VALIDATION REPORT

Produit: IDPPR

ID Gene™ Peste des Petits Ruminants Duplex

Developed in collaboration with cirad

Method	Real time RT-PCR – Duplex - Qualitative
Species	Caprine, Ovine
Matrices	Caprine and Ovine whole blood; swabs (oral, nasal, ocular or rectal) tissues or organs
Reference	IDPPR

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Manufacturer:

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1 INTRODUCTION

Peste des petits ruminants (PPR) is an acute and highly contagious virus disease of small ruminants such as sheep and goats with high morbidity and sometimes high mortality. It is endemic in Africa except in southern african countries, it is epidemic in the Middle East and Asia. The causative agent of the disease, Peste des Petits Ruminants virus (PPRV), is classified as a member of the genus *Morbillivirus* in the family *Paramyxoviridae*.

IDvet Genetics has developed a molecular biology detection tool to detect all genotypes of the peste des petits ruminants virus. This kit is a duplex real time reverse transcription PCR assay. It simultaneously amplifies a target DNA gene and an endogenous internal control.

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

2 KIT OVERVIEW

The **ID Gene™ Peste des Petits Ruminants Duplex**, reference: **IDPPR**, is developed, manufactured, controlled and conditioned at the headquarters of ID.vet Genetics: 310 rue Louis Pasteur 34790 Grabels.

2.1 Kit description

The IDPPR kit allows for detection of PPR virus RNA by real time RT-PCR.

Target gene "PPR": conserved gene of all Peste des petits ruminants virus.

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

Type of PCR: Duplex Real-time -RT-PCR, allowing for a qualitative analysis.

Validated species: sheep and goat.

Matrices: Caprine and Ovine whole blood, swabs (oral, nasal, ocular or rectal), tissues or organs.

Kit format: 50 or 100 reactions.

2.2 Kit composition, storage and stability

Our kits are composed of liquid reagents and lyophilized biological matrices.

The IDPPR kit contains the reagents listed in the table below.

Reference	*Component*	*Volume*	*Description*
TPC-PPR	*Target Positive Control*	*550 µl 1 vial*	*Inactivated PPRV vaccine, diluted in a virus-free whole blood and calibrated between 1 and 100 times the method detection limit (MDL).* *Freezed-dried pellet to be reconstituted in **550 µl** distilled or nuclease-free water.*
ARM-PPR	*Amplification Reaction Mixture*	*400 µl 1 or 2 tubes (white caps)*	*Ready-to-use reaction mix containing the reverse transcriptase,Taq polymerase and oligonucleotides for amplification and detection of PPRV and of the endogenous non-target positive control.*

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).

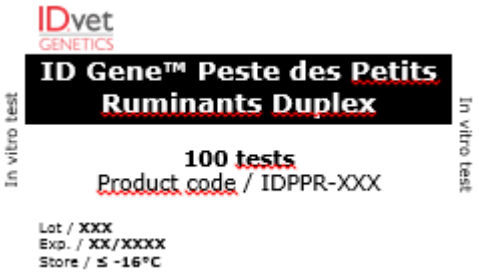
The kit is stable at ≤ -16°C from the manufactured date until the expiration date indicated on the label.

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.

2.4 Projet labels

Box label



Tube labels

3 PRESENTATION OF VALIDATION DATA

3.1 Validation protocol

The **ID Gene™ Peste des Petits Ruminants Duplex** kit was evaluated and validated by the OIE laboratory
CIRAD-BIOS
UMR AnimalS, health, Territories, Risks, Ecosystems (ASTRE)
Campus International Baillarguet, Montpellier, FRANCE.

3.2 PCR Characterization

3.2.1 Analytical specificity

3.2.1.1 *In silico* specificity

The specificity of the IDPPR RT-PCR was evaluated *in silico*. Alignements of the target PCR system (primers and probes) were performed with the databases available at NCBI (National Center for Biotechnology Information).

The alignment *in silico* of the target gene displayed 100% homology. The alignment shows no strong 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 12 PPR Virus reference isolates supplied by **CIRAD.**

Identification	Genotype	IDPPR Results
Maroc 2008	IV	Detected
Ethiopie 1994	III	Detected
Nigeria 1975	II	Detected
Ghana2014-1	IV	Detected
Ghana2014-2	IV	Detected
Ghana2014-5	IV	Detected
Côte d'ivoire 1989	I	Detected
Nigeria 75/1 inactivated vaccine	II	Detected
Samako, Mali 2014	II	Detected
Samako, Mali 2015	II	Detected
Dialan Cp2, Mali	II	Detected
Mali 1999	II	Detected

Conclusion:
The IDPPR kit successfully detected all the PPR 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	IDPPR 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 2	France	Not detected
BTV 4	France	Not detected
BTV 8	France	Not detected
BTV 9	France	Not detected
BTV 16	France	Not detected
H7N1	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
IBR	France	Not detected
IDB	France	Not detected
NDV	France	Not detected
Canine Distemper virus	Brésil	Not detected
Dolphin morbillivirus	France	Not detected
Measles virus	France	Not detected
Capripox virus - Sheep poxvirus	France	Not detected
Rinderpest virus	France	Detected
Parasites		
Leishmania infantum	France	Not detected
Neospora caninum	France	Not detected

N.B. : The IDPPR kit cross-reacts with the Rinderpest virus (bovine plague). In 2011, a global eradiction of Rinderpest was declared. The cross reaction between PPRV and Rinderpest virus has therefore no relevance for the current screening.

Conclusion:

The IDPPR kit is specific for its target, Peste des petits ruminants virus.

3.2.2 Analytical sensitivity

Experimental determination of the terms 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 flanking the supposed LD_{PCR} value were used. The LD_{PCR} was performed in repeatable conditions with intra-assay replicates and inter-assay (independent experiment).

The LD_{PCR} is determined from a PLS-PPR plasmid that is titrated with droplet digital PCR technique (ddPCR). The quantification on the ddPCR was performed 10 times. The plasmid quantification is represented by averaging 10 analyses.

The LD_{PCR} was determined with a PCR program called "rapid" (55min ± 10 min depending on the thermocycler used). Another program called “classic” can also be used.
The program is the following:

	Rapid amplification program
Step 1: Single time	**Reverse Transcription Phase** 10 min at 45°C
Step 2: Single time	**Activation of the polymerase:** 2 min at 95°C
Step 3: Repeat 40 times	**DNA denaturation:** 10 seconds at 95°C **Annealing:** 30 seconds at 60°C

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

Number of dilutions	**Number of replicates / dilution**	**Number of independent experimental runs**
6	4	2

Rapid program results:

Number of copies/PCR	**Positives in run 1**	**Positives in run 2**	**Total number of runs**	**Detection frequency**
100	4	4	8	100%
50	4	4	8	100%
25	4	4	8	100%
12.5	4	4	8	100%
6.25	4	4	8	100%
3.125	4	3	7	87.5%

Conclusion:
The 95% limit of detection of the IDPPR RT-PCR with the rapid amplification program is around **7 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%.

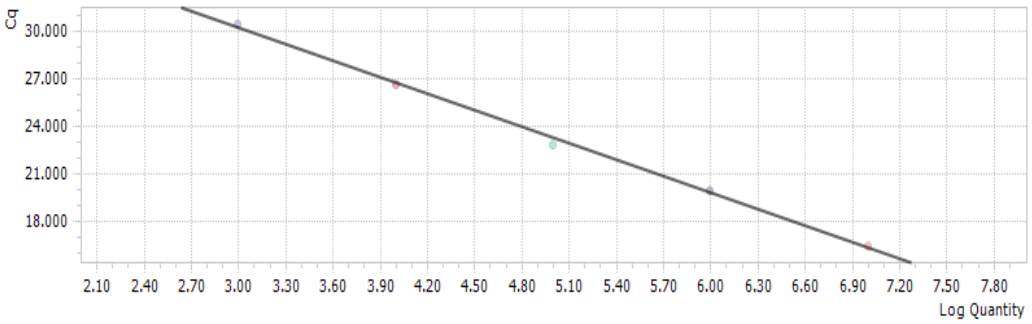
Experimental determination of the efficiency (E) and R^2

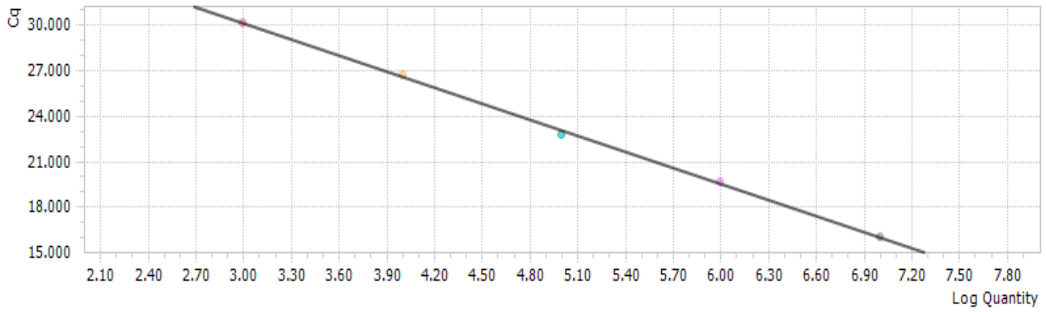
A range of 4 points of nucleic acid dilution made from the previously calibrated PPR plasmid is prepared to implement the following experimental design on the LightCycler® 96 instrument.

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	-3.48	40.66	1.94
Run 2	-3.53	40.72	1.92
Average	**-3.465**	**40.69**	**1.93**





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, IDvet Genetics decided to perform the approach of the method detection limit by spiking a calibrated PPR virus in a negative caprine whole blood and ocular swabs samples.

3.3.1 Methodology

A negative whole blood and ocular swabs sample are spiked with PPR virus vaccine, calibrated at 1.6 x 10^8copies / PCR with a droplet digital PCR. It is important to know that Methodology does not take into account the influence of target inclusion in the matrix analysis.

3.3.2 Experimental modality to approach the method detection limit (MDL)

The MDL. is evaluated by determining the last dilution where the 4 replicates are found positives.The limit estimated is not the absolute method limit of detection which is at a lower concentration of nucleic acid used for the target sequence control.

Experimental design for the experimental estimation of the MDL:

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 program.

3.3.2.1 MDL of MAGFAST384 and SPIN50/SPIN250 results on whole blood :

	copies/PCR	800	400	200	100	50
MAGFAST384	Replicate 1	32.2	34.3	34.6	36.1	-
	Replicate 2	33.4	34.7	34.4	38.2	36.8
	Replicate 3	33.3	34.3	34.0	37.0	36.7
	Replicate 4	32.9	34.6	34.5	37.2	37.0
	Average Cq	32.9	34.5	34.4	37.1	36.8
	% detection	100%	100%	100%	100%	75%
SPIN50/250	Replicate 1	33.0	34.2	-	-	-
	Replicate 2	35.0	36.4	-	-	-
	Replicate 3	32.8	33.0	-	-	-
	Replicate 4	30.1	35.2	35.8	-	-
	Average Cq	32.7	34.7	35.8	-	-
	% detection	100%	100%	25%	0%	0%

Conclusion:

The limit of detection of the method on whole blood with:

- MAGFAST384 and the rapid amplification program is **100 copies/PCR.**
- SPIN50/250 and the rapid amplification program is **400 copies/PCR.**

3.3.2.2 MDL MAGFAST384 and SPIN50/SPIN250 results on ocular swabs:

	copies/PCR	800	400	200	100	50
MAGFAST384	Replicate 1	32.2	34.5	33.7	35.0	-
	Replicate 2	33.4	34.3	36.3	34.5	-
	Replicate 3	32.7	34.5	36.0	37.4	35.9
	Replicate 4	33.0	34.8	35.2	36.9	-
	Average Cq	32.8	34.5	35.3	35.9	35.9
	% detection	100%	100%	100%	100%	25%
SPIN50/250	Replicate 1	33,7	35,6	35,3	-	-
	Replicate 2	33,7	34,9	35,9	32,6	-
	Replicate 3	33,0	34,8	35,2	36,9	-
	Replicate 4	32,7	34,5	36,0	37,5	35,9
	Average Cq	33.3	35.0	35.6	35.6	35.9
	% detection	100%	100%	100%	75%	25%

Conclusion:

The limit of detection of the method on ocular swabs with:

- MAGFAST384 and the rapid amplification program is **100 copies/PCR.**
- SPIN50/250 and the rapid amplification program is **200 copies/PCR.**

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 CIRAD-BIOS UMR AnimalS, health, Territories, Risks, Ecosystems (ASTRE).
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

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

- 88 blood samples from an experimental infection study achieved by the OIE reference laboratory CIRAD-BIOS UMR AnimalS, health, Territories, Risks, Ecosystems (ASTRE) are used to evaluate the diagnostic sensitivity and specificity of the IDPPR PCR kit.

Twelve samples were excluded from the analysis please see the appendix 4.1 for details.

- 80 ocular swab samples from the OIE reference laboratory CIRAD-BIOS UMR AnimalS, health, Territories, Risks, Ecosystems (ASTRE)

Six samples were excluded from the analysis please see the appendix 4.2 for details.

- 43 rectal swab samples from the OIE reference laboratory CIRAD-BIOS UMR AnimalS, health, Territories, Risks, Ecosystems (ASTRE)

Two samples were excluded from the analysis please see the appendix 4.3 for details.

- 37 rectal swab samples from a herd sheep with a negative status for PPR

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

3.4.2.1 Results of whole blood samples panel:

		PCR status with a validated method		
		Positive	Negative	Total
IDPPR method results	Positive	39	0*	39
	Negative	0*	37	37
	Total	39	37	76
		Se = (100%)	Sp = 100%	

Raw data of the study are detailed in the appendix 4.1.

Conclusions:

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

3.4.2.2 Results of ocular swabs panel:

		PCR status with a validated method		
		Positive	Negative	Total
IDPPR method results	Positive	48	0*	48
	Negative	0*	20	20
	Total	48	20	68
		Se = 100%	Sp = 100%	

Raw data of the study are detailed in the appendix 4.2.

Conclusions:

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

3.4.2.3 Results of rectal swabs panel:

		PCR status with a validated method		
		Positive	Negative	Total
IDPPR method results	Positive	41	0*	41
	Negative	0*	37	37
	Total	41	37	78
		Se = 100%	Sp = 100%	

Raw data of the study are detailed in the appendix 4.3.

Conclusions:

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

3.5 Robustness

Robustness is evaluated on RNA from a PPR sample extraction calibrated at 10x MDL and on the PLS-PPR plasmid diluted at 3x LD_{PCR}. The samples are performed in 4 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	**Reverse Transcription Step** 10 min at 44°C	**Reverse Transcription Step** 10 min at 45°C	**Reverse Transcription Step** 10 min at 46°C
Step 2: Single time	**Activation of the polymerase:** 2 min at 94°C	**Activation of the polymerase:** 2 min at 95°C	**Activation of the polymerase:** 2 min at 96°C
Step 3: 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 MDL	29.8	29.8	30.1	0.50%
3x LD_{PCR}	30.9	31.1	31.3	0.53%

Conclusion:

For amplification temperature variations of ± 1°C, the IDPPR kit has a coefficient of variation below 0.6%.

3.5.2 Variations in RNA volume

3 PCR amplifications were performed on a single ABI®7500 thermocycler with RNA volume ±10% with the rapid amplification program *(i.e.* the most critical conditions).

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

	Average of 8 Cq values for each RNA volume tested			
	-10%	Set volume	+10%	CV
10x MDL	31.36	31.21	31.06	0.48%
3x LD_{PCR}	31.15	30.97	31.26	0.46%

Conclusion:

For variations of the volume of RNA ± 10%, the IDPPR kit has a coefficient of variation below 0.5%.

3.6 RT-PCR stability

The shelf-life of the reagents present in the kit were evaluated by the technique of accelerated ageing. The stability study was performed by keeping all the reagents at 4°C for 14 weeks. The results obtained will allow for the prediction of the 12 months stability at ≤ -16°C. A real time stability study is ongoing .

3.5.1 Accelerated ageing stability principle

To make this prediction. the following formula derived from the Arrhenius equation is commonly used:

Predicted Stability = Accelerated Stability x $2^{\Delta T/10}$

The ΔT is defined by the difference in temperature between the expected storage temperature and the tested temperature.

Note that when the ΔT is high, the reliability of the predited stability is low. A correction factor should therefore be applied on the predicted data indicated on the manufacturer datasheet of the different component of the kit. The corrected and calculated predictions are shown in the table below:

The calculated predictions are shown in the table below:

Study starting from the manufacture of the IDPPR 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	

3.6.1.1 Data analysed for stability evaluation

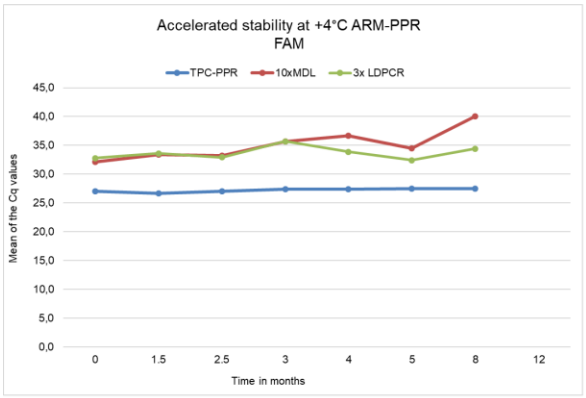
The real time and accelerated stability studies are performed in the most criticial conditions with the rapid amplification program. They have to meet the following requirements to be compliant:

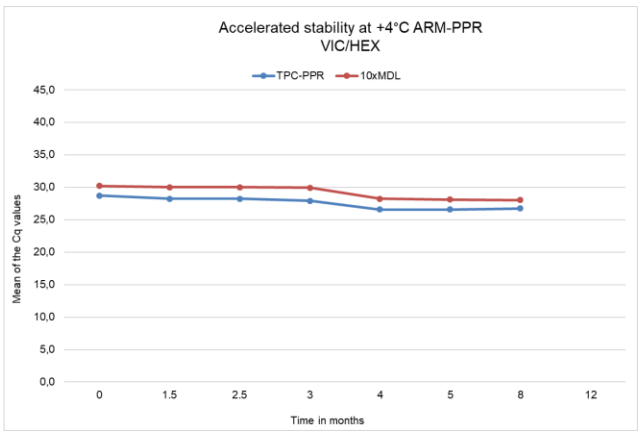
3 times the LD_{PCR} or 21 copies / PCR.

10 times the MDL or 1 000 copies / PCR (MAGFAST384)

The analysis of the target positive control (TPC–PPR) will allow visualisation of possible stability failure.

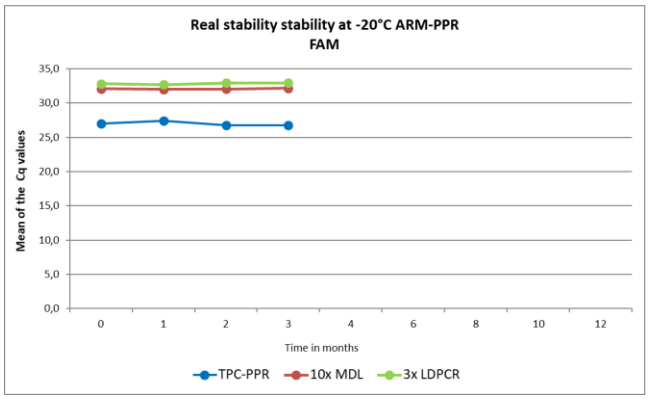
Stability study at +4°C during 10 weeks

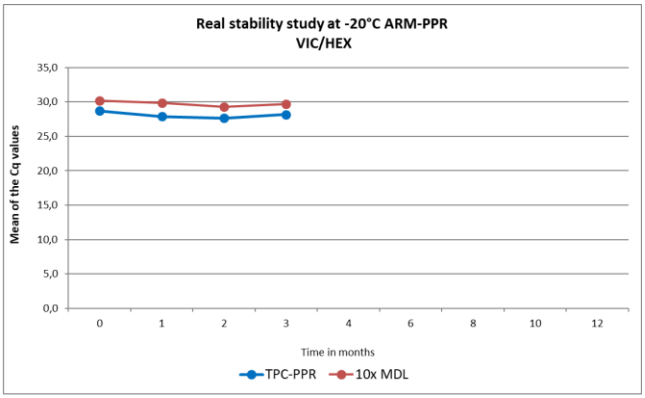




3.6.1.2 Preliminary data for the real time stability study

Stability at -20°C during 3 months





3.7 Cross-contamination tests:

3.7.1 MAGFAST384 methods on the IDEAL™

The following study was performed to ensure no cross-contamination upon extraction using magnetic beads with the IDEAL™ robot.
A range of PPRV-positive whole blood were diluted into negative whole blood and loaded alternately with 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

3.7.1.1 MAGFAST384 results on the IDEAL™

26.6	NEG	NEG	NEG	34.8	NEG	NEG	NEG	33.5	NEG	NEG	26.9
28.6	NEG	26.6	NEG	NEG	NEG	26.8	NEG	NEG	NEG	26.8	29.2
30.7	NEG	NEG	NEG	-	NEG	NEG	NEG	35.5	NEG	NEG	30.8
32.1	NEG	28.7	NEG	NEG	NEG	28.4	NEG	NEG	NEG	28.8	32.6
32.7	NEG	NEG	NEG	-	NEG	NEG	NEG	-	NEG	NEG	35.2
-	NEG	30.6	NEG	NEG	NEG	30.4	NEG	NEG	NEG	30.9	35.7
-	NEG	NEG	NEG	-	NEG	NEG	NEG	-	NEG	NEG	-
-	NEG	32.9	NEG	NEG	NEG	32.6	NEG	NEG	NEG	32.5	-

Conclusion:

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

3.7.2 MAGFAST384 methods with the KingFisher mL

The following study was performed to ensure no cross-contamination upon extraction on magnetic beads with the KingFisher mL robot:
A negative whole blood sample was spiked with a PPR plasmid (Cq=22) and was deposited alternately with the negative whole blood sample according to the diagram below:

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

3.7.2.1 MAGFAST384 results on the KingFisher mL

22.1	NEG	NEG	NEG	22.4
NEG	NEG	21.8	NEG	NEG
22.2	NEG	NEG	NEG	22.3

Conclusion:

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

1. OVERALL CONCLUSION:

The ID Gene™ Peste des Petits Ruminants Duplex kit developed in collaboration with CIRAD complies with the AFNOR NF U47-600-2 requirements.

4 APPENDICES

Appendix 1: IDPPR instructions for use

Certified management system

ID Gene™ Peste des Petits Ruminants Duplex

Ref: IDPPR-50 / IDPPR-100
50 / 100 tests

Real-time RT-PCR assay for the qualitative detection of PPR virus
Suitable samples: Caprine and Ovine whole blood, swabs (oral, nasal, ocular or rectal), organ or tissue.

In-vitro Use

Developed in collaboration with cirad

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General information

- **Characteristics**

ID Gene™ Peste des Petits Ruminants (IDPPR) kit is a real-time RT-PCR kit that amplifies a target sequence in Peste des Petits Ruminants (PPRV) viral genome.

This kit is a qualitative duplex test. It simultaneously amplifies target RNA and an endogenous internal control.

The kit contains a target positive control (TPC-PPR) which is to be extracted in the same manner as the samples to validate the extraction and amplification of the target

This kit can be used to test caprine and ovine whole blood collected in EDTA, swabs and organ or tissue.

- **Kit composition and storage conditions**

The IDPPR kit contains the reagents shown below:

Reference	*Component*	*Volume*	*Description*
TPC-PPR	Target Positive Control	550 µl 1 vial	Inactivated PPRV vaccine, diluted in a virus-free whole blood, freeze-dried and calibrated at between 1 and 100 times the method detection limit (MDL). Freeze-dried pellet to be reconstituted in 550 µl distilled or Nuclease-free water.
ARM-PPR	Amplification Reaction Mix	400 µl 1 or 2 tubes (white cap)	Ready-to-use reaction mixture containing the reverse transcriptase, Taq polymerase and oligonucleotides for amplification and detection of PPRV and of the endogenous non-target positive control.

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 (not more than 3 cycles).

- **Material required but not provided in the kit**

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

Amplification Instrument:

- Real-time thermal cycler with channels capable of reading the following fluorophores: FAM, HEX or VIC and Cy5.

  Examples of compatible thermal cyclers: CFX96™, Chromo4™ (Biorad), LC®480 I, LC®480 II, LC®96 Roche, AB® 7500 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 (optical quality compatible with the thermocycler) and appropriate adhesive films or caps

Reagents:

- Distilled or Nuclease-free water (recommended)

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 controls

- **Positive controls**

The IDPPR kit contains the following positive controls:

- Target Positive Control (TPC-PPR):

This control consists of inactivated PPRV vaccine strain, diluted in a virus-negative whole blood, calibrated at between 1 and 100 times the MDL.
This control validates the efficiency of the extraction and amplification process.
This control is prepared and extracted in the same way as the samples.

- **Endogenous Non-Target Positive Control (NTPCen):**
This control is constitutively present in ruminant cells of the sample to be tested. 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 for PPRV. The volume occupied by the sample is replaced by a negative matrix or Nuclease-free water.

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

- **Extraction of viral RNA**

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

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

- **Extraction of the control**

The volume of the control to be extracted are described in the table below:
Important:

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

Control	*Volume*
TPC-PPR	50 µl

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

Amplification protocol

- **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-PPR) from the other samples.
2. Thaw the IDPPR 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-PPR** tube by vortexing. Centrifuge down briefly.
4. Distribute **8 µl of ARM-PPR** per well. Use PCR strips, or microplates adapted to the thermal cycler in use.
5. Add the following to the reaction mix :
   - 5 µl of RNA extracted from each sample to be analysed
   - 5 µl of RNA extracted from the TPC-PPR
   - 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	*Channel capable of reading*	*Quencher*
Sequence specific to PPRV	FAM	non fluorescent *
Sequence specific to ruminant cells NTPCen	HEX/VIC	non fluorescent * (compatible HEX/VIC)

Note: For devices requiring an internal reference for optical calibration, the amplification mix ARM-PPR contains ROX.
**Using a TAMRA™ quencher can improve the data analysis with some instruments.*

2. Choose between the two different amplification programs validated by IDvet Genetics:
- standard program (allows for PCR kits from different suppliers to be used in a single run)

or,

- rapid program

	Step	Standard program	Rapid program	Number of cycles
(1)	Reverse transcription	10 min at 45°C	10 min at 45°C	1
(2)	Polymerase activation	10 min at 95°C	2 min at 95°C	1
(3)	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 phase 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 thermocycler 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 for each fluorescence detector.
The Cq is also known as the Ct value (Cycle Threshold) or Cp value (Crossing Point).
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-PPR	Detected in FAM and VIC/HEX	Refer to the Cq value indicated in the quality control certificate
NTPCen	Detected in VIC/HEX in each sample	Presence of a characteristic curve
NEC	No detection if water used	Complete absence of a characteristic curve
	Detected in VIC/HEX if virus-negative sample used	Presence of a characteristic curve
NAC	No detection	Complete absence of a characteristic curve

Note: TPC-PPR may be used to monitor variations in analytical sensitivity as it is calibrated at between 1 and 100 times the MDL.

- **Suggested interpretation of results**

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

Sample	PPRV signal	NTPCen signal	Interpretation
Individual	Detected	Detected or not detected	Animal detected as positive for PPRV
	Not detected	Detected	Animal not detected for PPRV
	Not detected	Not detected	A problem occurred during sample distribution or extraction process / PCR reaction was inhibited

Non-validated samples:

- If the NTPCen is not detected but the sample is detected positive for PPRV, 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

or,

- the PCR reaction was 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 step on 5 µl of this dilution.
3. If the NTPCen is detected, interpret the sample according to table above.
4. If the NTPCen is 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

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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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:

Reference	*Description*	*Volume*	
		SPIN250	**SPIN50**
LMAP	Lysis buffer MAP	1000 ml	50 ml
RAV1	Lysis buffer to be reconstituted	5 x 35 ml	35 ml
Carrier RNA		5 x 1 mg	1 mg
SPIN COLUMN	Silica columns	250	50
COLLECTION TUBES	Collection tubes	750	150
RAW	Wash Buffer 1	150 ml	30 ml
RAV3	Concentrated wash buffer 2	50 ml	12 ml
RNase-free H_2O	RNA elution buffer	30 ml	13 ml
ELU	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
- Nuclease-free filter tips
- 1.5 ml Nuclease-free tubes
- Mini centrifuge delivering 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@id-vet.com.

Standard protocol

Consult the PCR amplification instructions before performing the extraction process to ensure that other extraction conditions are not required. If not, the protocol below could be performed for sample extraction.
The appendices of this document outlines possible pre-treatment, and volumes of samples and controls to extract for each matrix and for all the amplification kits validated by ID.vet Genetics.

1. **Reconstitute the following reagents:**

a) **for the SPIN50 and SPIN250 kits:**

- Lysis buffer RAV1–Carrier RNA:

Reconstitute the lyophilised Carrier RNA with 1 ml of RAV1 buffer. Vortex and transfer the Carrier RNA solution dissolved into the tube labelled RAV1.
The RAV1-Carrier can then be stored at +4°C for 1 month or at ≤ -16°C for long-term storage. It is recommended to aliquot the RAV1-Carrier 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 RAV3 :

Reconstitute the RAV3 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 RAV3 :

Reconstitute the RAV3 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 RAV1-Carrier 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 8,000 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 8,000 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 RAW buffer** into each column. Close the column and **centrifuge for 1 min at 8,000 g.** Discard the collection tube.

   Keep the column and replace a new collection tube.
5) Pipette **600 µl** (reconstituted) **RAV3 buffer** into each column. Close the column and **centrifuge for 1 min at 8,000 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 for DNA extraction** or of **RNase-Free H_2O for RNA extraction.**

   Close the column and **incubate at room temperature for 1 min.**
8) **Centrifuge for 1 min at 8,000 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@id-vet.com. We will provide you with additional information.

For all questions or technical support, please contact us at the following address: support.genetics@id-vet.com
Material and Safety Data Sheets (MSDS) and protocols are available upon request: info@id-vet.com
For additional information, visit www.id-vet.com

APPENDIX : Protocol for RNA extraction using the SPIN50/SPIN250 kit and samples analysis using the ID Gene™ Peste des Petits Ruminants Duplex kit

Matrices: Whole blood (collected with anticoagulant), swabs (oral, nasal, ocular or rectal) , organ or tissue.

This appendix outlines extraction conditions for the **ID Gene™ Peste des Petits Ruminants Duplex** (IDPPR) amplification kit for whole blood, swabs, organ or tissue.

This appendix also describes the volume to be extracted for positive control (TPC-PPR) needed for the IDPPR test.

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

a) Reagent required but not provided in the kit:

- 1X PBS (molecular biology quality)
- Glass beads 3 mm
- Mixer Mill-type grinder, Precellys®24, FastPrep® (for use of other devices, please contact us) with 2ml adapted tubes.

b) Samples and controls preparation:

- **Swabs :**

1. Express the fluid from the swab in 1mL of 1X PBS.
2. Vortex for 1 minute.
3. **100 µl of supernatant** are needed for the extraction (see **step c** below).

- **Organ or tissue :**

4. In a 2 ml tube, weigh 20 mg organ previously dissected.
5. Add 1 ml of 1X PBS.
6. Add 1 to 3 glass beads and homogenize thoroughly by vortexing.
1. If possible, grind:
   - Precellys® 24/ Precess 24™: for 40 seconds at 6000 rpm
   - FastPrep®: for 45 seconds at 6 M/s
   - Mixer Mill / TissueLyser : for 2 minutes at 30 Hz.
2. Centrifuge **for 2 minutes at 1500 g.**
3. **100 µl of supernatant** are needed for the extraction (see below).

c) Extraction of viral RNA :

1. Prepare samples and controls for extraction as described below:

	Matrices			*Controls*	
Reagents	Whole blood	*Swabs*	*Organ / tissue*	*TPC-PPR*	*NEC*
Sample or control	100 µl	100µl	100µl	50 µl	100 µl of water or negative sample
Lysis buffer (RAV1-Carrier)	600 µl	600µl	600µl	600 µl	

1. Vortex **immediately for 15 seconds.**
2. Add **600 µl of absolute Ethanol** (homogenize by pipetting). Rapidly centrifuge the tube before opening. The solution obtained is **the lysate sample.**
3. Continue with **step 3** of the standard protocol.

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IDvet GENETICS

Certified management system

ID Gene™ Mag Fast Extraction Kit

Ref: MAGFAST384

384 extractions

Fast extraction of nucleic acids with magnetic beads
All matrices, all pathogens

For *in vitro* use

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General information

- **Characteristics**

The ID Gene™ Mag Fast Universal Extraction Kit (MAGFAST384) is a fast extraction and purification system of nucleic acid with magnetic bead, for use on all matrices and for all animal health pathogens. This kit is compatible with the following automated extraction systems:

- IDEAL™ (IDvet)
- MagMax™ Express-96 Magnetic Particles Processor (Thermo Scientific™)
- KingFisher ™ 96 (Thermo Scientific™)
- KingFisher ™ Flex (Thermo Scientific™)
- KingFisher ™ mL (Thermo Scientific™)

- **Kit composition and storage conditions**

The MAGFAST384 kit contains the following reagents:

Reference	*Description*	*Volume*
LYS-FAST	Lysis buffer ready for use	60 ml
MAGBEADS	Magnetic bead solution	4,5 ml
BB-FAST	Binding buffer	180 ml
WASH 1	Wash buffer 1	125 ml
WASH 2	Wash buffer 2	125 ml
ELU	Elution buffer	60 ml

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

- **Consumables and reagents required but not provided in the kit**

Consumables compatible with the following robots: MagMax™ Express-96, KingFisher™ 96, KingFisher™ Flex and IDEAL™

Consumables	*References*	*Description*
Plastic tip Combs for rod magnet proctection	IDTIP	96 Tip Comb for DW magnets (60 items / box)
Elution microplates	IDELU	96 KF plate (200µl, 60 plates / box)
Deepwell plates 	IDWELL	Microtiter Deepwell 96 Plate, V-bottom, polypropylene (60 plates / box)

Consumables compatible with the KingFisher™ mL:

Consumables	*References*	*Description*
Plastic tip Combs for rod magnet proctection	97002111	KingFisher™ mL Tip Comb (800 items)
Strips	97002121	KingFisher™ mL Tube, (20x45 items)
Combs + strips for 240 analysis 	97002141	KingFisher ™ mL Combi 240 (tubes and tip combs for 240 samples)

Other consumables and equipment:

- Precision pipettes capable of delivering volumes of 1 µl to 1000 µl with
- Nuclease-free filter tips
- Nuclease-free micro-tubes

Reagents:

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

Risks and precautions

Some components of the MAGFAST384 kit contain hazardous substances. Wear protective gloves/protective clothing/eye protection/face protection (P280). Material and Safety Data Sheets (MSDS) and Certificates of Analysis are available on request: info@id-vet.com.

Extraction Protocol

Consult the PCR amplification instructions before beginning the extraction process to ensure that other extraction conditions are not required. If not, the protocol below could be performed for sample extraction.
The appendices of this document outlines possible pre-treatment, and volumes of samples and controls to extract for each matrix and for all the amplification kits validated by IDvet Genetics.

1. Reconstitute the following reagents:

a) **MAGBEADS-BB-FAST bead solution**

Each reaction requires **10 µl of MAGBEADS and 300 µl of BB binding buffer**.
Prepare a fresh solution of MAGBEADS-BB-FAST before each extraction.
The MAGBEADS-BB solution can be prepared for all of the test samples. Allow for 10% extra volume.
Example: To extract 10 samples, mix 110 µl of MAGBEADS with 3300 µl of BB binding buffer.
Mix thoroughly the solution just before using.

b) **WASH buffer 3 (WASH 3): 80% Ethanol**

Each reaction requires 300 µl of WASH 3 for wash step 3. Prepare a sufficient volume of 80% ethanol. Allow for 10% extra volume.
Example: To extract 10 samples, prepare 3300 µl of 80% ethanol by diluting 2640 µl absolute ethanol with 660 µl of distilled or nuclease free water

2. Prepare the consumables for the extraction:

a) **KingFisher™ mL:** Prepare the appropriate number of strips for the number of samples to be extracted. Identify each strip with sample name and position on the tray.

b) **KingFisher™ 96, KingFisher™ Flex and IDEAL™:** Prepare and identify 4 deepwell plates (Lysis, Wash1, Wash2, and Wash3) and 1 microplate for elution. Plan one well by sample and control to extract.

3. Distribute the buffers as described below:

	Position			
Step	KingFisher™ mL	IDEAL™/ KingFisher™ 96/Flex	*Reagent*	*Volume*
Lysis	Well A	Deepwell 1	LYSFAST	125 µl
Wash 1	Well B	Deepwell 2	WASH 1	300 µl
Wash 2	Well C	Deepwell 3	WASH 2	300 µl
Wash 3	Well D	Deepwell 4	WASH 3	300 µl
Elution	Well E	Microplate for elution	ELU	80 µl

4. Distribute the samples as described below:

Position		
KingFisher™ mL	IDEAL™/ KingFisher™ 96/Flex	*Sample or control volume*
Well A	Deepwell 1	Maximum 125 µl*

*The test sample volume depends on the amplification kit to be used after extraction. This volume is outlined in the appendices of this document.

5. Add the MAGBEADS-BB solution as described below:

Position			
KingFisher mL	IDEAL™/ KingFisher 96/Flex	*Reagent*	*Volume*
Well A	Deepwell 1	MAGBEADS-BB	310 µl (or 300 µl of BB buffer + 10 µl of MAGBEADS)

6. Load robots and run the extraction program:

a) **KingFisher™ mL:**
1. Protect the robot magnets with the plastic tip combs.
2. Load the tray with the strips in the robot.
3. Select the **ID Gene™ MAG** program and press "START" 2 times to begin the extraction.

b) **KingFisher™ 96 and KingFisher™ Flex:**
1. Select the ID Gene™ MAGFAST program. Contact us for use with other extraction programs.
2. Press "START" and follow the directions for loading plates. When the loading is completed, press "START" to begin the extraction.

c) **IDEAL™ :**
1. Select the IDGeneFAST program. Contact us for use with other extraction programs.
2. Press "Load protocol" then "Loading", and follow the instructions for loading plates. When the loading is completed, confirm to begin the extraction.

7. At the end of the extraction program:

a) **KingFisher™ mL:**
Transfer eluates from the E Wells into labelled micro-tubes.

b) **IDEAL™, KingFisher™ 96 and KingFisher™ Flex:**
Collect the elution plate and cover with an adhesive film for conservation.
Discard the other plastic consumables (strips, deepwells and protective combs).

8. Keep the eluates at 5°C ± 3°C if the PCR is to be performed immediately. Maintain at <-16°C for long-term storage.

Technical support and documentation

The ID Gene™ amplification kit is constantly being improved.
If the kit you are interested in using or the matrices to be analyzed are not included in the annexes, please contact us at: info@id-vet.com.
We will provide you with additional information.

For all questions or technical support, please contact us at the following address: support.genetics@id-vet.com
Material and Safety Data Sheets (MSDS) and protocols are available upon request: info@id-vet.com
For additional information, visit www.id-vet.com

APPENDIX 8 : Protocol for RNA extraction using the MAGFAST384 kit

for sample analysis using the ID Gene™ Peste des Petits Ruminants Duplex kit

Matrices: Whole blood (collected with anticoagulant), swabs (oral, nasal, ocular or rectal), organ or tissue.

This appendix outlines extraction conditions for the **ID Gene™ Peste des Petits Ruminants Duplex** (IDPPR) amplification kit for whole blood, swabs, organ or tissue.

This appendix also describes the volume to be extracted for positive control (TPC-PPR) required for the IDPPR test.

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

a) **Reagent required but not provided in the kit:**

- 1X PBS (molecular biology quality)
- Glass beads 3 mm
- MixerMill-type grinder, Precellys®24, FastPrep® (for use of other devices, please contact us) with 2ml adapted tubes.

b) **Samples and controls preparation:**

- **Swabs :**

1. Express fluid from the swab in **1mL of 1X PBS**.
2. Vortex for 1min.
3. **50 µl of supernatant** are needed for the extraction (see **step c.** below).

- **Organ or tissue :**

4. In a 2 ml tube, **weigh 20 mg** organ previously dissected.
5. Add **1 ml of 1X PBS**.
6. Add 1 to 3 glass beads and homogenize thoroughly by vortexing.
7. If possible, grind:
   - Precellys® 24/ Precess 24™: for 40 seconds at 6000 rpm
   - FastPrep®: for 45 seconds at 6 M/s
   - MixerMill / TissueLyser : for 2 minutes at 30 Hz.
8. Centrifuge **for 2 minutes at 1500 g.**
9. **50 µl of supernatant** are needed for the extraction (see below).

c) **Extraction of viral RNA :**

1. Prepare samples and controls for extraction as described below:

Position	*Reagent*	*Matrices*			*Controls*	
		Whole blood	*Swabs*	*Organ / tissue*	*TPC-PPR*	*NEC*
A Well / Deepwell 1	Lysis buffer (LYSFAST)	125 µl	125 µl	125 µl	125 µl	
	Sample or control	50 µl blood	50 µl lysate	50 µl lysate	50 µl TPC-PPR	50 µl water or negative sample
B Well / Deepwell 2	WASH 1	300 µl	300 µl	300 µl	300 µl	
C Well / Deepwell 3	WASH 2	300 µl	300 µl	300 µl	300 µl	
D Well / Deepwell 4	WASH 3	300 µl	300 µl	300 µl	300 µl	
E Well / *Elution microplate*	ELU	80 µl	80 µl	80 µl	80 µl	

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

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Appendix 4: Results of Diagnostic Specificity and Sensitivity

1. Results obtained by CIRAD on whole blood

Samples ID		CIRAD	IDPPR	
			PPR	NTPCen
25/01/16	50095	Neg	Neg	24.51
	50101	Neg	Neg	25.91
	50103	Neg	Neg	24.10
	50132	Neg	Neg	26.11
	50096	Neg	Neg	23.92
	50108	Neg	Neg	26.04
	50327	Neg	Neg	23.10
	50330	Neg	Neg	25.26
26/01/16	50095	Neg	Neg	23.20
	50101	Neg	Neg	26.39
	50103	Neg	Neg	26.12
	50132	Neg	Neg	27.14
	50096	Neg	Neg	23.07
	50108	Neg	Neg	31.62
	50327	Neg	Neg	24.80
	50330	Neg	Neg	25.13
27/01/16	50095	Neg	Neg	25.61
	50101	Neg	Neg	25.47
	50103	Neg	Neg	24.84
	50132	Neg	Neg	24.75
	50096	Neg	Neg	25.11
	50108	Neg	34.66	27.14
	50327	Neg	Neg	24.77
	50330	Neg	Neg	27.73
28/01/16	50095	Neg	Neg	25.22
	50101	Neg	35.98	25.15
	50103	Neg	Neg	25.97
	50132	Neg	Neg	26.36
	50096	34.09	Neg	27.90
	50108	Neg	Neg	31.69
	50327	Neg	Neg	29.53
	50330	Neg	Neg	34.77
29/01/16	50095	Neg	Neg	26.45
	50101	Neg	Neg	28.96
	50103	Neg	Neg	25.51
	50132	Neg	Neg	28.04
	50096	35.34	35.90	29.36

	50108	32.96	31.14	27.72
	50327	33.09	32.76	25.80
	50330	32.86	33.77	28.12
30/01/16	50095	Neg	34.70	25.08
	50101	Neg	36.23	30.43
	50103	Neg	35.92	26.57
	50132	Neg	34.06	26.42
	50096	Neg	34.11	27.74
	50108	33.49	29.50	26.95
	50327	32.62	30.46	25.20
	50330	35.13	36.85	30.47
31/01/16	50095	34.76	33.48	26.77
	50101	34.57	35.97	29.86
	50103	32.81	33.47	26.40
	50132	33.89	32.69	26.68
	50096	31.67	33.46	24.62
	50108	32.64	30.90	28.25
	50327	33.75	33.98	30.08
	50330	32.00	33.74	26.11
01/02/16	50095	33.48	37.32	28.48
	50101	30.80	30.15	26.24
	50103	32.95	35.03	29.29
	50132	32.08	31.99	26.64
	50096	32.63	35.75	27.25
	50108	30.87	31.60	27.12
	50327	33.13	33.75	27.56
	50330	29.96	37.62	27.70
02/02/16	50095	29.98	30.27	23.27
	50101	32.01	31.69	26.11
	50103	32.42	32.25	26.21
	50132	34.21	31.87	26.37
	50096	32.59	35.11	26.28
	50108	34.14	Neg	32.05
	50327	34.47	33.33	27.55
	50330	31.96	33.57	24.80
03/02/16	50095	30.79	30.65	23.78
	50101	31.25	30.16	24.58
	50103	32.57	32.21	25.28
	50132	33.84	32.85	26.55
	50096	34.96	Neg	28.70
	50108	Neg	Neg	33.69
	50327	34.74	33.77	28.37
	50330	33.31	34.87	26.76

04/02/16	50095	Neg	Neg	30.75
	50101	34.10	34.68	27.59
	50103	Neg	35.92	28.05
	50132	34.91	34.65	27.25
	50096	Neg	Neg	25.79
	50108	Neg	35.71	32.42
	50327	Neg	Neg	28.59
	50330	34.23	36.17	25.50

Non detected by IDPRR	Positive in limit of detection by IDPPR (negative with CIRAD PCR)

*12 samples are discordants between the two PCR methods. 3 of them are not detected by IDPPR while they have a positive status with in-house CIRAD PCR.At the same times, 9 samples have a negative status with CIRAD validated PCR and are positive with IDPPR (all of these samples are around the limit of detection).

2. Results obtained by CIRAD on ocular swabs

		CIRAD	IDPPR	
Samples ID			PPR	NTPCen
J21	61065	Neg	Neg	27.12
	61066	Neg	Neg	26.41
	61096	Neg	Neg	30.55
	61100	Neg	Neg	28.90
	61101	Neg	Neg	29.38
	61117	Neg	Neg	27.18
	61123	Neg	Neg	27.98
	61125	Neg	Neg	25.06
	61126	Neg	Neg	28.60
	61142	Neg	Neg	23.19
J23	61065	Neg	Neg	25.57
	61066	Neg	Neg	-
	61096	Neg	Neg	26.16
	61100	Neg	Neg	26.14
	61101	Neg	Neg	32.13
	61117	Neg	Neg	32.15
	61123	Neg	Neg	25.31
	61125	Neg	Neg	26.01
	61126	Neg	Neg	26.84
	61142	Neg	Neg	24.89
J25	61065	Neg	31.49	26.87
	61066	26,22	28.14	23.64
	61096	26,85	30.00	25.81

	61100	39,53	30.59	25.11
	61101	Neg	31.44	25.75
	61117	28,95	31.40	27.32
	61123	Neg	32.86	23.48
	61125	21,84	23.87	21.29
	61126	23,27	26.08	26.10
	61142	Neg	35.66	23.17
J27	61065	19,89	21.80	22.31
	61066	22,95	26.06	24.89
	61096	23,7	30.37	33.16
	61100	22,49	24.99	24.04
	61101	24,25	27.12	25.61
	61117	22,97	25.37	24.04
	61123	22,79	25.75	24.25
	61125	20,56	22.30	22.64
	61126	20,96	22.85	24.10
	61142	23,69	25.87	22.41
J29	61065	16,16	18.04	21.63
	61066	17,46	19.03	25.77
	61096	17,83	20.29	26.34
	61100	16,86	18.89	22.10
	61101	18,16	20.76	24.04
	61117	18,12	20.05	24.38
	61123	20,03	20.99	27.43
	61125	14,96	16.87	21.33
	61126	15,99	18.75	23.36
	61142	18,9	19.49	19.66
J31	61065	23,5	24.20	28.11
	61066	19,3	20.71	23.87
	61096	19,66	23.71	24.42
	61100	19,58	22.72	26.05
	61101	22,76	25.73	26.97
	61117	19,39	19.39	28.69
	61123	21,89	22.85	28.58
	61125	18,86	19.57	28.03
	61126	17,95	18.65	27.94
	61142	18,66	19.41	26.74
J33	61065	NV	NV	-
	61066	28,4	27.63	22.56
	61096	24,97	25.85	21.27
	61100	23,65	29.48	24.27
	61101	26,8	28.10	24.38
	61117	24,53	25.38	26.79

	61123			
	61125	24,69	25.60	22.11
	61126	20,61	20.23	27.31
	61142	24,98	26.87	20.62
J35	61065	27,75	28.76	26.43
	61066	Neg	30.74	24.66
	61096			
	61100	24,05	26.00	27.89
	61101	Neg	30.13	27.08
	61117	29,31	29.78	28.12
	61123			
	61125	28,05	28.46	24.27
	61126			
	61142			

Positive in limit of detection by IDPPR (negative with CIRAD PCR)

*6 discordants results were obtained with 6 samples using two different PCR methods. They have negative status with the CIRAD validated RT-PCR and are positive with the IDPPR kit (around the limit of detection).

3. Results obtained by CIRAD on rectal swabs

	CIRAD	IDPPR	
Sample ID		PPR	NTPCen
50095			
J4	36,22	Neg	28,28
J5	29,80	28,7	24,99
J6	26,69	25,55	24,39
J7	20,49	18,84	26,43
J8	23,74	22,34	25,68
J9	19,38	17,72	25,19
J10	18,49	17,07	23,86
J11	20,42	19,69	26,09
J12	20,09	18,97	26,85
J13	19,55	18,7	26,35
J14	25,69	-	-
50101			
J4	Neg	35,71	27,91
J5	34,18	35,28	25,83
J6	27,48	26,98	29,15
J7	25,01	24,84	24,31
J8	20,32	19,66	26,24
J9	19,47	18,4	25,37
J10	15,96	18,32	27,86

J11	19,90	18,18	27,63
J12	22,42	23,67	23,97
J13	24,68	29,5	30,02
J14	25,13	24,62	28,82
50103			
J4	34,31	33,42	25,05
J5	31,79	29,73	27,59
J6	26,69	25,58	28,09
J7	24,25	23,21	26,69
J8	21,88	20,95	28,13
J9	21,84	20,41	25,87
J10	19,56	20,34	27,27
J11	20,17	21,28	26,96
J12	18,77	18,66	27,84
J13	18,92	19,59	23,86
J14	17,23	18,5	30,48
50132			
J4	36,97	36,31	26,6
J5	31,66	31,83	28,96
J6	27,24	26,64	25,06
J7	23,91	25,74	25,45
J8	20,58	22	26,26
J9	18,21	17,67	22,49
J10	20,40	19,56	23,26
J11	20,06	21,08	26,77
J12	24,51	29,05	30,01
J13	25,29	26,88	28,67
J14	24,03	26,3	30,09
Negative herd			
1	Neg	Neg	34,31
2	Neg	Neg	31,79
3	Neg	Neg	26,69
4	Neg	Neg	24,25
5	Neg	Neg	21,88
6	Neg	Neg	21,84
7	Neg	Neg	19,56
8	Neg	Neg	20,17
9	Neg	Neg	31,66
10	Neg	Neg	27,24
11	Neg	Neg	23,91
12	Neg	Neg	20,58
13	Neg	Neg	18,21
14	Neg	Neg	20,4
15	Neg	Neg	20,06

16	Neg	Neg	20,94
17	Neg	Neg	24,38
18	Neg	Neg	27,95
19	Neg	Neg	31,08
20	Neg	Neg	29,8
21	Neg	Neg	26,69
22	Neg	Neg	20,49
23	Neg	Neg	23,74
24	Neg	Neg	25.79
25	Neg	Neg	32.42
26	Neg	Neg	28.59
27	Neg	Neg	25.50
28	Neg	Neg	27.90
29	Neg	Neg	31.69
30	Neg	Neg	29.53
31	Neg	Neg	34.77
32	Neg	Neg	25.08
33	Neg	Neg	30.43
34	Neg	Neg	26.57
35	Neg	Neg	26.42
36	Neg	Neg	29.29
37	Neg	Neg	26.64

Non detected by IDPRR	Positive in limit of detection by IDPPR (negative with CIRAD PCR)

*Two samples displayed discordant results using the two different RT-PCR methods. One of them is not detected by IDPPR while it has a positive status with in-house CIRAD PCR. The other one sample has a negative status with CIRAD validated PCR and is positive with IDPPR (these samples are around the limit of detection).