VALIDATION REPORT

Produit: SPIN50/250

ID Gene™ Spin Universal Extraction Kit

Method	Silica technology
Species	All
Matrices	Blood-Serum-Tissue
Reference	SPIN50/250

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Manufacturer:

IDvet Genetics

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OVERVIEW TABLE

Feature	Specifications
Applications	Ready-to-use, sequencing and PCR-grade DNA / RNA From extra small to extra large scale, low to high throughput No alcohol precipitation necessary Fast and easy procedure
Technology / Separation principle	Silica-membrane technology / Chaotropic salt binding
Purification of total RNA, miRNA, poly A+ mRNA, DNA or protein	Genomic DNA, mitochondrial DNA, viral DNA
Fragment size	100 bp–approx. 50 kb
Format	Spin columns
Processing	Manual (centrifugation or vacuum)
Main sample type	Whole blood, body fluids, tissue
Sample amount	1–200 µl
Elution volume	50–200 µl
Time per run or per prep	20 min/4-6 preps
Yield	30–50 µg

1. INTRODUCTION

No phenol–chloroform extraction is required. DNA binds specifically to the SPIN50/250 silica-gel membrane while contaminants pass through. PCR inhibitors, such as divalent cations and proteins, are completely removed in two efficient wash steps, leaving pure nucleic acid to be eluted in either water or a buffer provided with the ID Gene™ Spin Universal Extraction. DNA/RNA from blood, related body fluids, or tissue are ready to use in PCR and blotting procedures.

2. KIT OVERVIEW

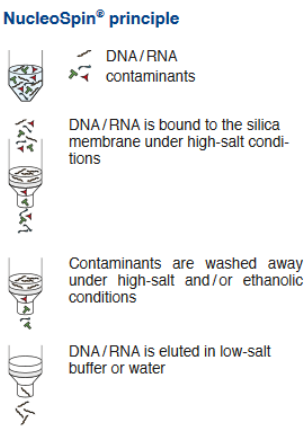
The **ID Gene™ Spin Universal Extraction Kit**, reference: **SPIN50/250**, is controlled and conditioned at the headquarters of IDvet Genetics:

310 rue Louis Pasteur 34790 Grabels.

2.1 Kit description

The ID Gene™ Spin Universal Extraction Kit allows the extraction of RNA/DNA in whole blood samples, body fluids and tissues.

With SPIN50/250, RNA viruses are lysed quickly and efficiently by Lysis Buffer RAV1 which is a highly concentrated solution of GITC. DNA viruses are usually more difficult to lyse and require ProteinaseK digestion. Lysis buffer and ethanol create appropriate conditions for binding of nucleic acids to the silica membrane of the SPIN50/250. Carrier RNA improves binding and recovery of low-concentrated viral RNA. Contaminations (potential PCR inhibitors) like salts, metabolites and soluble macromolecular cellular components are removed in simple washing steps with ethanolic buffers RAW and RAV3. The nucleic acids can be eluted in low salt buffer or water and are ready-for-use in subsequent reactions.



2.2 Kit composition, storage and stability

The ID Gene™ Spin Universal Extraction Kit contains the following reagents:

		Volume	
Reference	***Description***	**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 H2O	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.

2.3 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.

3. KIT OVERVIEW

3.1 Validation protocol

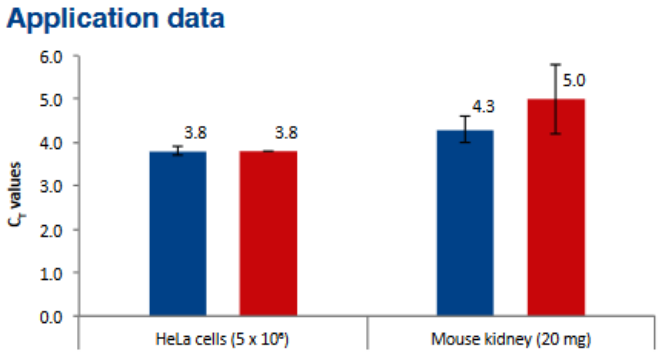
The ID Gene™ Spin Universal Extraction Kit was validated according to the requirements of the AFNOR NF U47-600-2 standard: Methods of analysis in animal health PCR.
Part 2: Requirements and recommendations for the development and validation of PCR in animal health.

3.2 Characterisation

3.2.1 Application Data

3.2.1.1 Efficient RNA recovery

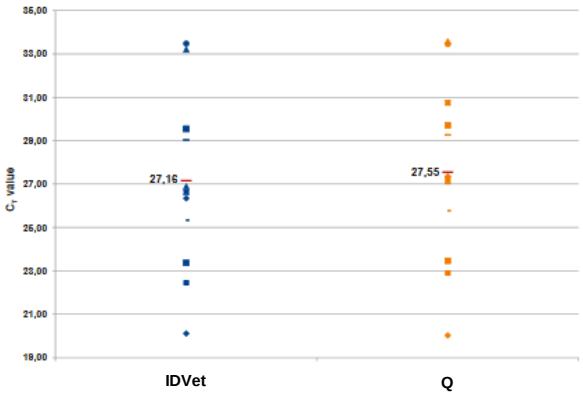
RNA was isolated from HeLa cells (5 x 106) and mouse kidney (20 mg) with the ID Gene™ Spin Universal Extraction Kit (blue) and a competitor kit (red). The RNA recovery was quantified by qRT-PCR. The CT values are practically the same, indicating similar or better yield for the MN kit



Conclusions:
Results indicate that the ID Gene™ Spin Universal Extraction Kit is characterized by a good yields.

3.2.1.2 High sensitive viral BTV RNA detection with ID Gene™ Spin Universal Extraction Kit

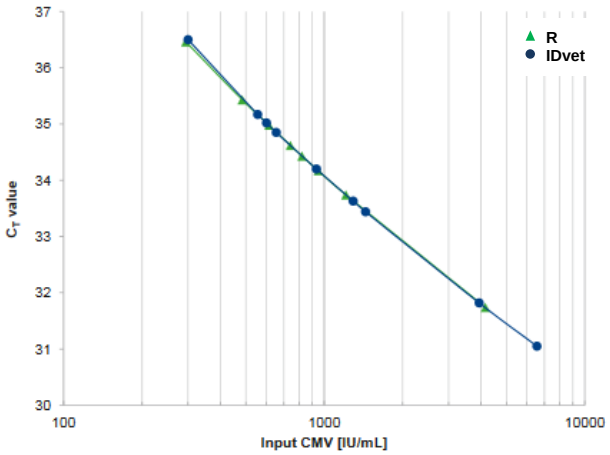
Viral BTV RNA was isolated from 12 different bovine EDTA-blood samples with different virus titer using ID Gene™ Spin Universal Extraction Kit (blue) and a competitor kit Q (orange). BTV RNA was quantified by Real-time RT-PCR.



Conclusions:
The average CT value is lower for ID Gene™ Spin Universal Extraction Kit indicating higher viral RNA yields.

3.2.1.3 Reliable viral CMV DNA recovery with ID Gene™ Spin Universal Extraction Kit

ID Gene™ Spin Universal Extraction Kit and a competitor kit R were used for viral CMV DNA isolation from different plasma samples with diverse virus titer. The viral DNA were quantified by qPCR in Roche LightCycler® 480.



Conclusions:
ID Gene™ Spin Universal Extraction Kit shows consistent reliable CMV DNA recovery.

3.2.1.4 Comparaison between ID Gene™ Mag Blood Extraction kit and ID Gene™ Spin Universal Extraction Kit

The national expert BVDV reference laboratory ISAE in Niort (France) provided a BVDV strain titrated to $10^{6.6}$TCID$_{50}$/ml (C24V Oregon). The TCID$_{50}$ is the infectious cytopathogenic dose that, injected into a tissue culture, can destroy 50% of cells.

A negative matrix of blood, serum or ear notches is thus spiked with BVDV virus, titrated at TCID$_{50}$/ml. It is important to know that Methodology 2 does not take into account the influence of inclusion of the target in the analysis matrix.

Experimental modality to approach the limit of detection method

The LD$_{METHOD}$ is estimated as the last dilution level where 8 repetitions are positive. The limit estimated is thus not the absolute limit of detection of the method which is at a lower concentration of target sequences.

Experimental design for the experimental estimation of the LD$_{METHOD}$:

Minimum number of sessions	Minimum number of operators	Minimum number of replicates per dilution
2	1	4

LD_{METHOD} MAG192/MAG384 results on individual whole blood samples

	$TCID_{50}$/ml	100	50	25	12.5	6.25
Session 1	Replicate 1	30.4	30.7	32.2	34.9	34.0
	Replicate 2	30.1	30.8	32.5	32.4	36.6
	Replicate 3	29.7	31.1	31.9	32.7	35.2
	Replicate 4	30.1	31.1	31.8	32.5	33.4
	Average Cq	30.1	30.9	32.2	33.2	34.8
	% detection	100%	100%	100%	100%	100%
Session 2	Replicate 1	29.9	30.6	32.0	33.0	34.6
	Replicate 2	30.0	30.8	31.1	32.7	32.7
	Replicate 3	29.8	30.9	31.6	34.1	34.4
	Replicate 4	30.1	31.0	31.3	32.9	33.8
	Average Cq	30.0	30.9	31.5	33.2	33.9
	% detection	100%	100%	100%	100%	100%

LD_{METHOD} SPIN50/SPIN250 results on individual whole blood samples

	$TCID_{50}$/mL	100	50	25	12.5	6.25
Session 1	Replicate 1	30.4	31.3	32.3	33.2	35.2
	Replicate 2	30.8	30.6	32.0	35.1	37.0
	Replicate 3	30.1	31.1	31.6	33.4	35.1
	Replicate 4	30.6	31.2	32.0	32.7	34.1
	Average Cq	30.5	31.0	32.0	33.6	35.4
	% detection	100%	100%	100%	100%	100%
Session 2	Replicate 1	30.8	31.9	33.0	33.1	33.7
	Replicate 2	30.6	31.6	31.6	33.2	33.4
	Replicate 3	30.3	31.6	31.8	32.7	33.2
	Replicate 4	30.4	31.3	32.8	33.7	37.4
	Average Cq	30.5	31.6	32.3	33.2	34.4
	% detection	100%	100%	100%	100%	100%

Conclusion:

The limit of detection of the method on individual whole blood with SPIN50/SPIN250 or MAG192/384 **is the same: 7 $TCID_{50}$/ml.** ID Gene™ Spin Universal Extraction Kit have the same efficiency than MAG192/384.

3.2.1.5 Comparaison between ID Gene™ Mag Blood Extraction kit and ID Gene™ Spin Universal Extraction Kit

Experimental modality to approach the limit of detection method:

A negative spleen sample is doped with Q fever culture (LNR France), titrated on culture. It is important to know that Methodology 2 does not take into account the influence of inclusion of the target in the analysis matrix.

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

Minimum number of sessions	Minimum number of operators	Minimum number of replicates per dilution
2	1	4

LD_{METHOD} MAG192/MAG384 results on individual spleen samples

	bacteria/mg	100	50	25	12.5	6.25
Session 1	Replicate 1	30.6	31	32.5	34.4	34.2
	Replicate 2	30.3	32.5	32.2	32.9	36.4
	Replicate 3	29.5	31.4	31.5	32.5	35.3
	Replicate 4	29.9	31.8	32.2	32.7	33.3
	Average Cq	30.1	31.7	32.2	33.2	34.8
	% detection	100%	100%	100%	100%	100%
Session 2	Replicate 1	29.6	31	32.8	33.3	34.8
	Replicate 2	30.3	31.4	32.6	32.4	32.5
	Replicate 3	29.5	31.8	32.3	34.2	34.2
	Replicate 4	30.4	31.1	32.6	32.8	34.0
	Average Cq	30.0	31.3	32.6	33.2	33.9
	% detection	100%	100%	100%	100%	100%

LD_{METHOD} SPIN50/SPIN250 results on individual spleen samples

	bacteria/mg	100	50	25	12.5	6.25
Session 1	Replicate 1	30.8	31.8	33.0	33.3	35.8
	Replicate 2	30.4	31.3	32.6	35.6	34.9
	Replicate 3	31.0	31.6	31.8	34.3	34.1
	Replicate 4	30.8	31.5	32.8	33.5	34.6
	Average Cq	30.7	31.5	32.6	34.16	34.8
	% detection	100%	100%	100%	100%	100%
Session 2	Replicate 1	31.1	32.1	33.4	33.6	33.9
	Replicate 2	31.3	32.2	32.0	33.8	33.4
	Replicate 3	30.5	31.7	31.9	33.6	33.6
	Replicate 4	30.6	32.0	33.8	34.7	38.4
	Average Cq	30.9	32.0	32.8	33.9	34.81
	% detection	100%	100%	100%	100%	100%

Conclusion:

The limit of detection of the method on individual spleen with SPIN50/SPIN250 or MAG192/384 **is the same: 7 bacteria/mg.** ID Gene™ Spin Universal Extraction Kit have the same efficiency than MAG192/384.

4. OVERALL CONCLUSIONS:

The **ID Gene™ Spin Universal Extraction Kit** isolates total DNA or RNA from blood, body fluids, and tissues.
The advantages are:

- Quickly process multiple samples. Six samples preparations are completed in 25 minutes,
- Prevent RNA loss. Precipitation or solvent extraction steps are not required.
- Obtain concentrated RNA. RNA is eluted in a short volume (50-200µL).
- Obtain high sensitivity, reproducibility, and specificity for a large numbers of applications.

After the differents assays realized, ID Gene™ Spin Universal Extraction Kit prove these performances. It was validated thanks to the French animal's health norm (UF 47-600), on more than ten pathogens

AppendiceS

Certified management system

ID Gene™ Spin Universal Extraction Kit

Ref: SPIN50/SPIN250

50/250 extractions

Nucleic acid extraction on silica 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 columns, that is usable on all matrices and all pathogens encountered in animal health care.

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

The SPIN50/SPIN250 kits contain the following reagents:

		Volume	
Reference	***Description***	**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 H2O	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 with nuclease-free filter tips
- 1,5 ml Nuclease-free tubes
- Mini centrifuge capable of up to 11000g

Reagents :

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

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 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) for the SPIN50 et SPIN250 kits:

- Lysis buffer RAV1–Carrier RNA:

Reconstitute the lyophilised Carrier RNA with 1 ml of RAV1 buffer. Vortex and transfer this 1 ml of dissolved Carrier RNA into the tube marked RAV1. Homogenise by mixing.
The RAV1-Carrier can then be stored at +4°C for 1 month or at ≤ -16°C for longer periods. It is recommended to aliquot the RAV1-Carrier and respect 3 freeze-thaw cycles. At thawing, the buffer may have crystals. It is therefore recommended to heat the buffer to approximately + 60 ° C for improved homogenization.

b) for the SPIN50 kit:

- Wash buffer RAV3 :

Reconstitute the RAV3 buffer by adding **50 ml absolute Ethanol** to the tube. Homogenise by mixing.
The reconstituted buffer can be stored for 1 year at 21°C ± 5°C.

c) <u>for the SPIN250 kit :</u>

- <u>Wash buffer RAV3 :</u>

Reconstitute the RAV3 buffer by adding **200 ml absolute Ethanol** to the tube. Homogenise by mixing.
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**
   - **<u>150 µl*</u> 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.
   This gives **<u>the sample lysate.</u>**

3. Extract the samples and controls as described below:

1) Prepare and label as many SPIN Columns (blue columns) as there are samples and controls to analyse.
2) Transfer **660 µl of lysate onto each column**. Close the column and **centrifuge for 1 min at 8000g**. Discard the collection tube.
   <u>Keep the column and the collection tube.</u>
3) Pipette **the remainder of the lysate onto the same column** and **centrifuge for 1 min at 8000g.** Discard the collection tube.
   <u>Keep the column and replace a new collection tube.</u>

<u>Note:</u> check that all the lysate has passed through the column. If it has 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 8000g.** Discard the collection tube.
   <u>Keep the column and replace a new collection tube.</u>
5) Pipette **600 µl** (reconstituted) **RAV3 buffer** into each column. Close the column and **centrifuge for 1 min at 8000g.** Discard the collection tube.
   <u>Keep the column and replace a new collection tube.</u>
6) **Centrifuge for 3 min at 11000g**. Discard the collection tube. **<u>Keep the column.</u>**
7) Place the column in a labelled 1,5 ml tube and **add 100 µl of ELU for DNA extraction** or of **RNase-Free H2O for RNA extraction.**
   Close the column and **incubate at room temperature for 1 min**.
8) **Centrifuge for 1 min at 8000g**. Discard the column. **<u>Keep the labelled micro-tube that contains the eluate.</u>**

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

<u>Technical support and documentation</u>

For all questions or technical support, please contact us at the following address: Genetics@id-vet.com
Safety Data Sheets (MSDSs) and protocols are available on request at info@id-vet.com
For further information, visit www.id-vet.com

APPENDIX 1: Protocol for RNA extraction with the SPIN50/SPIN250 kit and analysis of samples with the ID Gene™ BVD/BD Triplex kit

Matrices: Whole blood (collected with anticoagulant), serum or ear notch sample.

This appendix outlines extraction conditions for the **ID Gene™ BVD/BD Triplex** (IDBVD) amplification kit for whole blood, serum or ear notch samples (individual samples or pools of 10 or 20).

This appendix also describes the volumes to extract for the positive controls (TPC-BVD or TPC-EN-BVD) the negative control (NTPC-BVD) and the negative extraction control (NEC) needed for the IDBVD test.
Important: Controls must be extracted at the same time as the test samples.

a) Reagent required but not provided in the kit

- Proteinase K (20 mg/ml) for the extraction of nucleic acids from samples of ear biopsies: **reference Macherey-Nagel n° 740506** available from IDvet Genetics. Refer to the manufacturer's instructions for reconstitution and storage.

b) Extraction of viral RNA from whole blood collected with anticoagulant, or serum

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

If testing pooled samples: prepare **homogeneous mixtures of whole blood or serum beforehand** (10 or 20 samples)

Reagents	*Matrices (individual or mixture)*		*Controls*	
	Whole blood	*Serum*	*TPC-BVD*	*NEC*
Sample or control	**100 µl**	**100 µl**	**50 µl of TPC-BVD**	**100 µl water or negative sample**
Lysis buffer (RAV1-Carrier)	600 µl		600 µl	
NTPC-BVD	**20 µl**		**20 µl**	

Note: Depending on the number of samples to be analysed, it is possible to make a homogenous mix of the following reagents: ***(600 µl RAV1-Carrier + 20 µl NTPC-BVD) x (number of samples and controls + 10%)***
It is recommended to prepare 10% extra volume to avoid running out of mix.
Pipette ***620 µl*** *of the reagent mix into each tube containing a sample or control.*

2. Vortex **immediately for 15 seconds.**

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

This gives **the sample lysate.**

4. Continue with paragraph 3 of the standard protocol.

c) Extraction of viral RNA from ear notch samples

Important: ear notch samples are solid samples that require enzymatic lysis by addition of Proteinase K (20 mg/ml). (reference n°740506).

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

Reagents	*Individual ear notch sample*	*Controls*		
		TPC-EN-BVD	*NEC water*	*NEC biopsy*
Sample or control	**Ear notch to analyse**	**50 µl of TPC-EN-BVD**	**50 µl of water**	**Negative ear notch**
Lysis buffer (RAV1-Carrier)	600 µl	600 µl		
Proteinase K (20 mg/ml)	**20 µl**	**20 µl**		

Note: Depending on the number of samples to be analysed, it is possible to make a homogenous mix of the following reagents: ***(600 µl RAV1-Carrier + 20 µl Proteinase K (20 mg/ml)) x (number of samples and controls + 10%)***
It is recommended to prepare 10% extra volume to avoid running out of mix.
Pipette ***620 µl*** *of the reagent mix into each tube containing a sample or control.*

1. Vortex **immediately for 15 seconds.**

2. Incubate the samples and controls for **30 min at room temperature**. Briefly centrifuge tube contents before opening.

If testing pooled samples:

1. Perform lysis for each individual sample beforehand as described above.
2. Pool samples by taking **60 µl** of each individual lysate (maximum 10).

   In the case of a pool with less than 10 samples, take an equivalent volume of each individual lysate in order to obtain a final volume of 250 µl.
3. Homogenize the pool by vortex.

3. Add **20 µl of NTPC-BVD** to each sample (individual or pool) and control (TPC-EN-BVD, NEC)

4. Add **600 µl of absolute Ethanol** (homogenize by pipetting). Rapidly centrifuge the tube before opening.

This gives **the sample lysate.**

5. Continue with paragraph 3 of the standard protocol.

APPENDIX 2: Protocol for RNA extraction with the SPIN50/SPIN250 kit and analysis of samples with the ID Gene™ Bluetongue Duplex kit

Matrices: Whole blood (collected with anticoagulant).

This appendix outlines extraction conditions for the **ID Gene™ Bluetongue Duplex** (IDBTV) amplification kit for whole blood samples.
This appendix also describes the volume to extract for positive control (TPC-BTV) needed for the IDBTV test.
Important: Controls must be extracted at the same time as the test samples.

a) Extraction of viral RNA from whole sample collected with anticoagulant

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

		Controls	
Reagents	***Whole blood***	***TPC-BTV***	***NEC***
Sample or control	**100 µl**	**50 µl**	**100 µl of water or negative sample**
Lysis buffer (RAV1-Carrier)	600 µl	600 µl	

2. Vortex **immediately for 15 seconds.**
3. Add **600 µl absolute ethanol** (homogenize by pipetting). Rapidly centrifuge the tube before opening.
   This gives **the sample lysate.**
4. Continue with paragraph 3 of the standard protocol.

APPENDIX 3: Protocol for RNA extraction with the SPIN50/SPIN250 kit and analysis of samples with the ID Gene™ Schmallenberg Duplex kit

Matrices: Whole blood (collected with anticoagulant) or serum.

This appendix outlines extraction conditions for the **ID Gene™ Schmallenberg Duplex** (IDSBV) amplification kit for whole blood or serum samples.

This appendix also describes the volume to extract for positive control (TPC-SBV) needed for the IDSBV test.

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

a) Extraction of viral RNA from whole sample collected with anticoagulant or serum

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

	Matrices		*Controls*	
Reagents	***Whole blood***	***Serum***	***TPC-SBV***	***NEC***
Sample or control	**100 µl**	**100 µl**	**50 µl**	**100 µl of water or negative sample**
Lysis buffer (RAV1-Carrier)	600 µl		600 µl	

2. Vortex **immediately for 15 seconds.**
3. Add **600 µl absolute ethanol** (homogenize by pipetting). Rapidly centrifuge the tube before opening.
   This gives **the sample lysate.**
4. Continue with paragraph 3 of the standard protocol.

APPENDIX 4: Pre-treatment of samples and DNA extraction with the SPIN50/SPIN250 kit and analysis of samples with the ID Gene™ Paratuberculosis Duplex kit

Matrices: Ruminant faeces.

This appendix outlines the pre-treatment of samples and extraction conditions for the **ID Gene™ Paratuberculosis Duplex** (IDMAP) amplification kit for ruminant faeces samples (individual samples or pools of up to 10).

This appendix also describes the preparation and extraction of target positive control (TPC-MAP), non-target positive control (NTPC-MAP) and the negative extraction control (NEC), needed for the IDMAP test

▪ Materials and reagents required but not provided in the kit

- **ID Gene™ Easy Preparation of Faeces Sample (EZPREP) kit** for simplifying pre-treatment of faeces samples

- Nuclease-free 2 ml, 15 ml and 50 ml tubes

- Zirconium or glass powder 0.1 mm

- Mixer Mill-type grinder, Precellys24 (otherwise contact us) with 2ml adapted tubes.

- It is recommended to use **LMAP lysis buffer** instead of RAV1-Carrier lysis buffer for improved extraction from faeces. Reference IDvet Genetics: LMAP1000 (1000 ml) or LMAP500 (500 ml).

▪ Pre-treatment of faeces samples and controls

Solid Faeces samples require a particular pre-treatment prior to extraction of Map DNA.

IDVet Genetics propose 2 validated methods:

- A **simplified method using the ID Gene™ Easy Preparation of Faeces Sample (EZPREP) kit**, refer to the kit instructions and continue with the step 5 of the standard method
- A **standard method**, described below:

1. Weigh **5 g** faeces in a 50 ml tube.
2. Add **30 ml** distilled water.
3. Vortex for **30 sec** and allow to settle for **10 min (± 2 min)**.
4. Take **1,5 ml** (from the 15 ml mark level) and transfer to a labelled 2 ml tube.

   For pools of 5 or 10 samples: After step 3,

   - Take **2 ml** of each homogenised sample and place in a 15 ml tube (for pools of 5 samples) or 50 ml (for pools of 10 samples).
   - Vortex the mixture for **15 sec**.
   - Take **1,5 ml** and transfer to a labelled 2 ml tube.

5. Centrifuge for **10 min at 11 300 g**.
6. Tip off the supernatant.
7. To the pellet, add zirconium powder up to the 0,25 ml mark (approx).
8. Add **700 µl LMAP buffer** and **20 µl of the NTPC-MAP** to each sample.
9. Grind for **10 min at at least 30 Hz** (1800 oscillations/minute).
10. Centrifuge for **3 min at 11 300 g**.
11. **500 µl of supernatant** are needed for the extraction (see below).

Preparation of controls: Controls should be prepared and extracted at the same time as the samples being tested.

- TPC-MAP (Target Positive Control): In a 2 ml tube, add **50 µl of TPC-MAP** and zirconium powder up to the 0,25 ml mark (approx). Continue treatment from step 8 of the classic pre-treatment method above.

- NEC (Negative amplification control):
If the NEC is prepared with a sample of faeces of known negative status, follow the classic method of pre-treatment.
If the NEC is prepared with water, follow this procedure from step 4 with 1,5 ml of water.

▪ Extraction of bacterial DNA from faeces supernatant

1. Transfer **500 µl of supernatant** for each samples and controls to a labelled 1,5 ml tube.
2. Add **500 µl absolute ethanol** (homogenize 5 times by pipetting). Rapidly centrifuge the tube before opening.
   This gives **the sample lysate.**
3. Continue with paragraph 3 of the standard protocol.

APPENDIX 5: Protocol for DNA extraction with the SPIN50/SPIN250 kit and analysis of samples with ID Gene™ avec les kits ID Gene™ for the detection of abortive diseases (IDANA, IDCHLM, IDNEO, IDTOXO, IDQF and IDQFCH)

This appendix outlines extraction conditions for the amplification kit following:

- **ID Gene™ Anaplasma phagocytophilum Duplex** (IDANA) / samples : whole blood, swab
- **ID Gene™ Chlamydophila spp Duplex** (IDCHLM) / samples : organ, swab (placental, vaginal, cervical)
- **ID Gene™ Neospora caninum Duplex** (IDNEO) / samples : brain, placenta, muscle
- **ID Gene™ Q Fever Triplex** (IDQF) / samples : swab, milk, gastric juice
- **ID Gene™ Q Fever-Chlamydophila spp Triplex** (IDQFCH) / samples : swab, spleen, milk, gastric juice
- **ID Gene™ Toxoplasma gondii Duplex** (IDTOXO) / samples: brain, placenta, heart muscle, swab.

This appendix also outlines the preparation and extraction of non- target positive control (exogenous control NTPC-QF of IDQF kit) and the negative extraction control (NEC) required for each test.

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

a) Reagent required but not provided in the kit

- Proteinase K (20 mg/ml) for the extraction of nucleic acids from samples: **reference Macherey-Nagel n° 740506** available from IDvet Genetics. Refer to the manufacturer's instructions for reconstitution and storage.

b) Extraction of DNA from samples

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

Reagents	*Matrices*		*Controls*
	Liquid samples	***Solid samples***	***NEC***
Sample or control	**100 µl**	**20 mg**	**100 µl water or negative sample**
Lysis buffer (RAV1-Carrier)	600 µl		600 µl
Proteinase K (20 mg/ml)	**20 µl**		**20 µl**
NTPC (for the IDQF kit which contains an exogenous control)	**20 µl**		**20 µl**

Note: Depending on the number of samples to be analysed, it is possible to make a homogenous mix of the following reagents: ***x (number of samples and controls + 10%)***
It is recommended to prepare 10% extra volume to avoid running out of mix.
Pipette ***620 µl*** *of the reagent mix into each tube containing a sample or control.*

or

(600 µl RAV1-Carrier + 20 µl Proteinase K (20 mg/ml) + 20 µl de NTPC) x (nombre d'échantillons et témoins à analyser + 10% de volume mort). *Pipette* ***640 µl*** *of the reagent mix into each tube containing a sample or control.*

2. Vortex **immediately for 15 seconds.**

3. Incubate the samples and controls for **30 min at room temperature**. Briefly centrifuge tube contents before opening.

4. Add **600 µl absolute ethanol** (homogenize by pipetting). Briefly centrifuge the tube before opening.
This gives **the sample lysate.**

5. Continue with paragraph 3 of the standard protocol.