 3 (80% EtOH)	300 µL		Deepwell IDWELL-96 #4
ELUTION		ELU	80 µL		IDELU-96

3. LOAD THE ROBOT AND LAUNCH THE EXTRACTION/PURIFICATION

- Protect the robot magnetic rods with the 96-tip plastic combs IDTIP-96 or other suitable combs.

IDEAL™ 96	KingFisher ™96/Flex
• Select the *IDGeneFAST* or *IDGeneFastv3* program. • Press *Load protocol* and *Loading* and follow the instructions given by the device interface. • Insert the prepared plates and, when loading is complete, press *Confirm* to begin extraction.	• Select the *ID Gene MAGFAST_96* program or equivalent • Press *Start*. • Follow the instructions to load the plates. When the loading of the plates is complete, press *Start* to begin the extraction.

4. RECOVER THE NUCLEIC ACID ELUATES

- Collect the elution microplate and cover with an adhesive film for storage.
  Transfer into identified microtubes can be performed, if more convenient for storage.
- Discard the (other) plastic consumables.

5. STORE THE ELUATES

The eluted nucleic acids can be stored:

- at 5°C (± 3°C) if the qPCR is performed within the same day as extraction.
- at 5°C (± 3°C) if RT-qPCR is performed within 2 hours after extraction.
- below -16°C for longer-term storage or, if possible, -70°C for better RNA preservation.

** For assays where an Exogenous Non-Target Positive Control (NTPC) is supplied with the ID Gene™ or ID Gene Lyo™ (RT-) qPCR amplification Kit, follow the LYS-FAST+NTPC reagent preparation procedure outlined in the dedicated pre-treatment protocol.*

*** Follow the MAGBEADS-BB-FAST reagent preparation procedure. ⚠ Mix the suspension thoroughly by inversion, or by successive pipetting, just before use.*

TECHNICAL SUPPORT AND DOCUMENTATION

For all questions, technical support, requests for MSDS and protocols, please contact us at the following address: info@innovative-diagnostics.com.

HISTORY OF REVISIONS

Any changes made to the instructions for use will be clearly described on the front page in a red box. The symbols ❯❯ are used throughout the manual to alert the user of the changes made.

TYPE OF MODIFICATION	MODIFICATION	CHANGE OF VERSION	UPDATE OF THE REFERENCE
Correction of anomalies in the document : writing, typography, changes to page layout	Minor	No	No
Update : clarifications and addition of details on test implementation	Minor	No	Yes
Technical modification: technical modification of the kit, its composition and/or its testing procedure	MAJOR	YES	Yes

VERSION	EDIT DATE	REFERENCE	TYPE OF REVISION	REVISION MADE
1224	12/2024	doc4794	**Substantial update**: Addition/ modification of information and new document layout	Substantial update: • Update of the robots and of associated plastic consumables which may be used with the MAGFAST product. • List of the currently available pre-treatment protocols now available online. • Extraction protocols now presented according to robot type. • Addition of some precautions of use. • Addition of details on the storage conditions for nucleic acid eluates The IFU version has been incremented but the operating procedure remains unchanged.
0222	06/2022	doc3317	**Technical modification** : Update of the document following technical modification of the kit	• BB-FAST, WASH 1, WASH 2 now provided without ethanol. To be reconstituted with absolute ethanol before first use. • Pre-treatment protocols now all provided upon request (refer to list in Annex) • Innovative Diagnostics now mentioned as the manufacturer of the IDvet product range

APPENDIX 8 : Pre-treatment of samples and DNA extraction using the MAGFAST384 kit with an analysis using the ID Gene™ Capripox virus Triplex kit and ID Gene™ LSD DIVA Triplex kit

This appendix outlines extraction conditions for the **ID Gene™ Capripox virus Triplex** (IDCPV) and the **ID Gene™ LSD DIVA Triplex** (IDLSDIVA) amplification kit for whole blood, swabs (oral or nasal) or tissues (skin lesions).

This appendix also describes the volumes to be extracted for target positive control (TPC-CPV and TPC-LSDIVA), non-target positive control (NTPC-CPV and NTPC-LSDIVA) and negative control (NEC) needed for IDCPV and IDLSDIVA test.

Important: Controls must be extracted at the same time as samples.

a) Reagent required but not provided in the kit:

- For the extraction of DNA from whole blood, tissues or swabs (oral or nasal):

Proteinase K **reference Macherey-Nagel n°740506** available from IDvet Genetics. Refer to the manufacturer's instructions for reconstitution and storage.

- For the extraction of DNA from tissues: 1X PBS (molecular biology quality), 2 ml Nuclease-free tubes, glass beads 3 mm, MixerMill-type grinder, Precellys®24 (otherwise contact us) with 2ml adapted tubes.

b) Pre-treatment of samples and controls

Sample preparation and pre-treatment depends on the type of sample, and are described in the table below:

	Whole blood and swabs (oral or nasal)	Tissues (skin lesions)
Step 1 : Samples pre-treatment	1. **Dispense 100 µl** of liquid sample in a 2 ml tube, (swab supernatant or whole blood collected with anticoagulant) 2. Add : - 125µL of Lysis buffer **LYS-FAST** - 20µL of **Proteinase K** [1] (20 mg/ml) - 20µL of **NTPC-CPV** or **NTPC-LSDIVA** 3. Vortex 4. Incubate for **30 minutes at 21°C** (± 5°C) 5. **The whole** cell lysate solution is needed for the extraction	1. In a 2 ml tube, weigh **20 mg of tissue**, previously dissected, with precision scale. 2. Add : - 125µL of Lysis buffer **LYS-FAST** - 20µL of **Proteinase K** [1] (20 mg/ml) - 20µL of **NTPC-CPV** or **NTPC-LSDIVA** 3. - Add 1 to 3 glass beads and homogenize thoroughly by vortexing (for 20 to 30 seconds) or - Add zirconium powder up to the 0.25 ml tube mark and grind: - Precellys® 24/ Precess™ 24: for 40 seconds at 6000 rpm - FastPrep™: 45 seconds at 6 M/s - MixerMill / TissueLyse: for 2 minutes at 30 Hz 4. Incubate for **30 minutes at 56°C** (± 5°C) 5. Centrifuge for **2 minutes at 1500 g** 6. **The whole** cell lysate is needed for the extraction
Step 2 : Controls sample	Controls should be prepared and extracted at the same time as the samples being tested - TPC (Target Positive Control): For the **TPC-CPV** and the **TPC-LSDIVA** follow the method of whole blood and swab pre-treatment from **step 1.2** above with 50 µl of TPC. - NEC (Negative Extraction Control): If the NEC is prepared with a sample of known negative status, follow the method of pre-treatment from **step 1.1** above. If the NEC is prepared with of Nuclease-free water, follow the method of pre-treatment with 50µL of water from **step 1.2** above.	

1)Note: Depending on the number of samples to be analysed, it is possible to make a homogenous mix of the following reagents:
(125 µl of LYS-FAST + 20 µl of Proteinase K + 20 µL of NTPC-CPV or NTPC LSDIVA) x (number of samples and controls + 10%)
It is recommended to prepare 10% extra volume to avoid running out of mix.
Pipette 165 µl of the reagent mix into each tube containing a sample or control.

ver1217_0874

c) Extraction of DNA from samples and controls

1. Prepare the samples and controls to be extracted as described below:

		Samples			Controls	
Position	**Reagents**	**Whole blood**	**swabs**	**Tissues**	**TPC**	**NEC**
Well A / Deepwell 1	**Samples**	**Total lysate**			**Total lysate**	
Well B / Deepwell 2	WASH 1	300 µl			300 µl	300 µl
Well C / Deepwell 3	WASH 2	300 µl			300 µl	300 µl
Well D / Deepwell 4	WASH 3	300 µl			300 µl	300 µl
Well E / Elution Microplate	ELU	80 µl			80 µl	80 µl

2. Continue with **Step 5** of the extraction protocol.

Certified management system

ID Gene™ Spin Universal Extraction Kit

Ref: SPIN50/SPIN250

50/250 extractions

Nucleic acid extraction on silica spin-columns

All matrices, all pathogens

For in vitro use

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www.innovative-diagnostics.com - E-mail: info@innovative-diagnostics.com

SPIN50/SPIN250 ver0419_EN_2641

General Information

- **Characteristics**

ID Gene™ Spin Universal Extraction Kit (SPIN50/SPIN250) is an extraction system using silica spin-column.

This extraction system is designed to efficiency extract nucleic acid from all matrices and all pathogens involved in animal health.

- **Composition of the kit and storage conditions**

The SPIN50/SPIN250 kits contain the following reagents:

		Volume	
Reference	*Description*	SPIN250	SPIN50
LMAP	Lysis buffer MAP	2 x 100 ml	50 ml
LYS-SPIN	Lysis buffer to be reconstituted	5 x 35 ml	35 ml
CARRIER-SPIN		5 x 1 mg	1 mg
SPIN COLUMN	Silica columns	250	50
COLLECTION TUBES	Collection tubes	750	150
WASH1-SPIN	Wash Buffer 1	150 ml	30 ml
WASH2-SPIN	Concentrated wash buffer 2	50 ml	12 ml
H2O-SPIN	RNA elution buffer	30 ml	13 ml
ELU-SPIN	DNA elution buffer	30 ml	13 ml

All components must be stored at 21°C (± 5°C).

- **Materials, consumables, and reagents required but not provided in the kit**

Materials and Consumables:

- Precision pipettes capable of delivering volumes from 1 µl to 1000 µl with Nuclease-free filter tips
- 1.5 ml Nuclease-free tubes
- Mini centrifuge (rotation up to 11 000g)

Reagents:

- Absolute Ethanol (molecular biology quality)
- Distilled or nuclease-free water (recommended)

Risks and Precautions

Some components of the kit SPIN50 / SPIN250 contain hazardous substances. Wear protective gloves / protective clothing / eye protection / face protection (P280). Safety Data Sheets (MSDS) and Certificates of Analysis are available on request from info@tinnovative-diagnostics.com.

Standard protocol

The appendices of this document describe, for each validated matrix, the pre-treatment of samples and controls, to be applied before nucleic acid extraction, to obtain optimal results with PCR amplification using IDvet range of products.

In absence of a dedicated pre-treatment appendix, the standard protocol below can be applied for sample and controls extractions.

1. Reconstitute the following reagents:

a) for the SPIN50 and SPIN250 kits:

- Lysis buffer LYS-CARRIER-SPIN:

Reconstitute the lyophilised CARRIER-SPIN with 1 ml of LYS-SPIN buffer. Vortex and transfer the CARRIER-SPIN solution dissolved into the tube labelled LYS-SPIN.

The LYS-CARRIER-SPIN can then be stored at +4°C for 1 month or at ≤ -16°C for long-term storage. It is recommended to aliquot the LYS-CARRIER-SPIN to avoid more than 3 freeze-thaw cycles. When thawing, the buffer may have crystals. It is therefore recommended to heat the buffer to approximately +60 ° C to improve buffer homogenization.

b) for the SPIN50 kit:

- Wash buffer WASH2-SPIN:

Reconstitute the WASH2-SPIN buffer by adding **48 ml absolute Ethanol** to the tube. Homogenise by inversion.

The reconstituted buffer can be stored for 1 year at 21°C (± 5°C).

c) **for the SPIN250 kit:**

- Wash buffer WASH2-SPIN:

Reconstitute the WASH2-SPIN buffer by adding **200 ml absolute Ethanol** to the tube. Homogenise by vortexing.

The reconstituted buffer can be stored for 1 year at 21°C (± 5°C).

2. Prepare a lysate of samples and controls as described below:

1) Prepare one labelled microtube for each sample or control to lysate, then distribute the following reagents:

- 600 µl LYS-CARRIER-SPIN buffer

- 150 µl* maximum of sample.

**** Refer to the appendices of this manual describing the volumes of sample and control to extract, according to pathogen and sample type being analysed.***

2) Vortex **immediately for 15 sec.**

3) Add **600 µl absolute ethanol** (homogenise by pipetting). Briefly centrifuge the tube before opening.

The solution obtained is **the lysate sample**

3. Extract the samples and controls as described below:

1) Prepare and label as many SPIN COLUMNS (blue columns) as samples and controls to analyse.

2) Transfer **660 µl of lysate onto each column.** Close the column and **centrifuge for 1 min at 11 000 g**; Empty the collection tube.

Keep the column and the collection tube.

3) Pipette **the remaining lysate sample onto the same column** and **centrifuge for 1 min at 11 000 g.** Discard the collection tube.

Keep the column and replace a new collection tube.

Note: check that all the lysate has passed through the column. If not, pipette the remaining lysate up and down over the column and centrifuge again.

4) Pipette **500 µl of WASH1-SPIN** into each column. Close the column and **centrifuge for 1 min at 11 000 g.** Discard the collection tube.

Keep the column and replace a new collection tube.

5) Pipette **600 µl** (reconstituted) of **WASH2-SPIN buffer** into each column. Close the column and **centrifuge for 1 min at 11 000 g.** Discard the collection tube.

Keep the column and replace a new collection tube.

6) **Centrifuge for 3 min at 11 000 g.** Discard the collection tube. **Keep the column.**

7) Place the column in a labelled 1.5 ml tube and **add 100 µl of ELU-SPIN for DNA extraction or of H2O-SPIN for RNA extraction.**

Close the column and **incubate at room temperature for 1 min.**

8) **Centrifuge for 1 min at 11 000 g.** Discard the column. **Keep the labelled microtube that contains the eluate.**

4. The eluates should be kept at 5°C (± 3°C) if the PCR is to be performed immediately or at below -16 ° C for long-term storage.

Technical support and documentation

The ID Gene™ amplification kits are constantly being improved.

If the kit you are interested in using or the matrices to be analyzed are not included in the appendices, please contact: info@innovative-diagnostics.com. We will provide you with additional information.

For all questions or technical support, please contact us at the following address: info@innovative-diagnostics.com

Material and Safety Data Sheets (MSDS) and protocols are available upon request: info@innovative-diagnostics.com

For additional information, visit www.innovative-diagnostics.com

APPENDIX 7 : Pre-treatment of samples and DNA extraction using the SPIN50/SPIN250 kit for the analysis using ID Gene™ Capripox virus Triplex kit and ID Gene™ LSD DIVA Triplex kit

MATRICES: WHOLE BLOOD, SWABS (ORAL OR NASAL) OR TISSUES

December 2020

Modification of the extraction volume of positive controls from 50µl to 100µl, to homogenize the extraction protocol.

This appendix outlines extraction conditions for the **ID Gene™ Capripox virus Triplex** (IDCPV) and **ID Gene™ LSD DIVA Triplex** amplification kit for whole blood, swabs (oral or nasal) or tissues (skin lesions).

This appendix also describes the volumes to be extracted for target positive control (TPC-CPV and TPC-LSDIVA), non-target positive control (NTPC-CPV and NTPC-LSDIVA) and negative control (NEC) needed for IDCPV and IDLSDIVA tests.

Important: Controls must be extracted at the same time as samples.

a) Reagent and equipment required but not provided in the kit:

For the extraction of DNA from whole blood, tissue or swabs:

- Proteinase K **reference Macherey-Nagel n°740506** available from Innovative Diagnostics. Refer to the manufacturer's instructions for reconstitution and storage.
- 1X PBS (molecular biology quality).

For the extraction of DNA from tissue:

- Single-use scalpel.
- 2 ml Nuclease-free tubes, glass beads 3 mm, Mixer Mill-type grinder, Precellys®24 (otherwise contact us) with 2 ml adapted tubes

b) Pre-treatment of samples and controls

Sample preparation and pre-treatment depends on the type of sample, and are described in the table below:

	Whole blood and swabs (oral or nasal)	Tissues (skin lesions)
Step 1: Pre-treatment of samples	1. **For swab samples:** In a 2 ml tube, dispense 100 µl of swab supernatant **For whole blood samples:** In a 2 ml tube, deposit 100 µl of whole blood collected with anticoagulant and add 100 µl of 1X PBS. Vortex 2. Add: - 600 µl of **LYS-CARRIER-SPIN** buffer - 20 µl of **Proteinase K** [1] (20mg/ml) - 20 µl of NTPC-CPV or NTPC-LSDIVA 3. Vortex 4. Incubate for 30 min at 21°C (± 5°C) 5. Add 600 µl of absolute ethanol (homogenize by pipetting) 6. Rapidly centrifuge the tube before opening 7. Whole cell lysate is needed for the extraction	1. In a 2 ml tube, weigh 20 mg of tissues, previously dissected, add 200 µl of 1X PBS and Vortex. 2. Add 1 to 3 glass beads and homogenize thoroughly by vortexing (for 20 to 30 seconds) **or** Add zirconium powder up to the 0.25 ml tube mark and grind with: - Precelly® 24/ Precess™ 24: for 90 seconds at 6000 rpm - FastPrep™: for 2 x 45 seconds at 6 M/s - Mixer Mill / TissueLyser: for 5 minutes at 30 Hz 3. Centrifuge for 2 minutes at 1500 g 4. Pipette 140 µl of supernatant and add: - 600 µl of **LYS-CARRIER-SPIN** buffer - 20 µl of **Proteinase K** [1] (20mg/ml) - 20 µl of **NTPC-CPV** or **NTPC-LSDIVA** 5. Vortex 6. Incubate for 30 min at 56°C (± 5°C) 7. Add 600 µl of absolute ethanol (homogenize by pipetting) 8. Rapidly centrifuge the tube before opening 9. Whole cell lysate is needed for the extraction
Step 2: Preparation of controls	Controls should be prepared and extracted at the same time as the samples being tested - **TPC** (Target Positive Control): For the TPC-CPV and the TPC-LSDIVA follow the method of whole blood and swab pre-treatment from **step 1.2**. above with **100 µl of TPC.** - NEC (Negative extraction control): If the NEC is prepared with a sample of known negative status, follow the method of pre-treatment from **step 1.1** above. If the NEC is prepared with of Nuclease-free water, follow the method of pre-treatment with 100 µl of water from **step 1.2** above.	
(1) *Note: Depending on the number of samples to be analysed, it is possible to make a homogenous mix of the following reagents:* (600 µl of LYS-CARRIER-SPIN +Carrier buffer + 20 µl of Proteinase K + 20 µL of NTPC-CPV or NTPC-LSDIVA) x (number of samples and controls + 10%) *It is recommended to prepare 10% extra volume to avoid running out of mix.* *Pipette **640** µl of the reagent mix into each tube containing a sample or control.*		

Ver1220_2639

c) **Viral DNA extraction from samples or controls**

1. Prepare and label as many SPIN-COLUMNS (blue columns) as samples and controls to be analysed.
2. Transfer **660 µl of lysate into each column**. Close the column and **centrifuge for 1 min at 8000g**. Empty the collection tube. **Keep the column and the collection tube.**
3. Transfer **660 µl of lysate onto each column**. Close the column and **centrifuge for 1 min at 8000g**. Empty the collection tube. **Keep the column and the collection tube.**
4. Continue with the **step 3** of the standard protocol.

Ver1220_2639

c) Viral DNA extraction from samples or controls

1. Prepare and label as many SPIN Columns (blue columns) as samples and controls to be analysed.
2. Transfer **660 µl of lysate into each column**. Close the column and **centrifuge for 1 min at 8000g**. Empty the collection tube. **Keep the column and the collection tube.**
3. Transfer **660 µl of lysate onto each column**. Close the column and **centrifuge for 1 min at 8000g**. Empty the collection tube. **Keep the column and the collection tube**
4. Continue with the **step 3** of the standard protocol.

Certified management system

ID Gene™ Capripox Virus Triplex

Ref: IDCPV-50 / IDCPV-100

50 / 100 tests

Real-time PCR for the qualitative detection of Capripox viruses including Lumpy Skin Disease, Goatpox and Sheeppox

Suitable samples: Ruminant whole blood, swabs, tissues (skin lesions)

In-vitro Use

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General information

- **Characteristics**

ID Gene™ Capripox Virus Triplex (IDCPV) kit is a real-time PCR kit that amplifies a target sequence in Capripox (CPV) viral genome (including Lumpy Skin disease (LSD), Sheepox and Goatpox viruses).

This kit is a qualitative triplex test. It simultaneously amplifies the target DNA, endogenous and exogenous internal controls.

The kit contains the target positive control (TPC-CPV) and the non-target positive control (NTPC-CPV) which are to be extracted in the same manner as the samples to evaluate extraction efficiency and to detect the presence of PCR inhibitors.

This kit can be used to test ruminant whole blood collected in EDTA, swabs, tissues (skin lesions).

- **Kit composition and storage conditions**

The IDCPV kit contains the reagents shown below:

Reference	*Component*	*Volume*	*Description*
TPC-CPV	Target Positive control	550 µl 1 vial	Inactivated Neethling vaccine diluted in a virus-negative matrix, freeze-dried and calibrated between 10 and 100 times the detection limit of the method (MDL). Freeze-dried pellet to be reconstituted in **550 µl** distilled or Nuclease-free water.
NTPC-CPV	Non-Target Positive Control	2200 µl 1 vial	Non-pathogenic inactivated viral strain. Freeze-dried pellet to be reconstituted in **2200 µl** distilled or Nuclease-free water.
ARM-CPV	Amplification Reaction Mix	400 µl 1 or 2 tubes (white cap)	Ready-to-use reaction mix containing Taq polymerase and oligonucleotides for detection of CPV and of the endogenous and exogenous non-target positive controls.

All components should be stored at ≤ -16°C. It is recommended to prepare aliquots (minimum 100 µl) in order to avoid multiple freeze/thaw cycles (> 3 not recommended).

- **Required equipment, not provided in the kit**

All material used should be of suitable quality for molecular biology.

Amplification Instrument:

- Real-time thermal cycler capable of reading the following wavelengths: 525 nm (FAM), 548 nm (Yakima Yellow, equivalent to VIC) and 650 nm (Cy5).
  Examples of compatible thermal cyclers: CFX96, Chromo4 Biorad, LC480 I, LC480 II, LC96 Roche, 7500 AB and Rotor-Gene Q Qiagen.
  Please contact us regarding suitability with other thermal cyclers.

Consumables:

- Precision pipettes capable of delivering volumes of between 1 µl and 1000 µl
- Nuclease-free filtered tips
- 1.5 ml tubes
- 96-well PCR plates, strips or PCR micro-tubes (that have an optical quality compatible with the thermal cycler) and appropriate adapted adhesive film or caps

Reagents:

- Distilled or Nuclease-free water (recommended)

Contact genetics@id-vet.com for more information.

Remarks and precautions

The material used contains less than 0.1% hazardous or carcinogenic substances, thus MSDS sheets are not required. However, it is recommended to take appropriate precautions, as with any biochemical product, and to wear appropriate clothing.

Extraction and amplification controls

- **Positive controls**

The IDCPV kit contains the following positive controls:

- **Exogenous Non-Target Positive Control (NTPC-CPV):**

The exogenous positive control is a non-pathogenic inactivated DNA virus.
This control evaluates the efficiency of the extraction process and detects the presence of inhibitors in the amplification step.
This control is to be added to every sample as well as to the other controls (NEC) before extraction.

- Target Positive Control (TPC-CPV):

These controls consist of inactivated Neethling vaccine diluted in a virus-negative matrix, calibrated between 10 and 100 times the MDL.
These controls validates the extraction and amplification of the target.
These controls are prepared and extracted in the same way as samples.

- Endogenous Non-Target Positive Control (NTPCen):

This control is constitutively present in the cells of the test sample. Its function is to validate (1) cell lysis and (2) amplification of a non-target gene. It also confirms the presence of cells, and gives an indication of the quality of the sample.

- **Negative controls**

It is recommended to include the following negative controls in each run:

- Negative extraction control (NEC)

This control should be prepared and extracted in the same way as samples, but does not contain any target DNA. The volume occupied by the sample is replaced by a non-reactive matrix or Nuclease-free water.

- Negative control for amplification (NAC)

This control contains 8 µl of reaction mix (ARM-CPV) and 5 µl of Nuclease-free water. It is included in each analysis cycle to control for the presence of any aerosol contaminants.

Amplification protocol

- **Extraction of viral DNA**

The viral DNA must be extracted from the sample before being amplified by PCR.
For this, IDvet Genetics offers a range of extraction kits that conform to the French NF U47-600 standard:

Description	*Product name*	*Product code*
Magnetic bead extraction system	ID Gene™ Mag Universal Extraction Kit	MAG192/MAG384
	ID Gene™ Mag Fast Extraction Kit	MAGFAST384
Column extraction system	ID Gene™ Spin Universal Extraction Kit	SPIN50/SPIN250

- **Extraction of the controls**

The volumes of the control to extract are described in the table below:

Important:

- The volumes indicated are valid regardless of the extraction system.
- The controls must be extracted at the same time as the samples.

Control	*Volume*
TPC-CPV	**50 µl**
NTPC-CPV	**20 µl** to add to NEC and to each sample

Note: If the NEC is prepared with a negative sample matrix, refer to the extraction kit protocol for the matrix in question.

- **Preparation of the real-time PCR amplification reaction**

1. Prepare an experimental plan for the analysis of the samples and controls, being sure to distance the positive control (TPC-CPV) from the other samples.
2. Thaw the IDCPV kit, ideally at 5°C (± 3°C) in a refrigerated rack. Thaw at room temperature 21°C (± 5°C) only if the mix is to be used immediately after thawing.
3. Homogenise the contents of the **ARM-CPV** tube by vortexing. Centrifuge down briefly.
4. Distribute **8 µl of ARM-CPV** per well. Use PCR strips, or microplates adapted to the thermal cycler in use.
5. Add :
   - 5 µl of DNA extracted from each sample to be analyzed
   - 5 µl of DNA extracted from the TPC-CPV
   - 5 µl of extracted NEC
   - 5 µl of Nuclease-free water (NAC)
6. Cover the plate or strips with appropriate adhesive film or caps.

- **Programming the amplification phase**

1. Program the thermal cycler detectors to read the following wavelengths for each well:

Target	*Fluorophore*	*λ (nm)*	*Quencher*
CPV	FAM	495-525	non fluorescent
NTPC-CPV	Cy5	649	non fluorescent
NTPCen	VIC / Yakima Yellow	426-548	non fluorescent (compatible VIC/HEX)

Note: For devices requiring an internal normalization of the signal, the amplification mix ARM-CPV contains ROX.

2. Choose between the two 2 different amplification programs validated by IDvet Genetics:
   - Standard program (allows for PCR kits from different vendors to be used in a single session) or
   - Rapid program

Step	Standard program	Rapid program	Number of cycles
(1) Polymerase activation	10 min at 95°C	2 min at 95°C	1
(2) DNA denaturation/elongation	15 sec at 95°C 60 sec at 60°C	10 sec at 95°C 30 sec at 60°C	40

Note: The fluorescence is read at the end of the elongation step at 60°C.

3. Enter one or these programs in the thermal cycler and select a final volume of **13 µl per PCR**. If different volumes are combined in a single run, enter the largest volume on the plate.
4. Place the PCR plate, PCR strips or capillaries in the thermal cycler and start the program.

Validation and interpretation of results

- **Assay validation**

The analysis of results is based on the Cq (Quantification cycle) value of each sample that is obtained by each detector. The Cq is also known as the Ct value (Threshold cycle).

The test is validated according to criteria outlined in the table below. **Results should not be interpreted if any of these criteria are not met.**

Control	Expected result	Acceptability criteria
TPC-CPV	Detected in FAM	Refer to the Cq value given in the quality control certificate
NTPC-CPV	Detected in Cy5 in each sample	+ 3 Cq of control reported to Cq of NEC
NTPCen	Detected in VIC in each sample	Presence of a characteristic curve
NEC	Detected in Cy5 if water used	+ 3 Cq of control with respect to the Cq value indicated in the quality control certificate
	Detected in Cy5 and VIC if virus-negative sample used	Presence of a characteristic curve
NAC	No detection	Complete absence of a characteristic curve

- **Suggested interpretation of results**

For each sample, results may be interpreted according to the following criteria:

Sample	CPV signal	NTPCen signal	NTPC-CPV signal	Interpretation
Individual	Detected	Detected or Not detected	Detected or Not detected	Animal detected as positive for CPV
	Not detected	Detected	Detected	Animal not detected for CPV
	Not detected	Not detected	Not detected or $Cq_{NTPC-CPV} > Cq_{NEC} + 3$	A problem occurred during sample distribution or extraction process / PCR reaction was inhibited

Non-validated samples:

- If the NTPC-CPV or NTPCen are not detected but the sample is detected positive for CPV, consider the sample as positive.

- If the NTPCen is not detected: a problem occurred during sample distribution or during the extraction process. In this case, the sample is to be extracted again.

- If the NTPC-CPV is not detected:
   - A problem occurred during sample distribution or during the extraction process. In this case, the sample is to be extracted again.
   - Or the PCR reaction was inhibited. In this case, perform a new amplification run following the procedure below.

- If $Cq_{NTPC-CPV} > Cq_{NEC} + 3$ and no signal is detected for CPV, the reaction is inhibited. In this case, perform a new amplification run following the procedure below.

Procedure to follow if the PCR reaction was inhibited:

1. Dilute the extracted DNA 10 times in Nuclease-free water.
2. Repeat the amplification phase on 5 µl of this dilution.
3. If the NTPC and NTPCen are detected, interpret the sample according to table above.
4. If the NTPC and NTPCen are not detected, re-extract the sample or consider it uninterpretable.

Documentation and support

For questions or technical support, please contact: support.genetics@id-vet.com

For additional information, visit www.id-vet.com

July– *Juillet* 2025

Quality Control Sheet - Fiche de Contrôle Qualité

ID Gene™ Capripox Virus Triplex

Tests : 50 or/*ou* 100
Product Code / *Code Produit*: IDCPV
Batch / *N°de Lot* : 022
Date of Manufacture / *Date de Fabrication*: 07/2025
Expiry date / *Date d'expiration*: 06/2027
Instructions for use / *Mode d'emploi* : 1217

Kit components / *Composition du kit :*

Components / *Composants*	Description	Volume	Batch / *N° Lot*
ARM-CPV (white cap / bouchon blanc)	Amplification Reaction Mix / *Mélange Réactionnel d'Amplification*	400 µl vial / *flacon* *(x1: 50 reactions)* *(x2: 100 reactions)*	2411A3
TPC-CPV (freeze-dried / lyophilisé)	Target Positive Control / *Témoin Positif Cible*	550 µl (1 vial / *flacon*)	2310A1
NTPC-CPV (freeze-dried / lyophilisé)	Exogenous Non Target Positive Control / *Témoin Positif Non-Cible exogène*	2200 µl (1 vial / *flacon*)	2206A1

QC Results / *Résultats du CQ :*

Target Positive Control / *Témoin Positif Cible* (TPC-CPV) :

Expected Cq value for TPC-CPV (CPV signal in FAM™) / Valeur de *Cq attendue pour le* TPC-CPV (signal CPV en FAM™):
≤ 31 Cq*

Endogenous Non Target Positive Control / Témoin Positif Non Cible endogène (NTPCen) :

Mean Cq value for NTPCen (signal in VIC® /HEX™) / *Valeur moyenne de Cq pour le NTPCen (signal en VIC® /HEX™):*

25 Cq* on NEC-matrix (whole blood sample) / *sur NEC-matrix (sang total)*

Exogenous Non Target Positive Control / *Témoin Positif Non Cible exogène (NTPC-CPV) :*

Mean Cq value for NTPC-CPV (signal in CY5) / *Valeur moyenne de Cq pour le NTPC-CPV (signal en CY5) :*
≤ 29 Cq* on NEC-process (Nuclease-free water) /*pour NEC-process (eau Nuclease-free).*

*This mean Cq value was obtained in our Quality Control laboratory in our conditions: extraction of nucleic acids using the ID Gene™ Mag Fast Extraction Kit (MAGFAST384) and qPCR amplification using the ID Gene™ Capripox Virus Triplex kit with its rapid amplification program and a Life Technologies QuantStudio 5 Real-Time PCR System. Please note that Cq values are dependent on the matrices tested, the extraction methods used, and the thermal cyclers utilized. Innovative Diagnostics recommends that each laboratory determine its own threshold values from the controls (TPC/NEC-matrix/NEC-process). Please refer to the kit Instructions for use, chapter "Validation and interpretation of results".

**Cette valeur de Cq a été obtenue dans notre laboratoire de Contrôle Qualité dans nos conditions : extraction des acides nucléiques avec le kit ID Gene™ Mag Fast Extraction Kit (MAGFAST384) et amplification qPCR avec le kit ID Gene™ Capripox Virus Triplex en utilisant le programme d'amplification rapide et un thermocycleur Life Technologies QuantStudio 5 Real-Time PCR. Attention, les valeurs de Cq sont dépendantes des matrices testées, des méthodes d'extraction employées et des thermocycleurs utilisés. Innovative Diagnostics recommande à chaque laboratoire de déterminer ses propres valeurs seuils à partir des témoins (TPC/NEC-matrix/NEC-process). Veuillez-vous référer au mode d'emploi du kit, chapitre "Validation et interprétation des résultats".*

Quality Control Manager / *Responsable Contrôle Qualité :*

Marine Noguier

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DOC5158

October - *Octobre*, 2023

Quality Control Sheet - Fiche de Contrôle Qualité

Freeze-dried sentinel / Sentinelle lyophilisée TPC-CPV

10 tests (550 µl)

Product Code / *Code produit* : TPC-CPV

Batch / *N° de Lot* : TPC-CPV-2310A1

Manufacture date / *Date de Fabrication* : 10/2023

Expiry Date / *Date d'expiration* : 10/2028

A- Production and utilisation of the sentinel / *Production et utilisation de la sentinelle*

Inactivated CPV, diluted in a virus-negative whole blood matrix, freeze-dried and calibrated between 10 and 100 times the detection limit of the method (MDL). Results obtained using the «MAGFAST384 + IDCPV» methods and the rapid amplification program /

Virus CPV inactivé dilué dans une matrice de sang total négatif, lyophilisé et calibré entre 10 et 100 fois la limite de détection de la méthode (LD_M). Résultats obtenus selon la méthode « MAGFAST384 + IDCPV*» et le programme d'amplification rapide :*

	TPC-CPV Dilutions		
	Pur	10^{-1}	10^{-2}
Extraction 1	30,55	34,25	37,76
Extraction 2	30,45	33,71	-
Extraction 3	30,50	35,08	-
Extraction 4	30,58	34,27	36,28

Conclusion :

The sentinel TPC-CPV is calibrated between 10 and 100 times the detection limit of the method (MDL) /

La sentinelle TPC-CPV est calibrée entre 10 et 100 fois la limite de détection de la méthode (LD_M).

B- Quality Control of freeze-dried TPC-CPV / *Contrôle Qualité du TPC-CPV lyophilisé*

Cq values obtained with extraction of 50 µL of the TPC-CPV using the «MAGFAST384 + IDCPV» methods and the rapid amplification program /

Résultats en valeur de Cq pour l'extraction de 50 µL de TPC-CPV selon la méthode « MAGFAST384 + IDCPV *» avec le programme d'amplification rapide :*

	TPC-CPV Cq
Extraction 1	30,73
Extraction 2	30,80
Extraction 3	30,88

Quality Control Manager / *Responsable Contrôle Qualité* :

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Appendix n°6: Results of Diagnostic Specificity and Sensitivity

6.1 Results obtained by CODA-CERVA on whole blood

		CODA-CERVA	IDCPV with MAGFAST			IDLSDIVA with SPIN50/250		
		D5R	CPV	NTPCen	NTPC	CPV	NTPCen	NTPC
1	BG4 SUBCLINIC R8	27,7	26,91	24,67	30,57	26,91	24,67	30,57
2	BG4 SUBCLINIC R9	33,4	30,76	22,66	31,06	30,76	22,66	31,06
3	BG4 SUBCLINIC R10	NEG	NEG	24,70	31,46	NEG	24,70	31,46
4	BG4 SUBCLINIC R11	31,9	31,16	23,68	31,21	31,16	23,68	31,21
5	BG4 SUBCLINIC R12	31,7	30,71	23,99	30,83	30,71	23,99	30,83
6	BG4 SUBCLINIC R13	32,5	30,80	23,16	31,40	30,80	23,16	31,40
7	BG4 SUBCLINIC R14	29,1	27,52	23,21	30,76	27,52	23,21	30,76
8	BG4 SUBCLINIC R15	NEG	NEG	23,23	30,99	NEG	23,23	30,99
9	BG4 SUBCLINIC R16	30,0	28,44	25,86	30,96	28,44	25,86	30,96
10	BG4 SUBCLINIC R17	33,2	30,52	23,75	31,52	30,52	23,75	31,52
11	BG4 SUBCLINIC R18	31,2	29,28	23,47	31,11	29,28	23,47	31,11
12	BG4 SUBCLINIC R19	31,9	30,24	24,44	30,97	30,24	24,44	30,97
13	BG4 SUBCLINIC R20	33,3	30,65	27,27	30,94	30,65	27,27	30,94
14	BG4 SUBCLINIC R21	30,3	29,76	23,92	30,82	29,76	23,92	30,82
15	BG4 SUBCLINIC R22	NEG	NEG	23,73	31,08	NEG	23,73	31,08
16	BG4 SUBCLINIC R23	NEG	NEG	23,57	31,08	NEG	23,57	31,08
17	BG4 SUBCLINIC R24	30,1	30,43	24,50	30,97	30,43	24,50	30,97
18	BG4 SUBCLINIC R25	31,0	30,00	26,55	31,11	30,00	26,55	31,11
19	BG4 SUBCLINIC R26	NEG	NEG	23,95	31,28	NEG	23,95	31,28
20	BG4 SUBCLINIC R27	31,7	29,26	23,73	30,91	32,02	24,25	25,87
21	BG4 SUBCLINIC R28	NEG	NEG	24,00	31,47	NEG	24,00	25,96
22	BG1-R22CON 27DPV	34,63	35,00	25,27	30,25	35,00	24,38	26,20
23	BG1-R25CON 32DPV	37,81	35,00	23,26	29,76	35,00	24,86	27,58
24	BG1-R24CON 33DPV	36,53*	NEG*	24,28	29,78	33,26	23,56	26,53
25	BG1-R24CON 34DPV	36,54*	NEG*	23,35	30,16	35,00	23,23	29,89
26	BG2-R15-LAV4 28DPV	34,59	35,00	23,97	31,51	32,18	24,51	30,47
27	BG2-R15-LAV4 29DPV	37,76	35,00	22,93	30,16	31,24	24,16	30,30
28	BG2-R16-LAV4 27DPV	36,74	35,00	23,11	30,24	35,00	23,52	29,98
29	BG2-R16-LAV4 34DPV	33,44	35,00	24,30	30,10	30,68	23,99	29,96
30	BG2-R17-LAV4 34DPV	36,92	35,00	24,23	30,47	33,25	25,38	30,23
31	BG2-R18-LAV4 28DPV	33,58	32,80	24,70	30,57	32,05	22,79	30,81
32	BG2-R18-LAV4 31DPV	35,21	35,00	24,88	30,46	29,89	23,46	30,24
33	BG2-R19-LAV4 36DPV	35,97	35,00	23,57	30,62	35,00	23,43	29,95
34	BG2-R20-LAV4 27DPV	33,98	33,98	24,54	30,73	33,74	23,15	30,12
35	BG2-R26CON2 29DPV	35,54	35,00	23,52	30,22	31,74	22,66	30,06
36	BG2-R26CON2 35DPV	31,7	34,15	22,59	31,02	35,00	23,16	29,29
37	BG3-R2-LAV5 27DPV	32,5	35,00	21,22	30,15	NEG*	NEG	NEG
38	BG3-R2LAV5 10DPI	35,31	35,00	22,50	30,48	NEG*	23,08	30,54
39	BG3-R7-LAV5 7DPI	34,65*	NEG*	NEG	NEG	NEG*	21,36	30,53
40	BG3-R8-5DPV	NEG	NEG	21,98	30,75	NEG	23,01	31,25
41	BG3-R9-5DPV	NEG	NEG	22,51	30,19	NEG	21,70	30,44
42	BG3-R10-5DPV	NEG	NEG	22,66	31,04	NEG	20,94	29,20
43	BG3-R11-5DPV	NEG	NEG	23,23	30,57	NEG	NEG	NEG
44	BG3-R12-5DPV	NEG	NEG	22,91	30,48	NEG	23,43	30,41
45	BG3-R13-5DPV	NEG	NEG	21,55	29,98	NEG	21,86	29,86
46	BG3-R14-5DPV	NEG	NEG	22,00	31,50	NEG	21,55	30,16
47	BG3-R15-5DPV	NEG	NEG	21,16	30,21	NEG	25,62	34,16
48	BG3-R16-5DPV	NEG	NEG	22,88	30,77	NEG	21,77	NEG
49	BG3-R17-5DPV	NEG	NEG	22,06	31,03	NEG	23,04	NEG
50	BG3-R18-5DPV	NEG	NEG	21,45	31,06	NEG	22,44	32,05
51	BG3-R19-5DPV	NEG	NEG	22,65	31,55	NEG	21,67	30,89
52	BG3-R20-5DPV	NEG	NEG	23,06	32,13	NEG	23,08	31,80
53	BG3-R21-5DPV	NEG	NEG	23,62	30,72	NEG	22,10	29,84
54	BG3-R24-LAV8-6DPI	33,21	35,00	24,04	30,80	32,02	24,25	25,87
55	BG3-R24-LAV8-8DPI	35,95	35,00	24,12	30,92	35,00	24,00	25,96
56	BG3-R26-LAV8-7DPI	33,06	35,00	23,25	31,09	35,00	24,38	26,20

*With two extraction methods, 3 samples are detected with D5R PCR from CODA-CERVA but not detected with IDCPV kit. The 2 PCRs were not performed at the same time a potential degradation of the sample (therefore the DNAs) can explain the negative results obtained with IDCPV PCR. Furthermore the Ct/Cq values obtained with the D5R PCR are > 35; the positivity of the samples are therefore very low.

6.2 Results obtained by FLI on whole blood

Nr.	animal	sample type	Extraction and PCR FLI pan Capri	CPV	NTPCen	NTPC-CPV
				FAM	VIC	Cy5
1	A R-544	EDTA	30,18	29,75	28,51	29,84
2	C R-485	EDTA	33,76	35,39	28,51	30,04
3	A R-489	EDTA	30,85	31,08	27,61	29,62
4	A R-544	EDTA	29,82	27,59	26,64	29,27
5	C R-478	EDTA	30,56	31,49	27,85	29,41
6	C R-485	EDTA	31,99	32,52	28,17	29,06
7	C R-492	EDTA	28,12	31,45	28,09	29,71
8	A R-489	EDTA	28,89	28,71	30,27	35,72
9	neg. EDTA	spiked with Neethl. Vac. 10-2	25,68	24,68	28,32	29,23
10	neg. EDTA	spiked with Neethl. Vac. 10-3	30,02	29,21	28,66	29,53
11	neg. EDTA	spiked with Neethl. Vac. 10-4	33,42	32,67	28,38	29,68
12	neg. EDTA	spiked with Neethl. Vac. 10-5	36,72	36,17	28,3	29,83
13	neg. EDTA	spiked with Neethl.10-2/field 10-1	21,32	20,17	27,44	29,37
14	neg. EDTA	spiked with Neethl.10-3/field 10-1	21,08	20,22	27,17	29,37
15	neg. EDTA	spiked with Neethl.10-4/field 10-1	21,21	20,20	27,19	29,26
16	neg. EDTA	spiked with Neethl.10-5/field 10-1	21,34	20,02	27,04	29,25
17	Z-253 iv.	EDTA	31,32	33,04	29,91	29,74
18	Z-254 iv.	EDTA	26,35	27,72	28,16	29,79
19	Z-255 iv.	EDTA	27,76	29,72	28,22	29,86
20	Z-256 in.	EDTA	30,06	31,90	27,79	30,04
21	Z-256 in.	EDTA	29,06	30,34	28,27	29,71
22	Z-259 Ko.	EDTA	29,99	30,06	28,36	30,01
23	Z-259 Ko.	EDTA	28,15	28,20	28,96	31,61
24	Z-260 Ko.	EDTA	29,85	29,91	29,33	29,77

6.3 Results obtained by FLI on swabs

Nr.	animal	sample type	Extraction and PCR FLI pan Capri	CPV	NTPCen	NTPC
				FAM	VIC	Cy5
1	A R-487	nasal swab	28,82	28,83	30,51	29,57
2	A R-489	nasal swab	20,12	21,65	25,81	29,1
3	C R-478	nasal swab	30,33	30,43	31,84	29,56
4	A R-487	nasal swab	26,28	24,69	28,13	29,25
5	C R-478	nasal swab	29,48	29,36	33,53	29,63
6	C R-483	nasal swab	23,85	22,21	27,1	29,32
7	C R-485	nasal swab	23,55	23,04	26,63	29,26
8	C R-492	nasal swab	22,12	21,61	26,22	28,98
9	Z-256 in.	nasal swab	24,29	24,12	27,21	29,15
10	Z-254 iv.	nasal swab	27,24	27,46	27,27	29,64
11	Z-255 iv.	nasal swab	29,43	30,14	27,62	29,5
12	Z-256 in.	nasal swab	19,77	19,78	26	29,2
13	Z-257 in.	nasal swab	18,14	20,27	25,54	29,28
14	Z-258 in.	nasal swab	23,2	24,04	27,3	29,14
15	Z-256 in.	nasal swab	18,12	18,35	23,88	29,07
16	Z-257 in.	nasal swab	18,64	19,24	24,82	29,06
17	Z-258 in.	nasal swab	18,99	19,41	25,87	29,22
18	Z-256 in.	mouth swab	28,7	29,20	29,23	29,51
19	Z-256 in.	mouth swab	27,67	28,02	28,69	29,43
20	Z-257 in.	mouth swab	28,43	28,51	29,83	29,64
21	Z-256 in.	mouth swab	26,64	27,09	27,65	29,5
22	Z-257 in.	mouth swab	28,34	28,00	32,43	29,08
23	Z-256 in.	mouth swab	26,8	27,73	30,47	29,35
24	Z-257 in.	mouth swab	27,08	27,85	29,27	29,46
25	Z-258 in.	mouth swab	30,4	31,11	31,28	29,67

6.4 Results obtained by FLI on tissues or organs

Nr.	animal	sample type	Extraction and PCR FLI pan Capri	CPV	NTPCen	NTPC
				FAM	VIC	Cy5
1	A R-489	skin	**22,17**	21,31	25,03	28,88
2	A R-489	skin	**21,62**	22,00	25,24	29,01
3	A R-489	lung	**29,78**	29,55	24,89	29,02
4	C R-478	skin	**28,31**	28,72	27,58	29,76
5	C R-478	lung	**24,08**	24,15	25,72	29,9
6	C R-478	spleen	**30,54**	31,02	21,65	30
7	C R-478	trachea	**32,43**	32,54	27,15	29,56
8	C R-483	skin crust	**23,18**	22,95	23,14	29,17
9	Z-253	skin	**34,21**	34,38	26,08	29,49
10	Z-253	skin	**28,1**	28,13	27,57	29,63
11	Z-253	lung	**22,01**	23,17	25,7	29,52
12	Z-253	spleen	**33,8**	34,57	23,26	30,15
13	Z-254	skin	**32,43**	32,49	28,65	29,63
14	Z-254	skin	**26,54**	27,00	27,41	29,13
15	Z-254	liver	**34,4**	33,90	26,01	30,27
16	Z-254	lung	**28,75**	30,14	25,47	29,69