HANDBOOK

Bio-T kit® BVDV/BDV Universal

Cat. N° BIOTK042 - 100 reactions
Cat. N° BIOTK090 - 400 reactions

**Detection of Ruminant Pestiviruses (BVDV/BDV)
by real-time RT-PCR (qRT-PCR)
with Exogenous internal positive control (IC) and
Endogenous internal positive control (IPC)**

RUMINANTS

Sample types

- Whole blood (on EDTA)
- Serum*
- Ear notches*
- Milk (individual or bulk tank milk)
- Organs (fetal spleen, lung, rectum or injured abomasum)
- Faeces
- Semen
- Alveolar bronchial washing (ABW)
- Deep Nasopharyngeal swab (DNS)
- Individual analysis or by pool up to 1000 according to the matrix

* Bio-T kit® BVDV/BDV Universal has been validated by the French reference lab for BVDV (LE-BVDV, ANSES Niort) for the serum and ear notches samples.

Recommended nucleic acids (NA) extractions

- Magnetic beads extraction (e.g.: BioSellal – BioExtract® SuperBall® Cat. N° BES384)
- Silica membrane columns extraction (e.g.: BioSellal – BioExtract® Column Cat. N° BEC050 or BEC250 ; Indical- Cador® Pathogen 96 QIAcube® HT kit Cat. N° SP54161)
- Flash lysis without purification from ear notches and from serum with TLF buffer (Cat. N° TLF10ml) according to BioSellal validated protocol

Veterinary use only

DOCUMENTS MANAGEMENT

The Bio-T kit® BVDV/BDV Universal has two technical handbooks:

- The extraction handbook shared between the Bio-T kit® BVDV/BDV Universal and Bio-T kit® BVDV Genotyping, displaying BioSellal's validated or recommended extraction protocols for each type of sample.
- The Bio-T kit® BVDV/BDV Universal qRT-PCR handbook, presenting the instruction information to perform the qRT-PCR.

The last versions in use for each handbook are indicated on the certificate of analysis (CA) provided with the Bio-T kit® BVDV/BDV Universal.

Besides these two handbooks, the validation file and a performances confirmation handbook are available on request, contact BioSellal (contact@biosellal.com).

MODIFICATIONS MANAGEMENT

BioSellal indicates modifications done to this document by highlighting them using the rules presented in the Table below:

MODIFICATIONS MANAGEMENT			
Type of modification Highlighting color	**Minor modifications**	**Type 1 Major modifications**	**Type 2 Major modifications**
Impact on revision / version	**Change of revision date** **No change of version**	**Change of revision date** **+ change of version**	**Change of revision date** **+ change of version**
Examples of modifications	Corrections: typographical, grammatical or turns of phrase	EPC reference modification	Modification of Master Mix composition
	Addition of new sample type for extraction	Exogenous IPC reference modification	Modification of validated extraction protocol
	Addition of information giving more details or alternative protocol		
	Addition/Suppression of optional information		

PRESENTATION

Recommendations for sampling, shipping and storage of samples

Real-time RT-PCR is a powerful technique allowing the detection of few amounts of pathogen genome. Genome can be rapidly degraded depending on the pathogen nature (bacteria, parasites, enveloped viruses...), the genome nature (DNA / RNA) and the sample type (presence of DNase / RNase). Thus, BioSellal recommends the following instructions to guarantee an optimal diagnosis.

Sampling

To prevent cross-contamination between samples leading to false positive results, it is mandatory to use disposable materials for single use and to avoid direct contact between specimens.

Shipping

For organs, it is mandatory to ship immediately after sampling or by default to store it at ≤ -16°C. Shipment has to be done within 24h under cover of positive cold.
For other matrices, it is mandatory to ship within 7 days after collection. Ear notches and milk have to be store under cover of positive cold. The shipment must be done, if possible, under cover of the positive cold.

Storage after reception

It is recommended to immediately analyze samples after receipt or freezing at ≤ -16 ° C for a few months and ≤ -65°C beyond 1 year.

RUMINANTS Line

This kit belongs to the RUMINANTS line which gather a set of kits sharing common extraction and qRT-PCR protocols. It is compatible with BioSellal's other kits except with the ones belonging to the PIG and AVIAN lines. (information available via contact@biosellal.com).

Description of the Bio-T kit® BVDV/BDV Universal

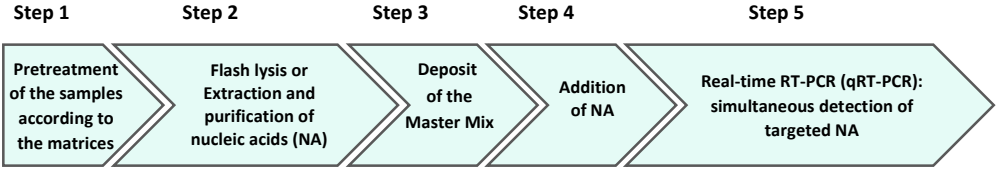
The **Bio-T kit® BVDV/BDV Universal** (Cat. N° BIOTK042/BIOTK090) contains a ready to use **one-step RT-PCR Master Mix** allowing the detection **in the same reaction well of**:

- **Ruminant Pestiviruses (BVDV/BDV**) with a 6-FAM labelling,
- **An Endogenous internal positive control IPC** (mRNA), with a Cy5 labelling, to assess the presence of sufficient amount of host cells, sample integrity, nucleic acids extraction quality and absence of RT-PCR inhibitors,
- **An Exogenous internal positive control IC (DNA)** with a VIC labelling, to add at the extraction step to assess nucleic acids extraction quality and absence of RT-PCR inhibitors. **Exogenous IC adding is mandatory with serum, faeces and milk matrices.**

This kit is based on qualitative detection (detected or non detected) from whole blood, serum, ear notches, organs, milk, faeces, semen, alveolar bronchial washing and deep nasopharyngeal swab samples (Individual analysis or by pool up to 1000 according to the matrix). It was developed and validated according to the **French regulatory standard NF U47-600-2 edited by AFNOR** and the specification of **the French Expert Laboratory for the BVD (ANSES-Niort) for ear notches and serum matrices.**

Extraction protocols validated or recommended by BioSellal are described in the Bio-T kit® BVDV/BDV Universal extraction handbook.

Description of the whole process



<table>
<tr><th colspan="2">Extraction handbook shared between the Bio-T kit® BVDV/BDV Universal and the Bio-T kit® BVDV Genotyping</th><th colspan="3">qRT-PCR handbook of the Bio-T kit® BVDV/BDV Universal</th></tr>
<tr><td>Whole blood
Serum
Milk
Organs*
Ear notches*
Faeces*
Semen*
Alveolar bronchial washing (ABW)*
Deep Nasopharyngeal swab (DNS)*</td><td>BioExtract® SuperBall®
BioExtract® Column</td><td rowspan="3">Ready-to-use Master Mix (MMBVDU-A)</td><td rowspan="3">Samples
NC/NCS
Process positive control
EPC (EPCBVDU-A)</td><td>Dyes:
FAM/VIC/Cy5
Passive reference:
ROX
Program:
Classical program with RT
Standard ramping</td></tr>
<tr><td>Whole blood
Serum
Ear notches*</td><td>Cador® Pathogen 96 QIAcube® HT Kit</td><td></td></tr>
<tr><td>Serum
Ear notches</td><td>Flash lysis</td><td>Dyes:
FAM/VIC/Cy5
Passive reference:
ROX
Program:
TLF program with RT
Standard ramping</td></tr>
</table>

* pretreatment mandatory

Kit contents and storage

Table 1. Description of the kit contents

Description	Reference	Volume / tube BIOTK042 100 reactions	Volume / tube BIOTK090 400 reactions	Presentation	Storage
Master Mix (MM) Ready to use	MMBVDU-A	1000 µl	4x1000 µl	Transparent cap tube Bag A	≤-16°C Protected from light, « MIX » Area
Exogenous Internal Positive Control (IC)	ICBVDU-A	500 µl	4 x 500 µl	Purple cap tube Bag B	≤-16°C « Extraction » Area
External Positive Control (EPC) Positive PCR control of BVDV/BDV	EPCBVDU-A	200 µl		Red cap tube Bag C	≤-16°C « Addition of Nucleic acids » Area
Water RNase/DNase free	Aqua-A	1 ml		Blue cap tube Bag C	5°C ± 3 or ≤-16°C « Addition of Nucleic acids » Area

Kit reagents are stable until the expiration date stated on the label, subject to compliance with good storage conditions.

List of consumables and reagents not included in kit

Table 2. Consumables and reagents not included in kit

Consumables/ Reagents	Description	Provider	Cat. N°
BioExtract® Column	DNA/RNA column extraction kit (50)	BioSellal	BEC050
	DNA/RNA column extraction kit (250)		BEC250
BioExtract® SuperBall®	DNA/RNA Magnetic beads extraction kit (4 x 96)		BES384
Cador® Pathogen 96 QIAcube® HT kit	DNA/RNA extraction kit Plate format (x5)	Indical	SP54161
TLF Buffer	Flash Lysis Buffer	BioSellal	TLF10ml
Flash Lysis Heat block for ear notches	Heat block adapted for Allflex tubes	BioSellal	BTLF
Flash Lysis Heat block for serum	Heat block adapted for 96-well plate	Supplier of your choice	
Metallic foil seal	96-well Plate Foil Seal	Agilent Technologies	5067-5154
PBS Buffer¥	Phosphate Buffered Saline 1X	Supplier of your choice¥	

For consumables related to the thermal cycler, refer to the user manual of the device.

¥ The use of molecular biology grade PBS is very important to obtain optimal results for sample extraction with protocols validated by BioSellal (Flash Lysis, BioExtract® Superball ® and BioExtract® Column). Thus, BioSellal recommends using a commercial PBS with a Molecular Biology grade quality.

List of reagents to confirm laboratory performances

Synthetic RNA of BVDV/BDV (titrated in number of copies/RT-PCR) used by BioSellal for the validation of the kit can be used to confirm the performance of your thermal cycler(s). To confirm the performance of your complete method, viral suspension of BVDV/BDV (quantified in TCID50/ml) used by BioSellal in the validation file could be provided. Internal reference material (MRI) for serum, blood and ear notches are also available to confirm the per
formance of the complete method over the time (extraction + RT-RT-PCR).

BioSellal sells these reagents under the following references:

Table 3. Optional reagents*

Reagent	Description	Provider	Cat. N°
BVDV/BDV RNA	**Quantified BVDV/BDV RNA** (48 x 10^4 copies/qRT-PCR)	BioSellal	cARN-BVDV-001*
BVDV/BDV RNA for positive control	**BVDV/BDV RNA** (for at least 12 wells to replace the EPC for RNA positive control)	BioSellal	CTL-BVDV-001*
BVDV/BDV Viral suspension	**Quantified BVDV/BDV viral suspension** ($1.10^{4.5}$ TCID50/ml)	BioSellal	SV-BVDV-001*
MRI serum	**MRI for Serum sample** (1 to 100 x LD_{METHOD})	BioSellal	SER-BVD-001*
MRI blood	**MRI for Blood sample** (1 to 100 x LD_{METHOD})	BioSellal	SG-BVDV-001*
MRI ear notches	**MRI for ear notches**	BioSellal	BA-BVDV-001*

* These reagents are available only on demand, please contact BioSellal (contact@biosellal.com).

Main critical points

- Wear appropriate personal protective equipment (lab coat, disposable gloves frequently changed).
- Work in dedicated and separate areas to avoid contamination: "Extraction" (unextracted samples storage, extraction equipment area), "Mix" (ready to use MM storage, qRT-PCR plates preparation), "Nucleic acids Addition" (Nucleic Acids storage and addition of extracted nucleic acids and controls in the qRT-PCR plate), "PCR" (final area containing the thermal cycler(s)).
- Use dedicated equipment for each working area (gloves, lab coat, pipettes, vortex, ...).
- Use filter tips.
- Before use, thaw all components at room temperature.
- **One-step RT-PCR Master-Mix is less stable than PCR Master-Mix. To guarantee its optimal performance, it is mandatory to extemporaneously defrost the tubes just before the use, to vortex it, to keep it at 5°C ± 3 during the deposit and to refreeze it immediately afterwards.**
- Vortex and spin briefly (mini-centrifuge) all reagents before use.
- Avoid the repetition of freezing-thawing cycles for samples, lysates, extracted nucleic acids.
- **Genomes of pathogens detected by the RUMINANTS line kits can be DNA or RNA. Working with RNA is more demanding than working with DNA** (RNA instability and omnipresence of the RNases). For these reasons, special precautions must be taken:
    - Always wear gloves, change them frequently, especially after contact with skin or work surfaces.
    - Treat all surfaces and equipment with RNases inactivation agents (available commercially).
    - When wearing gloves and after material decontamination, minimize the contact with surfaces and equipment in order to avoid the reintroduction of RNases.
    - Use “RNase free” consumable.
    - It is recommended to store the RNA at ≤ 5°C ± 3 during the manipulation and then freeze it as soon as possible, preferably at ≤ -65°C or by default at ≤ -16°C.
    - Open and close tubes one by one in order to limit the opening times and avoid any contact with RNases present in the environment (skin, dust, working surfaces…).

DETECTION OF BVDV/BDV BY qRT-PCR WITH BIOTK042/BIOTK090 KITS

Global Procedure

1) **Establish qRT-PCR plate setup** defining each sample position and **including the following controls**:

- **Negative Control Sample (NCS)**: water (or PBS) replaces the sample from the first step of sample preparation.
  This control is mandatory for each extraction series.

- **Negative Amplification Control (NC)**: 5 µl of water RNase/DNase free (Aqua-A tube, **blue** cap) replaces sample nucleic Acids extract on qRT-PCR plate.
  This control is recommended when using the kit for the first time or to verify the absence of Master Mix contamination.

- **External Positive Control of BVDV/BDV (EPC)** : Synthetic DNA (tube **EPCBVDU-A**, **red** cap), containing specific target of BVDV/BDV.
  This control is mandatory.
  This control could be replaced by an RNA external positive control sold separately (Cat N° CTL-BVDV-001).

⚠ CAUTION: *EPC tube handling represents nucleic acids contamination hazard, it is thus recommended to open and handle it in a restricted area, away from other PCR components and to take precautions to avoid cross-contamination with nucleic acids extracts during deposit on the qRT-PCR plate.*

- If available, **a Process Positive Control (MRI),** a weak positive sample, of the same nature of analyzed sample, is extracted in parallel with tested samples. After qRT-PCR, MRI Ct values will be monitored on a Shewhart control card. Obtaining conform Ct values validates the whole process. In this case, the use of the EPC, provided with the kit, is not mandatory.

- **RNA external positive control of BVDV/BDV:** (tube CTL-BVDV-001, green cap), containing sample extracted RNA positive for BVDV/BDV.
  This RNA control is less stable than EPC. To guarantee its optimal use, the optimal storage and handling are mandatory. Thus, it is strongly recommended to store it at ≤ -65°C (for 6 month) or by default at ≤ -16°C (for 3 month), make ready-to-use aliquot, avoid the repetition of freezing-thawing cycles for samples (≤ 2).

2) qRT-PCR plate preparation

In the "MIX" dedicated area

1. After thawing, vortex and rapid centrifugation, **transfer 10 µl Master Mix MMBVDU-A (**transparent cap) in each well of interest (samples and controls).

⚠ *NOTE: One-step RT-PCR Master-Mix is less stable than PCR Master-Mix. To guarantee its optimal performance, it is mandatory to extemporaneously defrost the tubes just before the use, to vortex it, to keep it at 5°C ± 3 during the deposit and to refreeze it immediately afterwards.*

In the "Nucleic Acids addition" dedicated area

2. **Add 5 µl of extracted nucleic acids (or NCS, water, MRI or EPC**: **EPCBVDU-A red** cap tube) in each well of interest. Make sure to pipet out in the bottom of the well, in the Master Mix, and to avoid the formation of bubbles.

Note: if the exogenous IC was not added during sample extraction, it can be added directly in the qRT-PCR plate:

- Add 1 µl of IC (**purple** cap) with the extracted nucleic acids
- Or add directly the IPC (1 µl per reaction) in an aliquot of Master Mix before the deposits of 11 µl of this mix into each well of interest. Then add 5 µl of extracted nucleic acids.

The reaction volume will be increased to 16 µl, without impacting the performances of the qRT-PCR.

3. **Seal the plate with an optically clear sealer** or **close the strip caps**.

In the "PCR" amplification dedicated area

4. **Define the thermal cycler parameters** (see Table 4, Table 5, Table 6, Table 7, Table 8)

5. It is recommended to **spin the plate down prior to place it in the thermal cycler**, to prevent drops in the well pit walls.

6. Start the qRT-PCR program. Approximated run time:
    - 80 min for classical program with RT
    - 90 min for PIG/AVIAN program with RT
    - 120 min for TLF program with RT (mandatory for serum)

3) Thermal cycler settings

This kit was developed and validated on ABI PRISM® 7500 Fast (Applied Biosystems) in standard ramping and confirmed on AriaMx™ (Agilent Technologies, Fast ramping by default). CFX96 thermal cycler (Bio-Rad), LightCycler 480 (Roche) and Rotor-Gene Q (Qiagen) could also be used (see the validation file). All other thermal cyclers with at least 3 channels (FAM, VIC, Cy5) are also compatible, contact our technical support for more information.

Table 4. Thermal cycler configuration

	ABI PRISM® 7500 Fast	AriaMx™
Mode	Quantitation – Standard curve	Quantitative PCR, Fluorescence Probe
Ramping	Standard Ramping	Fast Ramping by default
Passive Reference	**ROX§**	**ROX§**

§ Optional depending on your thermal cycler.

Table 5. Thermal cycler Settings

Target	Detectors		Final Volume / well
	Reporter	Quencher	
BVDV/BDV	FAM	NFQ-MGB or None*	**15 µl** = 10 µl Master Mix + 5 µl extracted nucleic acids or controls†
Exogenous IC	VIC	NFQ-MGB or None*	
Endogenous IPC	Cy5	NFQ-MGB or None*	
To assign to samples and controls†			

* Depends on the thermal cycler model. Do not hesitate to contact the BioSellal Technical Support (tech@biosellal.com)
† Controls are NC (water), NCS (extracted water), EPC and extracted MRI.

For ear notches extracted with the Flash Lysis protocol and for silica membrane columns and magnetic beads extractions, BioSellal validated the use of the following program:

Table 6. CLASSICAL Amplification program settings with RT

Standard Ramping		
Cycles	**Time**	**Temperature**
1 cycle	20 min	50°C
1 cycle	5 min	95°C
40 cycles	15 sec	95°C
	30 sec* + data acquisition	60°C

* Set 31s for some thermal cyclers such as ABI PRISM® 7500.
NB: This amplification program is compatible with all Bio-T kit® except for ones belonging to the PIG and AVIAN LINES.
This program is also compatible with ear notches extracted with the Flash lysis protocol. However, in order to be able to analyze ear notches and serum on the same plate when they are extracted with the flash lysis protocol, a specific program was developed. In any case, the classical program with RT is not compatible with the serum lysates obtained with the Flash Lysis protocol.

For Flash Lysis extractions (serum and ear notches matrices), BioSellal validated the use of the following program:

Table 7. TLF Amplification program settings with RT		
Standard Ramping		
Cycles	**Time**	**Temperature**
1 cycle	20 min	50°C
1 cycle	5 min	95°C
40 cycles	15 sec	95°C
	1 min + data acquisition	60°C

For thermal cycler such as LightCycler®480 and LightCycler®96 (Roche Life Science), it is recommended to use the following program (For silica membrane column, magnetic beads and Flash lysis extraction):

Table 8. PIG/AVIAN Amplification program settings with RT		
Standard Ramping		
Cycles	**Time**	**Temperature**
1 cycle	20 min	50°C
1 cycle	5 min	95°C
40 cycles	**10 sec**	95°C
	45 sec + data acquisition	60°C

NB: This amplification program is compatible with all Bio-T kit® of the PIG and AVIAN LINES.

The data of these programs validation are available in the validation file of the Bio-T kit® BVDV/BDV Universal.

RESULTS INTERPRETATION

To analyze and interpret the signals obtained by qRT-PCR, the Threshold must be set up.
The threshold must be assigned carefully in order to obtain the most reproducible result between different manipulations according to the requirements defined in Annex C of the French Standard **NF U47-600 (part 1)**. A consistent set of positives controls, usually an In-house Reference Material (MRI) or the EPC, is used to set the threshold value above the baseline and in the exponential amplification phase of the plot.
The Threshold Cycle, named « Ct » or « Cq » (depending on thermal cyclers), corresponds to the intersection between the amplification curves and the threshold line. It allows the relative measurement of the concentration of the target in the PCR reaction when a calibrated extract is analyzed in the same series.
The qRT-PCR series is validated if the controls (EPC, MRI, NCS and NC) present valid results, then the result of each sample can be interpreted.

Main Scenarios

Controls Reading

Table 9. PCR Controls results interpretation

	Targets			Interpretation
	BVDV/BDV (FAM)	Exogenous IC (VIC)	Endogenous IPC (Cy5)	
NCS Negative Control Sample **MANDATORY**	Neg	Pos	Neg	Valid
	Pos / Neg	Pos	Pos/ Neg	If at least the target BVDV/BDV or endogenous IPC is positive: Contamination with a positive sample during extraction step or during qRT-PCR plate preparation.
NC Negative PCR Control **OPTIONAL**	Neg	Neg	Neg	Valid
	At least one of three targets Pos			Contamination with a negative or a positive sample during PCR plate preparation? or Master Mix / Water contamination?
EPC BVDV/BDV PCR external positive control **MANDATORY** *IN ABSENCE OF MRI*	Pos[1]	Neg	Neg	Valid
	Neg	Neg	Neg	Problem during qRT-PCR plate preparation: Master Mix error? EPC omission?
	Pos[1]	At least one of two targets Pos		Contamination with a sample during qPCR plate preparation?
Sample process positive Control MRI **RECOMMENDED** *IF AVAILABLE*	Pos[2]	Pos[3]	Pos[3]	Valid
	Neg	Neg / Pos[3]	Neg/ Pos[3]	Problem during qRT-PCR: Master Mix error? Nucleic acids extract omission or extract not in contact with Master Mix? Process drift: extraction (in case of exogenous IPC addition directly into qRT-PCR plate and not during extraction) Problem with MRI preparation? Degradation of the sample process positive control?

1 The Ct value obtained must be conform with the value indicated on the Certificate of Analysis (CA).
2 The Ct value must be included within control card limits.
3 The obtained Ct value depends on the thermal cycler, the sample type and the used extraction protocol. For IC, it must be, at most, within the range specified on the certificate of analysis (CA). IPC and IC values, obtained from the different matrices with the methods validated by BioSellal, are available in the validation file of Bio-T kit® BVDV/BDV Universal. BioSellal recommends that the laboratory determines its own maximum tolerated IC/IPC value based on its extraction method and thermal cycler.
BioSellal has determined maximum tolerated values for endogenous IPC on blood and ear notches (see Table 12).

Note:

Endogenous IPC targets a gene expressed by ruminant cells, thus it cannot be detected in NCS, NC and EPC. However, due to cross-reaction between ruminant GAPDH and human GAPDH, a slight signal can be observed for IPC in the controls, the Ct value of this signal must be lower than 35.

Sample Reading: In case of exogenous IC addition

Table 10. Different types of results obtained for the samples if the exogenous IC was added during the extraction or the qRT-PCR (mandatory for serum, faeces and milk matrices)			
	Targets		
BVDV/BDV (FAM)	Exogenous IC (VIC)	Endogenous IPC[3] (Cy5)	Interpretation
Neg	Conform Ct[1]	Pos Ct within the range of the validation file excepted for serum and milk matrices, and < tolerated values[2]	**Negative or Undetected**
	Non Conform Ct >35[1]		**Uninterpretable – Risk of low positive sample non- detection** Presence of inhibitors[4]?
Pos	Conform Ct[1]		**Positive or Detected**
	Non Conform Ct >35[1]		**Positive or Detected** Presence of inhibitors[4]? Obtained Ct value for BVDV/BDV can't be used to estimate viral load (possible underestimation)
Pos	Conform Ct[1]	Neg or Ct > tolerated value according to the matrice[2]	**Positive or Detected** Competition with target? Insufficient cell quantity? Problem during extraction?
	Non Conform Ct >35[1]		**Positive or Detected** Presence of inhibitors[4]? Problem during extraction? Obtained Ct value for BVDV/BDV can't be used to estimate viral load (possible underestimation)
Neg	Conform Ct[1]	Neg or Ct > tolerated value according to the matrice[2]	**Uninterpretable or Negative*** degradation of nucleic acids in the sample? Sampling problem: Insufficient cell quantity (absence of biopsy)? Problem during extraction? Omission of extracted nucleic acids or extrat not in contact with Master Mix?
	Non Conform Ct >35[1]		**Uninterpretable** Presence of inhibitors[4]? Problem during extraction?

1 Obtained Ct values depends on the thermal cycler, the analyzed matrix and the extraction methods used. For IC, it must be, at most, within the range specified on the certificate of analysis (CA). IPC and IC values, obtained from the different matrices with the methods validated by BioSellal, are available in the validation file of Bio-T kit® BVDV/BDV Universal. BioSellal recommends that the laboratory determines its own maximum tolerated IC/IPC value based on its extraction method and thermal cycler.

2 see Table 12 for details

3 For milk, faeces and serum sample, the Ct values for endogenous IPC is to not consider. Indeed, the number of cells thus the quantity of endogenous mRNA is variable depending on the sample.

4 In case of suspicion of inhibition, 1) Repeat qRT-PCR by pre-diluting nucleic acids to 1/10 or even 1/100 in DNase / RNase free water or 2) Resume analysis from extraction.

Samples Reading: in the absence of exogenous IC

Table 11. Different types of results obtained for the samples if the exogenous IC was not added during the extraction or the qRT-PCR (matrices except milk, faeces and serum)		
Targets		
BVDV/BDV (FAM)	Endogenous IPC (Cy5)	Interpretation
Neg	Pos Ct within the range of the validation file excepted for serum and milk matrices, and < tolerated values[2]	Negative or Undetected
Pos		Positive or Detected
Pos	Neg or Ct>35 Ct > tolerated value according to the matrice[2]	Positive or Detected Presence of inhibitors[1]? Competition with the main target? Extraction problem? Obtained Ct value for BVDV/BDV can't be used to estimate virale charge (possible underestimation)
Neg	Neg or Ct>35 Ct > tolerated value according to the matrice[2]	Uninterpretable or Guardedly Negative[3] Risk of low positive sample non- detection = repeat the analysis Presence of inhibitors[1]? Absence of sample (ear notches)? Nucleic acids degradation in the sample? Extraction problem? Nucleic acids extract omission or extract not in contact with Master Mix?

1 In case of inhibition suspicion, 1) Repeat the qRT-PCR with the dilution of extracted nucleic acids at 1/10 or 1/100 in the DNase/RNase free water. 2) Restart the analysis from the extraction step.

2 The obtained Ct value depends on the thermal cycler, the analyzed matrix and the extraction methods used. IPC values, obtained from the different matrices with the methods validated by BioSellal, are available in the validation file of Bio-T kit® BVDV/BDV Universal. BioSellal recommends you determine your own maximal IPC Ct value depending on your own extraction method and thermal cycler.

3 BioSellal describes maximum warning Ct values of endogenous IPC for two matrices (Table 12), values beyond which a negative result for BVDV/BDV is made guardedly negative, or uninterpretable in absence of IPC signal.

Maximum IPC Ct value for ear notches and blood sample for PI detection

BioSellal has determined maximum tolerated values for endogenous IPC on blood and ear notches. Above this value, a negative result for BVDV/BDV target is to be consider “Guardedly Negative” and “ Uninterpretable” if the IPC Ct value is negative.

These data were established for ear notches sample thanks to a stability study conducted by BioSellal. This study showed that the endogenous IPC was more sensitive to degradation by the RNases contained in the ear notches, than the genome of the BVDV / BDV virus.

Table 12 : Maximum tolerated values for endogenous IPC		
Analysis	**Ear notches**	**Whole blood**
Individual	Ct>31	Ct>31
Pool	Ct>28	Ct>31

NB: As a reminder, do not consider endogenous IPC values for milk, faeces and serum matrices for which cells quantity, and by consequence endogenous mRNA, is variable according to samples.

Notes:

biosellal

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