

## Protocols for KingFisher instruments

### Using extraction kit

#### "ADIAMAG"

Ref. NADI003 (200 extractions)

Ref. NADI003-XL (800 extractions)

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## Main change since previous version

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N/A	Not Applicable (first publication)
Correction	Correction of document anomalies
Technical change	Addition, revision and/or removal of information related to the product
Administrative	Implementation of non-technical changes noticeable to the user
Note: minor typographical, grammar and formatting changes are not included in the revision historic	

Release Date	Part Number	Change type	Change summary
2018/02	NEKF-17	Technical change	Modification of Influenza virus protocol with swab samples
2018/06	NEKF-18	Technical change	Addition of table §II-1 Addition of protocol for African Swine Fever virus (ASFV) detection Remove of "storage of sample"
2019/05	NEKF-19	Administrative	Modifications of references and name kit, buffers and the name of automate programs
2019/05	NEKF-19	Technical change	Addition of IHVN and VHSV protocols
2019/12	NEKF-20	Technical change	Modification of <i>Chlamydia abortus</i> by <i>Chlamydia spp.</i> Addition of protocols for: feline coronavirus (FIP), <i>Besnoitia besnoiti</i> , avian mycoplasmas and <i>Ornithobacterium rhinotracheale</i> . Modification of <i>A. phagocytophilum</i> protocol for blood of ruminant.
2021/01	NEKF-21	Technical change	Addition of protocols for <i>Leptospira</i> from water and urine Addition of IPNV protocols
2021/01	NEKF-21	Administrative	Modification of email by <a href="mailto:biox@biox.com">biox@biox.com</a> Reference NADI003-XL ADIAMAG XL (800R) added
2021/04	NEKF-22	Technical change	Addition of protocols for PRV from blood, swab and tissus. Addition of protocols for <i>Besnoitia besnoiti</i> from skin biopsy

## I. General information

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### 1. Purpose of the kit

ADIAMAG is a nucleic acids DNA and RNA extraction kit based on nucleic acids adsorption on magnetic beads. The kit is adapted to KingFisher™ mL, DUO and 96/Flex instruments. The kit contains all the buffers required to extract the DNA/RNA from different matrices.

After samples lysis (performed separately from the instrument and using appropriate buffers), the supernatants can be handled with the same automated purification process using KingFisher™ mL, KingFisher DUO or KingFisher™ 96/Flex instruments, irrespective of the pathogen and matrix used. The following table describes the protocol used with the different instruments.

Well/plate	Description
1	Nucleic acids release and binding to magnetic beads. Beads collection by magnetic rods and transfer into the step 2.
2	Beads release. W3 buffer washing step. Beads collection by magnetic rods and transfer into the step 3.
3	Beads release. W4 buffer washing step. Beads collection by magnetic rods and transfer into the step 4.
4	Beads release. Ethanol 80% washing step. Beads collection by magnetic rods, air drying step followed by transfer into the step 5.
5	Nucleic acids elution step with E6 buffer. Nucleic acid-free beads collection and transfer into the step 3.

**Contact us to obtain the files for your KingFisher™ instruments.**

## 2. Description of test

Adiagene validated the ADIAMAG kits with various matrices for the detection of different DNA/RNA pathogens in combination with the ADIAVET™ range. The following table summarises the validated protocols.

		Samples													
		EDTA blood	Sera, plasma	Ear notche	Skin biopsy	Tissue	Brain	Swab	Oral fluid	Faeces	Milk	tracheo – nasal swab	Culture swab	Water/urine	Semen
<b>Animal diseases</b>	<i>Influenza virus</i>					X		X							
	<i>Chlamydia</i>					X		X			X				
<b>Ruminant diseases</b>	BoHV-4							X							
	<i>Coxiella burnetii</i>					X		X		X	X				
	<i>Leptospira</i>					X		X						X	
	<i>Neospora caninum</i>					X	X	X							
	BVD virus	X	X	X		X*					X				
	Schmallenberg virus (SBV)	X	X			X	X								
	<i>Anaplasma phagocytophilum</i>	X				X		X							
	<i>Besnoitia besnoiti</i>	X			X										
	<i>Toxoplasma gondii</i>					X	X	X		X					
	Bluetongue virus (BTV)	X													
	<i>M. avium</i> subsp. <i>paratuberculosis</i>									X					
<b>Swine diseases</b>	<i>Mycoplasma hyopneumoniae</i>					X		X	X			X			
	Virus PRV, Aujeszky's disease					X	X	X				X			
	PRRS virus		X			X		X	X						
	<i>Actinobacillus pleuropneumoniae</i>					X		X							
	Classical Swine Fever Virus (CSFV)	X	X			X*		X							
	African Swine Fever Virus (ASFV)	X	X			X*		X							
<b>Avian diseases</b>	<i>Mycoplasma gallisepticum</i> <i>M. synoviae</i> <i>M. meleagridis</i> <i>M. iowea</i>							X							
	<i>Ornithobacterium rhinotracheale</i>							X							
<b>Contagious equine metritis (CEM)</b>	<i>Taylorella equigenitalis</i> , <i>Taylorella asinigenitalis</i> , <i>Klebsiella pneumoniae</i> <i>Pseudomonas aeruginosa</i>							X							
<b>Fish diseases</b>	Infectious hematopoietic necrosis virus (IHNV)					X							X		
	Viral hemorrhagic septicemia virus (VHSV)														
	Infectious pancreatitis necrosis virus (IPNV)					X							X		X
<b>Feline disease</b>	Feline coronavirus (FIP)	X						X							

\*If using the reference NADI003-XL, it is necessary to add the reference NADI004.

For the pool size, refer to the user manual of the ADIAVET™ kit concerned.

## II. Material & reagents

### 1. Composition of kit

The ADIAMAG kits contain the following buffers:

Designation of reagents	Type of reagent	NADI003 ADIAMAG 200 extractions	NADI003-XL ADIAMAG XL 800 extractions	NADI004 LB3 -125mL	Instruction
Lysis Buffer - LB1	Lysis buffer 1	1 x 20 mL	1 x 100 mL		Ready to use
Lysis Buffer - LB2	Lysis buffer 2	1 x 50 mL	1 x 125 mL		Ready to use
Lysis Buffer - LB3	Lysis buffer 3	1 x 125 mL		1x125 mL	Ready to use
ADIAMAG beads	Magnetics beads	2 x 1,5 mL	1 x 12 mL		Ready to use
Binding Buffer - B2	Binding buffer	1 x 180 mL	1 x 500 mL		Ready to use
Wash Buffer - W3	Wash Buffer	1 x 75 mL	1 x 300 mL		Ready to use
Wash Buffer - W4	Wash Buffer	1 x 75 mL	1 x 300 mL		Ready to use
Elution Buffer - E6	Elution Buffer	1 x 30 mL	1 x 125 mL		Ready to use
Proteinase K - PK	Enzyme	1 x 75 mg	4 x 75 mg		Lyophilized – to rehydrate
Proteinase Buffer - BPK	Rehydratation buffer	1 x 8 mL	1 x 15 mL		Ready to use

Refer to Appendix 1 for equivalence between the reagent's names of ADIAMAG Starter (ref. NADI001) or ADIAMAG Plus (ref NADI002) and "ADIAMAG" (ref NADI003, NADI004 and NADI003-XL).

### 2. Validity and storage

After reception, all the extraction reagents can be stored at room temperature (+18 to +25°C) and are stable for up to 1 year and at the most until the shelf-life of the kit. Leave the flasks closed to prevent evaporation.

Prior to use, resuspend the lyophilized proteinase K using 2.6 mL of "Proteinase Buffer - BPK". The Proteinase K solution should be stored at <-15°C.

In case the Lysis Buffer LB2 precipitate, preheated the solution at +70°C until it clears.

In case the Lysis Buffer LB1 and LB3 precipitate, preheat the solution at +30-40°C until it clears.

### 3. Equipment required, but not supplied in the kit

**Warning: The material should be Nuclease-free (e.g. autoclaved 25 minutes twice at +121 °C or once 60 minutes at +121 °C)**

- Class II Microbiological Safety Cabinet
- Centrifuge for microtubes and 20 mL tubes
- Grinder (Mixer Mill or Fast Prep)
- Incubator, heating bath or block heater
- Vortex
- 1 - 10 µL pipette, 20 - 200 µL pipette and 200 - 1000 µL pipette
- Nuclease-free filter tips
- Nuclease-free microtubes: 1.5 mL and 2 mL
- Sterile tubes of 10 or 15 mL
- Powder-free latex or nitrile gloves
- Razor blades
- 80% ethanol solution
- Sterile distilled water
- Sterile saline water (NaCl 8.5 g/L)
- 1X PBS buffer (recommended composition, NaCl 150 mM, Na<sub>2</sub>HPO<sub>4</sub> 5 mM, KH<sub>2</sub>PO<sub>4</sub> 1.7 mM, without Ca<sup>2+</sup>, without K<sup>+</sup> - another composition can be used after validation by the user)
- MEM medium + antibiotics (penicillin 100 IU/mL and streptomycin 100 µg/mL)

**Specific equipment for KingFisher instruments****KingFisher 96/Flex**

- DEEP WELL plates (Thermo scientific, 96 tests: ref. 10373480)
- ELUTION PLATES (Thermo scientific, 96 tests: ref. 10357939)
- TIPS (Thermo scientific, 96 tests: ref. 11744978)

**KingFisher DUO**

- DEEP WELL plates (Thermo scientific, 96 tests: ref. 10373480)
- TIPS-12 (Thermo scientific, 600 tests: ref. 97003500)

**KingFisher mL**

- KingFisher combi 240 (Thermo Scientific, 240 tests: ref :97002141)

**Specific equipment for *Mycobacterium avium* subsp. *paratuberculosis* detection**

- Pack ADIAFILTER (Bio-X Diagnostics, 100 tests: ref. ADIFIL100).
- Glass beads for grinding: ADIAPURE™ ALIQUOTED GLASS BEADS (Bio-X Diagnostics, 480 tests: ref ADIADPBA1-480) only for Mixer mill.
- Lysing Matrix B tubes (MP Biomedical, 100 extractions: ref. 116911.100) only for grinder Fast Prep.

**Specific equipment for SBV virus or Influenza virus detection from tissue**

- Lysing Matrix D tubes (MP Biomedicals, 100 extractions: ref. 116913.100) only for grinder Fast Prep.
- Metal beads 3 mm (e.g. Qiagen, 200 extractions: ref. 69997) only for grinder Mixer Mill.

### III. Use of samples and controls

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#### 1. Precautions

**Caution:**

Prepare buffers according to the §II.2

Buffers can contain toxic substances, please consult the MSDS safety data sheet.

Store reagents at the recommended temperature.

Only appropriately trained personnel should perform this extraction. Ensure the micropipettes used are calibrated. The quality of the obtained results depends on rigorous respect of good laboratory practices.

PCR generates large amount of DNA. A few molecules of amplified products are sufficient to generate a positive result. Hence, PCR tubes should not be opened after amplification.

Samples for analysis should be handled and disposed of as biological waste. **Take all measures of security and confinement required for the manipulation of the concerned biological agents.**

Before starting the process, read the entire protocol and scrupulously respect it.

#### 2. Storage of nucleic acid extracts

Extracted DNAs can be stored at 4°C for up to 24 hours after extraction. For long term storage, DNAs should be kept at <-15°C.

Extracted RNAs are highly sensitive to temperature. Extraction should be performed at room temperature as fast as possible to avoid degradation. Crude extracts can be stored at 4°C for a few hours after extraction. For long term storage, RNAs should be stored at <-65°C.

#### 3. Controls preparation

Several controls should be included for each extraction.

The mix of the different controls included in the AdiaVet kits allows validation of all the steps (extraction and amplification) for all the samples.

- The endogenous or exogenous internal control included in the ADIAVET™ kits allows validation of the extraction and amplification steps of each sample.
- The positive control included in the ADIAVET™ kits allows validation of the specific target amplification.

Other controls should or must be added.

##### A. Negative control of extraction (required)

To verify the absence of cross-contamination, at least one negative control has to be included per trial (e.g. AFNOR NF U47-600-1 guidelines recommends 1 negative control per 24 columns or 4 negative controls per trial of 96-wells plate). This control can be a negative sample or a buffer used for dilution.

##### B. Positive control of extraction (recommended)

A positive control (including the specific pathogen) can be added in each trial. It can be a positive sample available in the laboratory or a negative sample spiked with the specific pathogen. This positive control will be detected close to the limit of detection of the method and allow comparison of the results obtained in different assays

## IV. Preparation of the buffers and loading of the instruments

### 1. Preparation of the binding buffer

We recommend preparing the binding buffer just before use.

Carrefully mix the “ADIAMAG Beads” solution before each use.

During buffer preparation, always add one more reaction than needed (to cover the loss of solution during multi-pipetting).

Mix (per reaction):

- 600 µL of **Binding Buffer B2**
- 13 µL of **ADIAMAG Beads**

### 2. Loading of the instrument

According to the KingFisher™ instrument used, the loading is carried out as follow:

- KingFisher™ mL: one tube comb per sample
- KingFisher™ DUO: one 96-plate
- KingFisher™ 96/Flex: one 96-plate per step

Distribute the buffer in each well/line/plate as mentioned in the following table:

	Buffer to add	KingFisher™ mL	KingFisher™ DUO	KingFisher™ 96/Flex
Preparation of reagents	600 µL binding buffer (600µL Binding Buffer B2 + 13 µL ADIAMAG beads)	Well 1 	Line B 	Plate 1 
	350 µL Wash Buffer W3	Well 2 	Line C 	Plate 2 
	350 µL Wash Buffer W4	Well 3 	Line D 	Plate 3 
	350 µL Ethanol 80%	Well 4 	Line E 	Plate 4 
	60 µL or 100 µL* Elution Buffer E6	Well 5 	Line F 	Plate 5 
	Tip	On rail 	Line A 	Plate 6 
Loading of samples and run the instrument	Transfer the sample prepared according to §V, in the Well 1/Line B/Plate 1. Turn on the instrument. Select the program. Place the comb or plates in the instrument. Press Start. After extraction, collect the purified nucleic acids in Well 5/Line F/Plate 5.			

\*100 µL for the influenza virus, PRV, Besnoitia and fish diseases

### 3. Instrument programs

All instrument programs listed below are compatible with the PCR kit range ADIAVET™:

(summarizing the standard and short programs for the different automates)

	<b>Standard program (34 minutes)</b>	<b>Short program (21 minutes)</b>
<b>KingFisher™ 96/Flex</b>	KF96V3-2	KF96V4-2
<b>KingFisher™ DUO</b>	KFDUOV3-1	KFDUOV4-1
<b>KingFisher™ mL</b>	KFMLV3	KFMLV4

For program files request: [biox@biox.com](mailto:biox@biox.com)

*Warning, in the layout of our program, there may be a difference between real volume and programmed volume. This difference is validated.*

## V. Samples preparation before transfer on the device

### 1. From blood

	BVD, BTV, SBV, FIP, CSFV and ASFV viruses	<i>A. phagocytophilum</i> and <i>B. besnoitii</i>
Sample preparation	Take <b>100 µL</b> of sample.	Place <b>500 µL to 1 mL</b> of <b>bovine blood</b> or <b>100 µL</b> of <b>equine blood</b> in a microtube. Add <b>1 mL</b> of <b>sterile distilled water</b> . Mix and incubate 10 minutes on ice. Centrifuge 6 000 g/5 minutes. Discard the supernatant. Add <b>1 mL</b> of <b>sterile distilled water</b> . Centrifuge 6 000 g/5 minutes. Discard the supernatant
Lysis	Add <b>250 µL</b> of <b>Lysis Buffer LB2</b> . Mix.	Add <b>250 µL</b> of <b>Lysis Buffer LB2</b> <b>+ 10 µL</b> of <b>Proteinase PK</b> . <sup>1</sup> Mix. Incubate 15 minutes at room temperature.
Loading of the instrument	Transfer <b>200 µL</b> in the <b>binding buffer</b> (well 1/line B/plate 1). <sup>2</sup>	

<sup>1</sup>A pre-mix LB2 + PK can be prepared just before use and added to each sample.

<sup>2</sup> For 96-plate lysis, the binding buffer can be added directly to the lysed sample.

### 2. From sera

	BVD, SBV, CFSV, ASFV and PRRS viruses
Sample preparation	Take <b>100 µL</b> of sample.
Lysis	Add <b>250 µL</b> of <b>Lysis Buffer LB2</b> . Mix.
Loading of the instrument	Transfer <b>200 µL</b> in the <b>binding buffer</b> (well 1/line B/plate 1). <sup>1</sup>

<sup>1</sup> For 96-plate lysis, the binding buffer can be added directly to the lysed sample.

### 3. From oral fluids

	<b><i>M. hyopneumoniae</i> and PRRS virus</b>
<b>Sample preparation</b>	Take <b>100 µL</b> of sample.
<b>Lysis</b>	Add <b>250 µL</b> of <b>Lysis Buffer LB2</b> Mix.
<b>Loading of the instrument</b>	Transfer <b>200 µL</b> in the <b>binding buffer</b> (well 1/line B/plate 1). <sup>1</sup>

<sup>1</sup> For 96-plate lysis, the binding buffer can be added directly to the lysed sample.

### 4. From milk

	<b>BVD Virus, <i>C. burnetii</i> and Chlamydia</b>
<b>Sample preparation</b>	Take <b>100 µL</b> of sample.
<b>Lysis</b>	Add <b>100 µL</b> of <b>Lysis Buffer LB1 + 10 µL</b> of <b>Proteinase PK</b> . <sup>1</sup> Mix. Incubate 15 minutes at 56°C.
<b>Loading of the instrument</b>	Transfer <b>the whole volume</b> in the <b>binding buffer</b> (well 1/line B/plate 1).

<sup>1</sup>A pre-mix LB1 + PK can be prepared just before use and added to each sample.

### 5. From culture supernatant

	<b>PRV, IHNV, VHSV and IPNV</b>
<b>Sample preparation</b>	Take <b>100 µL</b> of sample.
<b>Lysis</b>	Add <b>100 µL</b> of <b>Lysis Buffer LB1</b> <b>+ 10 µL</b> of <b>Proteinase PK + 5 µL</b> of <b>EPC-Ext</b> . <sup>1</sup> Mix. Incubate 15 minutes at room temperature.
<b>Loading of the instrument</b>	Transfer <b>the whole volume</b> in the <b>binding buffer</b> (well 1/line B/plate 1).

<sup>1</sup> Specific EPC-Ext are included in each ADIAVET™ kit. See the ADIAVET™ kit of interest instruction manual to prepare, store and use this control.

A pre-mix LB1 + PK + EPC-Ext can be prepared just before use and added to each sample.

## 6. From ear notch samples

	<b>BVD virus</b>
<b>Sample preparation</b>	From <b>1 ear tissue sample</b> .
<b>Lysis</b>	Add <b>350 µL of Lysis Buffer LB3</b> . Mix. Incubate 15 minutes at room temperature. <sup>1</sup>
<b>Loading of the instrument</b>	Transfer <b>100 µL of individual or pooled<sup>2</sup> supernatant</b> in the <b>binding buffer</b> (well 1/Line B/plate 1).

<sup>1</sup> For new analysis, each individual supernatant can be stored at +4°C for 24 hours or at <15°C for long terme

<sup>2</sup> Up to 25 samples can be pooled together. Mix 50 µL of each sample and homogenize.

## 7. From skin biopsy

	<b>Besnoitia besnoiti</b>
<b>Sample preparation</b>	Place <b>50 mg</b> of skin biopsy in a microtube.
<b>Lysis</b>	Add <b>100 µL of Lysis Buffer LB1 + 10 µL of Proteinase PK + 100µl PBS Buffer 1X (or sterile saline water)</b> <sup>1</sup> Mix. Incubate a night at 56°C.
<b>Loading of the instrument</b>	Transfer <b>20 µL of individual supernatant</b> in the <b>binding buffer</b> (well 1/Line B/plate 1).

<sup>1</sup>A pre-mix can be prepared just before use and added to each sample.

## 8. From trachea- bronchial washing

	<b>M. hyopneumoniae</b>
<b>Sample preparation</b>	Transfer <b>1mL of trachea-bronchial washing</b> in microtube. Centrifuge 30 minutes at 10 000g. Discard the supernatant.
<b>Lysis</b>	Add <b>100 µL of Lysis Buffer LB1 + 10 µL of Proteinase PK.</b> <sup>1</sup> Mix and incubate 15 minutes at room temperature.
<b>Loading of the instrument</b>	Transfer <b>the whole volume</b> in the <b>binding buffer</b> (well 1/Line B/plate 1).

<sup>1</sup>A pre-mix LB1 + PK can be prepared just before use and added to each sample.

## 9. From water and urine

<i>Leptospira</i>	
<b>Sample preparation</b>	Transfer <b>10 mL</b> in tube. Centrifuge 30 minutes at 10 000 g or 10 minutes at 4500 g. Discard the supernatant. Add 1 mL PBS 1X. Mix.
<b>Lysis</b>	Transfer <b>100 µL</b> in microtube Add <b>100 µL</b> of <b>Lysis Buffer LB1</b> + <b>10 µL</b> of <b>Proteinase PK</b> . <sup>1</sup> Mix and incubate 15 minutes at 56°C.
<b>Loading of the instrument</b>	Transfer <b>the whole volume</b> in the <b>binding buffer</b> (well 1/Line B/plate 1).

<sup>1</sup>A pre-LB1 + PK can be prepared just before use and added to each sample.

## 10. From coelomic liquid

<b>IPNV</b>	
<b>Sample preparation</b>	Take <b>100 µL</b> of sample.
<b>Lysis</b>	Add <b>100 µL</b> of <b>Lysis Buffer LB1</b> + <b>10 µL</b> of <b>Proteinase PK</b> + <b>5 µL</b> of <b>EPC-Ext</b> . <sup>1</sup> Mix. Incubate 15 minutes at room temperature.
<b>Loading of the instrument</b>	Transfer <b>the whole volume</b> in the <b>binding buffer</b> (well 1/Line B/plate 1).

<sup>1</sup> Specific EPC-Ext are included in each ADIAVET™ kit. See the ADIAVET™ kit of interest instruction manual to prepare, store and use this control.

A pre-mix LB1 + PK + EPC-Ext can be prepared just before use and added to each sample.

## 11. From tissue or brain

### A. Sample preparation

#### a) BVDV, CSFV, ASFV viruses (lymphoïde tissues: spleen, ganglions, tonsil) and PRV virus (brain, lung) with Lysis buffer LB3

Place **20 mg** of sample in a microtube.

Continue according to the table below.

#### b) SBV (brain, spleen) and influenza virus

Grind<sup>1 or 2</sup> **0.1 g** of sample with **1 mL** of **PBS Buffer 1X** or **sterile saline water**.

Transfer **100 µL** of sample in a microtube.

Continue according to the table below.

<sup>1</sup> For example with a grinder like Mixer Mill: add a metal bead (3 mm) and grind 2 minutes at 30 Hz then centrifuge 6000 g/2 minutes.

<sup>2</sup> For example with a grinder like Fast Prep: in a Lysing Matrix D tube, grind twice 20 seconds at 6m/sec with a 5 minutes break on ice between both. Then centrifuge 2000 g/3 minutes.

#### c) IHNV, VHSV and IPNV (spleen, anterior kidney and heart or brain; eggs or sperm)

Homogenize, by stomacher, mixer or mortar and pestle with sterile sand, re-suspend in the original transport medium with a ratio of 10% w / v, centrifuge for 15 minutes at 4000 g.

or

Grind<sup>1 or 2</sup> **0.1 g** of sample with **1 mL** of **PBS Buffer 1X** or **sterile saline water**.

Transfer **100 µL** of sample in a microtube.

Add **5 µL** of **EPC-Ext** included in the ADIAVET™ kits specific of the pathogen.

*See the user manual of ADIAVET™ kits to prepare, store and use the control.*

Continue according to the table below.

<sup>1</sup> For example with a grinder like Mixer Mill: add a metal bead (3 mm) and grind 2 minutes at 30 Hz then centrifuge 6000 g/2 minutes.

<sup>2</sup> For example with a grinder like Fast Prep: in a Lysing Matrix D tube, grind twice 20 secondes at 6m/sec with a 5 minutes break on ice between both. Then centrifuge 2000 g/3 minutes.

#### d) *M. hyopneumoniae*, PRRSV (lung) and ASFV (lymphoid tissues: spleen, ganglions, tonsil) viruses

Place **20 mg** of sample in a microtube.

*NB: for the PRRS virus, pooled analysis (up to 3 samples) can be performed, n x 20 mg.*

Add **1 mL** of **sterile saline water**.

Grind (e.g. with a grinder like Mixer Mill, add a metal bead (3 mm) and grind 2 minutes at 30 Hz).

Centrifuge 6000 g/2 minutes.

Transfer **100 µL** of the supernatant of the grinded sample in a microtube.

Continue according to the table below.

#### e) *Actinobacillus pleuropneumoniae*

Vortex one biopsy in **1 mL** of **sterile saline water**.

Place **20 µL** of obtained liquid in a microtube.

Continue according to the table below.

#### f) *A. phagocytophilum*, *C. burnetii*, *Chlamydia*, *Leptospira*., *N. caninum* and *T. gondii* (tissue e.g. cotyledon of placenta, foetal tissues)

*The analysis from spleen is not recommended. Potential PCR inhibitors can disrupt the analysis.*

Rub within the tissue using dry swab.

Continue according to § 12.A.f.

**g) *N. caninum* and *T. gondii* (brain)**

Mix **one volume of brain** and **one volume of sterile saline water**.

Vortex.

Transfer **100 µL** of **the obtained liquid** in a microtube.

Continue according to the table below.

**B. Nucleic acids extraction and purification**

	<b>BVDV Virus</b>	<b>PRV, CSFV and ASFV viruses</b>	<b><i>M. hyopneumoniae</i>, SBV, PRRSV, influenza, IHN, VHSV, IPNV and ASFV viruses</b>	<b><i>A. pleuropneumoniae</i>, <i>N. caninum</i> and <i>T. gondii</i></b>
<b>Lysis</b>	Add <b>350 µL</b> of <b>Lysis Buffer LB3</b> <sup>4</sup> . Mix.		Add <b>100 µL</b> of <b>Lysis Buffer LB1</b> + <b>10 µL</b> of <b>proteinase PK</b> . <sup>1</sup> Mix.	
	Incubate 15 minutes at room temperature.	Grind <sup>2-3</sup> .	Incubate 15 minutes at room temperature.	Incubate 15 minutes at 56°C.
<b>Loading of the instrument</b>	Transfer <b>100 µL</b> in the <b>binding buffer</b> (well 1/Line B/plate 1).		Transfer <b>the totality</b> in the <b>binding buffer</b> (well 1/Line B/plate 1).	

<sup>1</sup>A pre-mix LB1 + PK can be prepared just before use and added to each sample.

<sup>2</sup> For example with a grinder like Mixer Mill: add a metal bead (3 mm) and grind 2 minutes at 30 Hz then centrifuge 6000 g/2 minutes.

<sup>3</sup> For example with a grinder like Fast Prep: in a Lysing Matrix D tube, grind twice 20 secondes at 6m/sec with a 5 minutes break on ice between both. Then centrifuge 2000 g/3 minutes.

<sup>4</sup> For PRV, add 5 µL of EPC-Ext included in the ADIAVET™ kit

## 12. From swab

### A. Sample preparation

#### a) *M. hyopneumoniae*, PRRSV virus and PRV virus.

Add **2 mL** of **sterile saline water** in the tube with the swab.  
Vortex.

*NB: for PRRSV virus, up to 3 swabs can be pooled together. Transfer the supernatant obtained in the tube with the next swab.*

Press each swab to collect as much liquid as possible.

Transfer the liquid in a 2 mL-microtube.

Place **100 µL** of **the obtained liquid** in a microtube.

Continue following the table below.

#### b) FIP (feline coronavirus).

Add **2 mL** of **sterile saline water** in the tube with the swab.

Vortex.

Press each swab to collect as much liquid as possible.

Transfer the liquid in a 2 mL-microtube.

Place **100 µL** of **the obtained liquid** in a microtube.

Add **5 µL** of **EPC-Ext** included in the ADIAVET™ kit of interest.

*See the ADIAVET™ kit of interest user manual to prepare, store and use this control.*

Continue according to the table below.

#### c) Avian Influenza Virus

Add 1 swab in a tube with **1 mL** of **MEM medium (+antibiotic if viral culture is needed) or sterile saline water**.

Vortex.

*Up to 10 swabs can be pooled together (mix 1 volume of each individual). Keep the individual mixtures at a temperature <-65°C.*

*In case of pools, some weak positive samples can be not detected*

Take **100 µL** of **the obtained liquid**.

Or

Add **1 to 5 swabs** into a tube with **2 mL** of **MEM medium (+antibiotic if viral culture is needed) or sterile saline water**.

Vortex.

*Up to 10 swabs can be pooled together (mix 1 volume of each individual).*

Take **100 µL** of **the obtained liquid**.

Continue according to the table below.

#### d) Swine Influenza Virus

Add 1 swab into a tube with **2 mL** of **MEM medium + antibiotic** (to allow subsequent viral culture) or **sterile saline water**.

Vortex.

Take **100 µL** of **the obtained liquid**.

Continue according to the table below.

#### e) *Actinobacillus pleuropneumoniae*

Vortex one swab in **1 mL** of **sterile saline water**.

Place **20 µL** of **the obtained liquid** in a microtube.

Continue according to the table below.

f) *A. phagocytophilum*, *C. burnetii*, *Chlamydia*, *Leptospira.*, *N. caninum*, *T. gondii*, BoHV-4 and ASFV

Vortex one swab in **1 mL** of **PBS Buffer 1X**.  
Place **100 µL** of **the obtained liquid** in a microtube.  
Continue according to the table below.

g) **Avian mycoplasmas and *Ornithobacterium rhinotracheale***

Cut 1 to 3 swabs in **1 mL** of **sterile saline water**.  
Place **100 µL** of the obtained liquid in a microtube.  
Continue according to the table below.

h) **CEM: *Taylorella equigenitalis*, *Taylorella asinigenitalis*, *Klebsiella pneumoniae* and *Pseudomonas aeruginosa***

Vortex one swab in **500 µL** of **PBS Buffer 1X**.  
Place **100 µL** of **the obtained liquid** in a microtube.  
Continue according to the table below.

**B. Nucleic acids extraction and purification**

	<i>M. hyopneumoniae</i> , FIP, PRRSV, PRV and influenza viruses	<i>A. pleuropneumoniae</i> , <i>A. phagocytophilum</i> , <i>C. burnetii</i> , <i>Chlamydia</i> , <i>Leptospira.</i> , <i>N. caninum</i> , <i>T. gondii</i> , BoHV-4, Avian mycoplasmas, <i>Ornithobacterium rhinotracheal</i> , CEMO and ASFV
<b>Lysis</b>	Add <b>100 µL</b> of <b>Lysis Buffer LB1</b> + <b>10 µL</b> of <b>proteinase PK</b> . <sup>1,2</sup>	
	Mix.	
	Incubate 15 minutes at room temperature.	Incubate 15 minutes at 56°C.
<b>Loading of the instrument</b>	Transfer <b>the totality</b> in the <b>binding buffer</b> (well 1/Line B/plate 1).	

<sup>1</sup>A pre-mix LB1 + PK can be prepared just before use and added to each sample.

<sup>2</sup> Add **5 µL** of **EPC-Ext** included in the ADIAVET™ kit of interest.

See the ADIAVET™ kit of interest user manual to prepare, store and use this control.

### 13. From faeces

#### A. Sample preparation

##### a) *M. avium* subsp. *paratuberculosis*

The quantity of faeces used can vary from 1 to 10 g (=X) as long as a weight/volume ratio of 1/7 is respected. Dilute 1 quantity of faecal sample in 7 volumes of sterile demineralised water (e.g.: 10 g of faeces diluted in 70 mL of sterile demineralised water).

PCR detection assays have higher sensitivity and reproducibility when high quantity of faecal sample are used.

**Environmental samples** (for example soil scrapings from different breeding areas, ...) have to be treated as faecal samples. Dilute 3-10 g of sample in water.

In order to increase the amount of faeces analysed per sample, a concentration step is possible using ADIAFILTER.

#### Sample preparation using ADIAFILTER concentration:

Biological samples:	Bovine faeces	Caprine and ovine faeces
<b>Dilution of sample</b>	Place <b>X g +/- 0.2 g of faeces</b> in a bottle with <b>sterile demineralised water</b> and vortex at least 15 seconds. Allow to settle 10 to 20 minutes.	Crush <b>X g +/- 0.2 g of faeces</b> in a bottle with <b>sterile demineralised water</b> . It is recommended to keep the mixture over night at room temperature for a complete homogenisation. Vortex at least 15 seconds. Allow to settle 10 to 20 minutes.
<b>Cleaning and concentration</b>	Transfer <b>10 mL of the obtained supernatant</b> on the ADIAFILTER. Centrifuge 5 minutes at 3 000 g. Discard the filter and the supernatant. <b>Note</b> : the pellet can be stored for a week at +4°C before extraction.	
<b>Disruption</b>	Add <b>500 µL of sterile demineralised water</b> to the pellet and vortex to obtain a homogeneous suspension. Transfer the obtained suspension in a 1.5mL-microtube containing <b>300 mg of glass beads</b> and disrupt for 10 minutes at 30 Hz with a Mixer Mill <sup>1</sup> . Centrifuge +5 minutes at 15 000 g. Transfer <b>100 µL of sample</b> into a microtube.	

<sup>1</sup> or transfer the obtained suspension in a Lysis Matrix B tube and disrupt 3 x 45 seconds at 4 m/sec with the Fast Prep

Continue following § 13.B.

#### Sample preparation without ADIAFILTER concentration:

Biological samples:	Bovine faeces	Caprine and ovine faeces
<b>Dilution of samples</b>	Place <b>X g +/- 0.2 g of faeces</b> in a bottle with <b>sterile demineralised water</b> and vortex at least 15 seconds. Allow to settle 10 to 20 minutes.	Crush <b>X g +/- 0.2 g of faeces</b> in a bottle with <b>sterile demineralised water</b> . It is recommended to keep the mixture over night at room temperature for a complete homogenisation. Vortex at least 15 seconds. Allow to settle 10 to 20 minutes.
<b>Disruption</b>	Transfer <b>1 mL of the obtained supernatant</b> in a 1.5mL-microtube containing <b>300 mg of glass beads</b> and disrupt 10 minutes at 30 Hz with a Mixer Mill <sup>1</sup> . Centrifuge 5 minutes at 15 000 g. Transfer <b>100 µL of sample</b> in a microtube.	

<sup>1</sup> or Transfer the obtained solution in a Lysis Matrix B tube and disrupt 3 x 45 seconds at 4 m/sec with the Fast Prep

Continue according to § 13.B.

**b) *C. burnetii***

Add **5 mL of PBS Buffer 1X** to **1 g of faeces**.  
 Homogenize, e.g. with a vortex, for at least 15 secondes.  
 Centrifuge 3000 g/2 minutes.  
 Transfer **100 µL of sample** in a microtube.  
 Continue according to the table below.

**c) *T. gondii***

Place **1 g** of faeces in a previously labelled 10 mL or 15 mL sterile tube.  
 Add **10 mL of PBS Buffer 1X** (*This preparation is stable for 24 hours at room temperature*).  
 Vortex until a homogenous solution is obtained.  
 Allow to settle 2 to 5 minutes.  
 Transfer **500 µL of supernatant** in a previously identified microtube.  
 Centrifuge at 3 000 g for 5 minutes. Discard the supernatant.  
 Homogenise the pellet with **1 mL of PBS Buffer 1X** (*This solution is stable for 24 hours at room temperature*).  
 Transfer **500 µL of supernatant** in a tube containing 300 mg of glass beads.  
 Disrupt 10 minutes at 30 Hz with a Mixer Mill (or transfer the obtained solution in a Lysis Matrix B tube and disrupt 3 x 45 seconds at 4 m/sec with the Fast Prep).  
 Centrifuge at 15 000 g 5 minutes.  
 Transfer **100 µL of supernatant** in a previously identified microtube.  
 Continue according to the table below.

**B. Nucleic acids extraction and purification**

	<b><i>M. avium</i> subsp. <i>paratuberculosis</i> with ADIAFILTER</b> <b><i>C. burnetii</i> and <i>T. gondii</i></b>	<b><i>M. avium</i> subsp. <i>paratuberculosis</i> without ADIAFILTER</b>
<b>Lysis</b>	Add <b>100 µL of Lysis Buffer LB1 +10 µL of proteinase PK + 5 µL of EPC-Ext.<sup>1</sup></b> . Mix and incubate 15 minutes <b>at +56°C</b> .	Add <b>100 µL of Lysis Buffer LB1 +10 µL of proteinase PK + 5 µL of EPC-Ext.<sup>1</sup></b> . Mix and incubate 15 minutes <b>at room temperature</b> .
<b>Loading of the instrument</b>	Transfer <b>the totality</b> in the <b>binding buffer</b> (well 1/Line B/plate 1).	

<sup>1</sup> Specific EPC-Ext are included in each ADIAVET™ kit. See the ADIAVET™ kit of interest instruction manual to prepare, store and use this control.  
 A pre-mix LB1 + PK + EPC-Ext can be prepared just before use and added to each sample.

## VI. Amplification

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For the amplification of extracted nucleic acids, please refer to “Amplification” and “Interpretation of results” paragraphs of the ADIAVET™ kit of interest user manual.

## VII. Index of symbols

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Symbol	Meaning
	Catalogue number
	Manufacturer
	Upper temperature limit
	Use by date
	Batch code
	Consult Instructions for Use
	Contains sufficient for <n> tests
	Keep away from sunlight
	For veterinary in vitro use only

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## VIII. Appendix 1

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The reagents of ADIAMAG Starter/Plus and the ADIAMAG extraction kits are similar. Only names and volumes of reagents are modified. The correspondence is shown below:

Name of reagents ADIAMAG PLUS (Ref. NADI001) ADIAMAG STARTER (Ref. NADI002)	Name of reagents ADIAMAG (Ref. NADI003)	Name of reagents ADIAMAG XL (Ref. NADI003-XL)	Name of reagents LB3 (Ref. NADI004)
Nucleomag B beads	ADIAMAG Beads	ADIAMAG Beads	
Lysis buffer T1	Lysis Buffer - LB1	Lysis Buffer - LB1	
Lysis buffer BQ1	Lysis Buffer - LB2	Lysis Buffer - LB2	
Lysis buffer RL1	Lysis Buffer - LB3		Lysis Buffer - LB3
Binding Buffer MB2	Binding Buffer - B2	Binding Buffer - B2	
Wash buffer MB3	Wash Buffer - W3	Wash Buffer - W3	
Wash buffer MB4	Wash Buffer - W4	Wash Buffer - W4	
Elution buffer MB6	Elution Buffer - E6	Elution Buffer - E6	
Proteinase pk	Proteinase K - PK	Proteinase K - PK	
Proteinase buffer PB	Proteinase Buffer - BPK	Proteinase Buffer - BPK	