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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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History of revisions

Version	Edit date	Reference	Type of revision	Revision made
0419	12/2020	DOC2641	Technical modification: Update of the document following technical modification of the kit And Update: Addition/ Modification of of protocols	• Added a History of revisions table. • Modification of the extraction volume of positive controls from 50µl to 100µl, to homogenize the extaction protocol. • Removal of reference IDBTV8.
	02/2020	DOC2222	Update: Addition/ Modification of of protocols	• New pre-treatment protocols available upon request (Appendix 10).
	09/2019	DOC1946	Update: Addition/ Modification of of protocols.	• Following addition of oropharyngeal fluid matrix to IDASF TPC lyophilized mastermix protocol, addition of the matrix in pre-treatment protocol ASF.
	08/2019	DOC1888	Update: Addition/ Modification of of protocols	• Addition of oropharyngeal fluid matrix to ASF-SPIN pre-treatment protocol (Appendix 10).
	06/2019	DOC1807	Update : Addition/ Modification of of protocols	• Addition of ASF-SPIN pre-treatment protocol (Appendix 10).
	06/2019	DOC1711	Technical modification: Update of the document following technical modification of the kit And Update: Addition/modification of protocols	• Change in the rotation speed for centrifugations during the extraction step (11 000 g instead of 8 000 g) • Improved protocol for extraction of BVDV nucleic acids from organs (Appendix 1) • Addition of BRU-SPIN pre-treatment protocol (Appendix 10)
1118	01/2019	DOC1558	Update : Addition/ Modification of of protocols	• Improvement of the table for viral extraction to better understand the volume of lysis buffer to be added to the TPC-PPR + NEC controls (Appendix 9)
	12/2018	DOC1469	Technical modification: Update of the document following technical modification of the kit Update : Addition/ Modification of of protocols	• Change in the names and labelling of the buffers • Clarification of the pre-treatment protocol for samples and controls for MAP DNA extraction (Appendix4)

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

MATRICES: WHOLE BLOOD (COLLECTED WITH ANTICOAGULANT), SERUM, MILK, EAR NOTCH SAMPLES OR ORGANS.

December 2020

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

April 2019

- Improved RNA extraction protocol from organs

November 2018

- Changed the names and labelling of the buffers

This appendix outlines extraction conditions for the **ID Gene™ BVD/BD Triplex** (IDBVD) amplification kit for whole blood, serum, ear notch samples or organs (individual samples or pools of up to 100).

This appendix also describes the volumes to extract for the positive controls (TPC-BVD or TPC-EN-BVD) non-target positive control (NTPC-BVD) and negative extraction control (NEC) needed for the IDBVD test.

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

a) Reagents required but not provided in the kit

1. For the extraction of RNA from ear notch samples and organs: Proteinase K (available for purchase at Innovative Diagnostics, **product code: Proteinase K**).

b) Extraction of viral RNA from whole blood, serum or milk

1. Prepare samples and controls as described below.
If testing pooled samples: prepare **homogeneous mixtures of whole blood or serum** (up to 100 samples).

Reagents	*Matrices (individual or pool)*			*Controls*	
	Whole blood	*Serum*	*Milk*	*TPC-BVD*	*NEC*
Sample or control	**100 µl**			**100 µl**	**100 µl water or negative sample**
LYS-CARRIER-SPIN	600 µl			600 µl	
NTPC-BVD	**5 µl**			**5 µl**	

Note: To simplify the distribution of the reagents, it is possible to prepare a homogenous mixture:
(600 µl LYS-CARRIER-SPIN + 5 µl NTPC-BVD) x (number of samples and controls + 10% extra volume)
*Distribute **605 µl** of the mix in each tube containing 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 **step 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.

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

Reagents	*Individual ear notch*	*Controls*		
		TPC-EN-BVD	*NEC (water)*	*NEC (ear notch)*
Sample or control	**Ear notch**	**100 µl**	**100 µl**	**Negative ear notch**
LYS-CARRIER-SPIN	600 µl	600 µl		
Proteinase K (20 mg/ml)	**20 µl**	**20 µl**		

Note: To simplify the distribution of the reagents, it is possible to prepare a homogenous mixture:
(600 µl LYS-CARRIER-SPIN + 20 µl PK) x (number of samples and controls + 10% extra volume)
*Distribute **620 µl** of the mix in each tube containing sample or control.*

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

3. Incubate samples and controls between **5 and 30 minutes at 21°C (± 5°C)**. Quickly centrifuge the tube before opening.

If testing pooled samples:

1. Perform lysis for each individual sample as described above.
2. Pool samples by collect **60 µl** of each individual lysate (maximum 25).
   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 vortexing.**

4. Add **5 µl of NTPC-BVD** to each sample (individual or pool) and control (TPC-EN-BVD, NEC)
5. Add **600 µl of absolute Ethanol** (homogenize by pipetting). Rapidly centrifuge the tube before opening.
   This gives **the sample lysate.**
6. Keep the lysates at +5°C (± 3°C) if the PCR is to be performed within 24 hours.
7. Continue with **step 3** of the extraction protocol.

d) Extraction of viral RNA from organs (spleen)

1. Weigh **20 mg** of dissected organ in a 2 ml tube.
2. Chop them finely with a sterile single use scalpel
3. Prepare the controls:
   For TPC-BVD, follow the below protocol with **100 µl of TPC.**
   For the NEC, follow the below protocol with 100 µl of RNase free water or negative matrix (known status)
4. Add in each sample and control:
   - 600 µl of LYS-CARRIER-SPIN
   - 5 µl of NTPC-BVD
   - 20 µl of proteinase K
5. Incubate 30 minutes at 70°C (± 5°C).
6. Make a rapid centrifugation
7. Prepare 600µL of the organ supernatant and controls for extraction.
8. Add **600 µl of absolute Ethanol** (homogenize by pipetting). Rapidly centrifuge the tube before opening.
   The solution obtained is **the lysate sample.**
9. Continue with step 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 (INDIVIDUAL OR POOL UP TO 10) OR ORGAN (I.E. SPLEEN)

December 2020

- Modification of the extraction volume of positive controls from 50µl to 100µl, to homogenize the extraction protocol.
- Removal of reference IDBTV8

This appendix outlines extraction conditions for the **ID Gene™ Bluetongue Duplex** (IDBTV) amplification kit for whole blood sample (individual or pool of up to 10) or organ (*i.e.* spleen).

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) Reagent required but not provided in the kit for the preparation of organs:

- 1X PBS (molecular biology quality),
- Nuclease-free 2 ml tubes,
- glass beads 3mm,
- Mixer Mill-type grinder, Precellys24, FastPrep (otherwise contact us) with 2ml adapted tubes.

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

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

   If testing pooled samples: Prepare **homogeneous mixtures of whole blood** (up to 10 samples)

Reagents	*Whole blood*	*Controls*	
		TPC-BTV	*NEC*
Sample or control	**100 µl**	**100 µl**	**100 µl of water or negative sample**
Lysis buffer (LYS-CARRIER-SPIN)	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.
   The solution obtained is **the lysate sample.**
4. Continue with **step 3** of the standard protocol.

c) Extraction of viral RNA from organs (i.e. spleen)

1. In a 2 ml tube, weigh 20 mg organ previously dissected.
2. Add 1 ml of 1X PBS.
3. Add 1 to 3 glass beads and well homogenize by vortex.
4. If possible, grind:
   - Precellys® 24/ Precess™ 24: for 40 seconds at 6,000 rpm
   - FastPrep™: for 45 seconds at 6 M/s
   - Mixer Mill / Tissue Lyse: for 2 minutes at 30 Hz.
5. Centrifuge **during 2 minutes at 1,500 g**.
6. **100 µl of supernatant** are needed for the extraction.
7. Prepare the organ supernatant and controls to extract as described below:

Reagents	*Organ supernatant*	*Controls*	
		TPC-BTV	*NEC*
Sample or control	**100 µl**	**100 µl**	**100 µl of water or negative sample**
Lysis buffer (LYS-CARRIER-SPIN)	600 µl	600 µl	

8. Vortex **immediately for 15 seconds.**
9. Add **600 µl of absolute Ethanol** (homogenize by pipetting). Rapidly centrifuge the tube before opening.
   The solution obtained is **the lysate sample.**
10. Continue with **step 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), SERUM OR BRAIN TISSUE.

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™ Schmallenberg Duplex** (IDSBV) amplification kit for whole blood, serum or brain tissue.

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) Reagent required but not provided in the kit

For the preparation of tissue: 1X PBS (molecular biology quality), Nuclease-free 2 ml tubes, glass beads 3mm, Mixer Mill-type grinder, Precellys®24, FastPrep® (otherwise contact us) with 2ml adapted tubes

b) 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**	**100 µl**	**100 µl of water or negative sample**
Lysis buffer (LYS-CARRIER-SPIN)	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.
   The solution obtained is **the lysate sample.**
4. Continue with **step 3** of the standard protocol.

c) Extraction of viral RNA from brain tissue

1. Finely dissect the brain tissue sample in a Petri dish using sterile scalpel and tweezers.
2. Weigh **100 mg** of tissue in a 2 ml microtube.
3. Add **1 ml** of 1X PBS.
4. Add 2 glass beads and vortex.
5. Grind with :
   - Precellys® 24/ Precess™ 24: for 40 seconds at 6,000 rpm
   - FastPrep®: for 45 seconds at 6 M/s
   - Mixer Mill / TissueLyser: for 2 minutes at 30 Hz.
6. Centrifuge for **2 minutes at 6,000 g**.
7. **100 µl of supernatant** are needed for the extraction.
8. Prepare tissue lysates and controls for extraction as described below:

		Controls	
Reagents	*Tissue supernatant*	*TPC-SBV*	*NEC*
Sample or control	**100 µl**	**100 µl**	**100 µl of water or negative sample**
Lysis buffer (LYS-CARRIER-SPIN)	600 µl	600 µl	

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

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

MATRICES: RUMINANT FAECES, BOOT SWABS, MYCOBACTERIAL CULTURE MEDIA.

December 2020

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

November 2018

- Clarification of the pre-treatment protocol for samples and controls.

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), boot swabs and mycobacterial culture.

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), required for the IDMAP test

a) 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, Precellys®24, FastPrep® (if using other devices please contact info@innovative-diagnostics.com) with 2ml adapted tubes.
- It is recommended to use **LMAP lysis buffer** instead of LYS-FAST buffer for improved MAP extraction. Reference Innovative Diagnostics: LMAP50 (50 ml) or LMAP1000 (1000 ml).
- Specifically, for the analysis of faeces sampling with boot swabs: Laboratory Mixer, among the following models or equivalent: Stomacher™80 Mark 2 or MiniMix 100 mL.

b) Pre-treatment of faeces samples and controls

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

Innovative Diagnostics offers 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 **step 5** of the classic method described below.
- A **classic method**, described below:

	Faeces	Faeces sampling with boot swabs:	Mycobacterial cultures
Step 1: Pre-treatment of samples	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. Pipette **1.5 ml** and transfer to a pre-labelled 2 ml tube. (**if EZPREP used continue from this step).** ***For pools of 5 or 10 samples:*** After step 3 above: • Collect **2 ml of each homogenised sample** individually and place in a 15 ml tube (for pools of up to 5 samples) or 50 ml (for pools of up to 10 samples). • Vortex the mixture of samples for **15 sec**. • Pipette **1.5 ml** and transfer to a pre-labelled 2 ml tube. 5. Centrifuge for **10 min at 11,300 g**. 6. Discard the supernatant.	1. After sampling, boot swabs are washed in a Lab Stomacher blender with **50 ml of 0.9 % of NaCl solution**. 2. **Transfer the solution** in a 50 ml tube and allow to settle for **10 min** (± 2 min). 3. Collect **1.5 ml of the solution** and transfer in a pre-labelled 2 ml tube. 4. Centrifuge for **10 min at 11,300 g.** 5. Discard the supernatant.	1. Homogenize the samples as follows: • Thoroughly **vortex the culture broth "TREK"** or, • resuspend **2 to 3 colonies in 500 µl of sterile water** and vortex vigorously.
Step 2: Preparation of controls	**Controls should be prepared and extracted at the same time as the samples to be tested.** **- TPC-MAP (Target Positive Control)**: In a 2 ml tube, add **100 µl of TPC-MAP and continue with Step 3 below**. **- NEC (Negative amplification control):** If the NEC is prepared with a sample of known negative status, follow the classic method of pre-treatment described above. If the NEC is prepared with water: In a 2 ml tube, add **500 µl of water and continue with Step 3 below**.		
Step 3: Sample preparation	1. To the pellet, **add zirconium powder** up to the 0.25 ml level mark (approx.). 2. **Add 700 µl LMAP** buffer and **20 µl of the NTPC-MAP** to each sample. 3. **Grind** using one of the device below: - Precellys ® 24/PRECESS 24: 6,800 rpm for 2 x 90 sec. - FastPrep®: 6.5 M/s for 4x45 sec. - Mixer/Mill®/TissueLyser Mixer: 30 Hz for 10 min. 4. **Centrifuge** for **3 min at 11,300g**. 5. **500 µl of supernatant** will be required for extraction.	1. To the pellet, **add zirconium powder** up to the 0.25 ml level mark (approx.). 2. **Add 700 µl LMAP** buffer and **20 µl of the NTPC-MAP** to each sample. 3. **Grind** using one of the device below: - Precellys ® 24/PRECESS 24: 6,800 rpm for 2 x 90 sec. - FastPrep®: 6.5 M/s for 4x45 sec. - Mixer/Mill®/TissueLyser Mixer: 30 Hz for 10 min. 4. **Centrifuge** for **3 min at 11,300g**. 5. **500 µl of supernatant** will be required for extraction.	1. In a pre-labelled 2 ml tube **add zirconium powder** up to the 0.25 ml level mark (approx.). 2. Add **500 µl** of TREK broth or **500 µl** of bacterial suspension to be tested. 3. Add **300 µl** of **LMAP buffer** and **20 µl of the NTPC-MAP** to each sample. 4. **Grind** using one of the device below: - Precellys ® 24/PRECESS 24: 6,800 rpm for 2 x 90 sec. - FastPrep®: 6.5 M/s for 4x45 sec. - Mixer/Mill®/TissueLyser Mixer: 30 Hz for 10 min. 5. **Centrifuge** for **3 min at 11,300g**. 6. **500 µl of supernatant** will be required for extraction.
Step 4 : Lysate obtention	In each tube containing **500 µl of supernatant (either from each sample, or from TPC-MAP, or from NEC)** add **500 µl of absolute ethanol**. Homogenize 5 times by pipetting up and down. Rapidly centrifuge the tube before opening. The sample thus obtained is **the lysate.** Continue with **step 3** of the standard protocol.		

APPENDIX 5: Protocol for DNA extraction with the SPIN50/SPIN250 kit and analysis of samples with the ID Gene™ African Swine Fever Duplex kit

MATRICES: WHOLE BLOOD, SERUM OR TISSUE HOMOGENATES (SPLEEN, LYMPH NODES, TONSIL, BONE MARROW AND KIDNEY).

INDIVIDUAL SAMPLES OR POOLS UP TO 20 SAMPLES.

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™ African Swine Fever Duplex** (IDASF) amplification kit for whole blood or tissue homogenates samples (individual samples or pools up to 20 samples).

This appendix also describes the volume to extract for positive control (TPC-ASF) needed for the IDASF test

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

a) Reagent required but not provided in the kit

For the preparation of organs: 1X PBS (molecular biology quality), Nuclease-free 2 ml tubes, glass beads 3mm, Mixer Mill-type grinder, Precellys®24, FastPrep (otherwise contact us) with 2ml adapted tubes

b) Extraction of viral DNA from whole sample collected with anticoagulant

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

   If testing pooled samples: prepare **homogeneous mixtures of whole blood or serum** (up to 20 samples).

	Matrices		*Controls*	
Reagents	***Whole blood***	***Serum***	***TPC-ASF***	***NEC***
Sample or control	**100 µl**		**100 µl**	**100 µl of water or negative sample**
Lysis buffer (LYS-CARRIER-SPIN)	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.
   The solution obtained is **the lysate sample.**
4. Continue with **step 3** of the standard protocol.

c) Extraction of viral DNA from organs

1. Weigh **20 mg** of dissected organ in a 2 ml tube.
2. Add **1 ml** 1X PBS.
3. Add 1 to 3 glass beads and vortex.
4. If possible, grind the mixture:

   - Precellys™ 24/ Precess™ 24: for 40 sec at 6000 rpm.
   - FastPrep™: for 45 sec at 6 M/s.
   - Mixer Mill™ / Tissue Lyser™: for 2 min at 30 Hz.

5. Centrifuge for **2 min at 1500 g**.
6. **100 µl of organ supernatant** are needed for the extraction.

	Matrix	*Controls*	
Reagents	***Organ supernatant***	***TPC-ASF***	***NEC***
Sample or control	**100 µl**	**100 µl**	**100 µl of water or negative sample**
Lysis buffer (LYS-CARRIER-SPIN)	600 µl	600 µl	

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

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d) Extraction of viral DNA from oropharyngeal fluids

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

	Matrices	*Controls*	
Reagents	***Oropharyngeal fluids***	***TPC-ASF***	***NEC***
Sample or control	**200 µl**	**100 µl**	**200 µl of water or negative sample**
Lysis buffer (LYS-CARRIER-SPIN)	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.
   The solution obtained is **the lysate sample.**
4. Continue with **step 3** of the standard protocol.

APPENDIX 6 : Pre-treatment of samples and DNA extraction with the SPIN50/250 kit for the analysis of samples with the ID Gene™ kits for the detection of abortive diseases (IDCHLM , IDQFCH, IDQF, IDNEO, IDANA, IDTOXO and IDCAMP)

December 2020

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

This appendix describes the pre-treatment of samples and specific DNA extraction, from samples that can be analysed with the PCR kits below:

- **ID Gene™ Chlamydophila spp Duplex** (IDCHLM)
- **ID Gene™ Q Fever Triplex** (IDQF)
- **ID Gene™ Q Fever-Chlamydophila spp Triplex** (IDQFCH)
- **ID Gene™ Neospora caninum Duplex** (IDNEO)
- **ID Gene™ Anaplasma phagocytophilum Duplex** (IDANA)
- **ID Gene™ Toxoplasma gondii Duplex** (IDTOXO)
- **ID Gene™ Campylobacter fetus Duplex** (IDCAMP)

This appendix also describes the preparation and extraction of target positive control (TPC-QF, TPC-CHLM, TPC-QFCH, TPC-NEO, TPC-TOXO, TPC-CAMP, TPC-ANA), non-target positive control (NTPC-QF) and the negative extraction control (NEC), needed for the abortive diseases tests.

The table below summarizes the different matrices that can be analyzed with the detection kits presented:

			Matrice type						
	Pathogen wanted	**Product reference**	Whole blood	Swabs	Tissues/ organs	Stomach juice	Faeces	Milk	Preputial washing
Abortive diseases	*Chlamydophila spp*	**IDCHLM / IDQFCH**		x	x				
	Coxiella burnetii	**IDQF / IDQFCH**		x	x	x	x	x	
	Neospora caninum	**IDNEO**			x				
	Anaplasma phagocytophilum	**IDANA**	x	x	x				
	Toxoplasma gondii	**IDTOXO**		x	x				
	Campylobacter fetus	**IDCAMP**		x					x

a) Reagent required but not provided in the kit :

- 1X PBS (molecular biology quality)
- Nuclease-free water
- Proteinase K **reference Macherey-Nagel n°740506** available from Innovative Diagnostics. Refer to the manufacturer's instructions for reconstitution and storage.
- Precision Scale
- Vortex
- A centrifuge for microtubes 1.5 ml and 2 ml up to 11300g
- Nuclease-free 2 ml, 15 ml and 50 ml tubes
- Zirconium or glass powder 0.1 mm or glass beads 3mm
- For pre-treatment of faeces matrix for *Coxiella burnetii* detection **(IDQF)** : **Simplified method using the ID Gene™ Easy Preparation of Faeces Sample (EZPREP) kit**

Especially for IDQF:

- A **simplified method using the ID Gene™ Easy Preparation of Faeces Sample (EZPREP) kit**

b) Preparation and pre-treatment of controls and samples

The pre-treatment and preparation of the sample to be tested depend on the sample type collected. Please follow the instructions described in the table below related to the sample type used:

	Whole blood	Swabs, milk, stomach juice, vaginal mucus	Tissue (brain/ muscle / placenta / spleen)	Preputial washing	Faeces
Step 1 : Preparation of samples	**1.** In a 2 ml tube, pipette 500 µl of blood sample collected in EDTA tube. **2.** Add 1 ml of nuclease free water and vortex. **3.** Centrifuge at 11300g for 5min. **4.** Discard the supernatant	**For swabs** (placental, vaginal, endocervical) : Express the swab in 1 ml of 1X PBS (pH 7.4) by vortexing for 30sec For Q Fever, it is possible to perform analysis of pools of up to 3 swabs from different animals. Perform volume-to-volume mixing from 3 individual eluates. The extraction will be performed with **100 µl** of the pool. **The other fluid samples** (milk, vaginal mucus, fetal fluid, stomach juice) can be used directly **1.** Collect **100 µl** of liquid sample	**1**. In a 2 ml tube, weigh 20 mg of solid sample, previously dissected, using a precision scale **2.** Add 1 ml of 1X PBS (pH 7.4) **3a.** Add zirconium powder up to the 0.25 ml mark (approx) 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 **Otherwise,** **3b.** Add 1 to 3 glass beads and vortex thoroughly **4.** Centrifuge at 1500 g for 2 min **5.** Collect **100 µl** of supernatant	**1.** Centrifuge the entire sample for 20 min at 4500g **2.** Discard the supernatant **3.** Resuspend the pellet in 500 µl of 1X PBS **4.** Vortex and collect **100 µl** of the sample	Innovative Diagnostics offers two validated methods: **A simplified method** using the ID Gene™ Easy Preparation of Faeces Sample (EZPREP) kit, refer to the kit instructions. **A standard method**, described below : **1.** Weigh 5g faeces in a 50 ml tube. **2.** Add 30 ml of distilled water. **3.** Vortex for 30 sec and leave the sample to settle for 10 min (± 2 min) **4.** In a labelled 2ml tube, pipette 1.5ml or press the EZ-DROP up to mark level 1.5 ml) **5.** Centrifuge at 11300g for 10min. **6.** Discard the supernatant by inversion
Step 2 : Preparation of controls	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-QF, TPC-NEO, TPC-CHLM, TPC-QFCH, TPC-CAMP, TPC-TOXO and TPC-ANA follow the protocol described in **step 3** with **100 µl of TPC** - NEC (Negative extraction control): If the NEC is prepared with a sample known negative status, prepared the NEC as described in **step1** of the protocol above. If the NEC is prepared with water continue treatment from **step 3 with 100 µl** of water nuclease-free				
Step 3 : Pre-treatment of samples	**5.** Add : - 600 µl of lysis buffer LYS-CARRIER-SPIN - 20µl of proteinase K (2) **6.** Vortex and centrifuge briefly **7.** Incubate 30 min at 70°C (± 5°C) **8.** The whole cell lysate will be needed for extraction	**2.** Add : - 600 µl of lysis buffer LYS-CARRIER-SPIN - 20 µl of NTPC-QF (1) - 20 µl of proteinase K (2) **3**. Vortex and centrifuge briefly **4.** Incubate 30 min at 70°C (± 5°C) **5.** The whole cell lysate will be needed for extraction	**6.** Add : - 600 µl of lysis buffer LYS-CARRIER-SPIN - 20 µl of NTPC-QF (1) - 20 µl of proteinase K (2) **7.** Vortex and centrifuge briefly **8.** Incubate 30 min at 70°C (± 5°C) **9.** The whole cell lysate will be needed for extraction	**5.** Add : - 600 µl of lysis buffer LYS-CARRIER-SPIN - 20 µl de proteinase K(2) **6.** Vortex and centrifuge briefly **7.** Incubate 30 min at 70°C (± 5°C) **8.** The whole cell lysate will be needed for extraction	**7.** Resuspend the pellet with: - 600 µl of lysis buffer LYS-CARRIER-SPIN - 20 µl of NTPC-QF(1) - 20 µl of proteinase K(2) **8.** Vortex and centrifuge briefly **9.** Incubate 30 min at 70°C (± 5°C) **10.** Centrifuge at 11,300g for 3 min **11.** The whole supernatant will be needed for extraction.

(1) : only for IDQF kit
(2) : Depending on the number of samples to be tested, it is possible to make a homogenous mix of the following reagents :
For IDQF : (600 µl of LYS-CARRIER-SPIN + 20 µl of Proteinase K + 20 µl of NTPC-QF) x (number of samples and controls + 10% extra volume) 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.
For the other abortive disease kit : (600 µl of LYS-CARRIER-SPIN + 20 µl de Proteinase K) x (number of samples and controls + 10% extra volume). Pipette 620 µl of the reagent mix into each tube containing a sample or control.

c) Extraction of DNA from samples and controls

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

Reagents	***Matrices***		***Controls***		
			TPC	***NEC water***	***NEC negative matrix***
Samples or controls	**Complete lysate**	**500µL of faeces supernatant**	**Complete lysate from 100 µl of TPC**	**Complete lysate from 100 µl of water**	**Complete lysate from 100 µl of negative matrix**
Absolute ethanol	600µl	500µl	600µl		

2. Homogenize by pipetting and rapidly centrifuge the tube before opening.
3. Continue with **step 3** of the standard protocol.

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.*

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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.

APPENDIX 8 : Pre-treatment of samples and DNA extraction using the SPIN50-250 kit for the analysis with ID Gene™ MG/MS Triplex amplification kit

MATRICES: TRACHEAL OR OROPHARYNGEAL SWABS, FTA® CARDS (INDIVIDUAL OR POOLS OF UP TO 3)

This appendix describes the pre-treatment of samples and specific DNA extraction of *Mycoplasma gallisepticum and Mycoplasma synoviae*, from tracheal or oropharyngeal swabs and FTA® cards that can be analysed with the **ID Gene™ MG/MS Triplex** (IDMGMS) kit.

This appendix also describes the volumes to extract for target positive control (TPC-MGMS), and negative control (NEC) needed for the IDMGMS test.

Reagent required but not provided in the kit:

- 1X PBS (molecular biology quality)
- Nuclease-free water
- Vortex
- Mini spin for micro tests tubes (volume of 1.5 ml and 2 ml)
- Nuclease-free tubes of 2 ml, 15 ml and 50 ml

1. Sample preparation

For tracheal or oropharyngeal swabs

Note: It is best to treat individually heavily soiled autopsy swabs.

For dry swabs:

- Resuspend individually each swab in 1ml of 1X PBS into an 15ml tube (or hemolysis tube or equivalent)
- Cut off by scissors or snapped off at the break point of the cotton bud from 1 to 3 swabs.
- Place the swabs into the 15 ml (or hemolysis tube).
- Vortex for 30 seconds. Collect 100 µl of the 1X PBS supernatant for the extraction, store the volume left at -20°C.

Note: Dry swabs should be transported at 5 ° C (± 3°C) within 24 hours after collection. It is not recommended to freeze dry swabs. They must be resuspended in PBS before storage at -20 ° C.

For FTA® cards

For individual samples

- Collect 3 sample discs from each sample spot of the FTA® card using a coring device (a 1.6 to 2 mm disc is recommended).

For pooled samples (up to 3)

- Collect 1 sample disc from each sample spot from each FTA® card using a coring device (a 1.6 to 2 mm disc is recommended).

  Pool up to 3 discs (1 disc per sample to be tested).

- Place the discs in a tube containing 1ml of 1XPBS
- Vortex for 30 seconds.
- Collect 100 µl of the supernatant for the extraction, store the volume left t at -20°C.

2. Control preparation

The negative and positive controls should be prepared and extracted at the same time as the samples being tested.

- Add 550 µl of Nuclease-free water to the freeze-dried TPC-MGMS. 100 µl will be necessary for the extraction.
- For the NEC (Negative extraction control):
  - If the NEC is prepared with a sample of known negative status, follow the classic method of pre-treatment.
  - If the NEC is prepared with water, follow this procedure with 100 µl of Nuclease-free water.

3. Continue with the step 1 of the standard extraction protocol

APPENDIX 9: 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.

December 2020

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

Janvier 2019

- Improvement of table for viral extraction to better understand the volume of lysis buffer to be added to the TPC-PPR + NEC controls.

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:**

1. In a 2 ml tube, weigh **20 mg** organ previously dissected.
2. Add 1 ml of 1X PBS.
3. Add 1 to 3 glass beads and homogenize thoroughly by vortexing.
   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.
4. Centrifuge **for 2 minutes at 1500 g**.
5. **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**	**100 µl**	**100 µl of water or negative sample**
Lysis buffer (LYS-CARRIER-SPIN)	600 µl	600µl	600µl	600 µl	600 µl

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

APPENDIX 10: Protocols for pre-treatment of samples/controls and nucleic acids extraction with the SPIN50/SPIN250 kit for analysis using other kits from the ID Gene™ qPCR product range.

The following pre-treatment and nucleic acid extraction protocols for the ID Gene™ Spin Universal Extraction Kit (SPIN50/SPIN250) to be used with ID Gene™ qPCR amplification kits are available upon request at info@innovative-diagnostics.com:

Pathogen	Species	Sample type	qPCR amplification kit	Reference of the pre-treatment and nucleic acids extraction protocols	Version	Modification done
African Swine Fever Virus (ASFV)	Swine, wild pig or warthog	• **Serum and whole blood** (individual samples or pools of up to 20**)** • **Oropharyngeal fluid** (individual samples or pools) • **Organ** (spleen, lymph node, tonsil, bone marrow and kidney, individual samples or pools of up to 20)	**ID Gene™ African Swine Fever Duplex kit** Product code: IDASF	**ASF-SPIN**	0619	For IDASF qPCR kit provided with a Positive Amplification Control (PAC) instead of a Target Positive Control (TPC). Kit now validated for oropharyngeal fluid
Brucella spp.	Ruminants	• **Placental, vaginal, cervical swabs** • **Milks** • **Organs and tissues**	**ID Gene™ Brucella spp Triplex kit** Product code: IDBRU	**BRU-SPIN**	0319	N/A, IDBRU qPCR kit now available
Influenza A virus: G1-like lineages of the H9 subtype	Birds	• **Swabs** (tracheal, oropharyngeal and cloacal swabs; Individual samples or pools of up to 5) • **Organ** (trachea, lung, and cecal tonsils; Individual samples or pools of up to 5) • **Nucleic acid storage card** (individual samples or pools of up to 5)	**ID Gene™ Influenza H9 Lineage G1-like Duplex kit** Product code: IDFLUH9G1	**FLUH9G1-SPIN**	1119	N/A, IDFLUH9G1 RT-qPCR kit now available
***Mycobacterium tuberculosis complex* (MTC)** *M. tuberculosis, M. bovis, M. africanum, M. microti, M.canetti, M. caprae, and M. pinnipedii*	Cattle, sheep, goats, deers, suids and badgers	• **Lymph nodes and peripheral tissues**	**ID Gene™ Mycobacterium tuberculosis complex Duplex kit** Product code: IDTUB	**TUB-SPIN**	0519	N/A, IDTUB qPCR kit now available

Pathogen	Species	Sample type	qPCR amplification kit	Reference of the pre-treatment and nucleic acids extraction protocols	Version	Modification done
Newcastle Disease Virus (NDV)	Birds	• **Swabs** (tracheal, oropharyngeal and cloacal swabs; Individual samples or pools of up to 5) • **Organ** (trachea, lung, spleen, liver, brain and cecal tonsils; Individual samples or pools of up to 5) • **Nucleic acid storage card** (individual samples or pools of up to 5)	**ID Gene™ Newcastle Disease Duplex kit** Product code: IDNDV	**NDV-SPIN**	1019	N/A, IDNDV RT-qPCR kit now available
Porcine Reproductive and Respiratory Syndrome Virus (PRRSV)	Swine	• **Whole blood, serum, cell culture supernatant** • **Organ or tissue** • **Bronchial swab** • **Oropharyngeal fluid**	**ID Gene™ PRRSV Triplex kit** Product code: IDPRRSV	**PRRSV-SPIN**	0819	N/A, IDPRRSV RT-qPCR kit now available
Influenza A virus	Birds	• **Swab** (tracheal, oropharyngeal and cloacal; Individual samples or pools of up to 5) • **Organ** (trachea, lung, spleen, liver; individual samples or pools of up to 5) • **Nucleic acid storage card** (individual samples or pools of up to 5)	**ID Gene™ Influenza A Duplex** Product code: (IDFLUA)	**FLUA-SPIN**	1219	N/A, IDFLUA RT-qPCR kit now available
Mycoplasma gallisepticum* (MG) and *Mycoplasma synoviae (MS)	Birds	• **Swabs** (tracheal, oropharyngeal. Individual samples or pools of up to 3) • **Nucleic acid storage card** (individual samples or pools of up to 3)	**ID Gene™ MG/MS TRIPLEX** Product code: IDMGMS	**MGMS-SPIN**	0120	N/A, IDMGMS RT-qPCR kit now available
Avian coronavirus, including all variants of Infectious Bronchitis Virus (IBV) and coronaviruses isolated from turkey, pheasant, and pigeon	Chicken, turkey, pheasant, pigeon	• **Swab** (tracheal, oropharyngeal and cloacal; Individual samples or pool of up to 5) • **Organ** (trachea, lung, kidney, cecal tonsils; Individual samples or pools of up to 5) • **Nucleic acid storage card** (individual samples or pool of up to 5)	**ID Gene™ Infectious Bronchitis Duplex** Product code: IDIBV	**IBV-SPIN**	0920	N/A, IDIBV RT-qPCR kit now available