HANDBOOK

Bio-T kit® *Mycoplasma bovis & Histophilus somni*

Cat. N° BIOTK052 - 50 reactions

Detection of *Mycoplasma bovis* (*M. bovis*) and *Histophilus somni* (*H. somni*) by real-time PCR (qPCR) with Endogenous Internal Positive Control (IPC)

RUMINANTS

Sample types

- Trans-tracheal aspiration liquid (TTA)
- Alveolar bronchial washing (ABW)
- Deep Nasopharyngeal swab (DNS)
- Organs (lungs)
- Individual analysis or by pool up to 3 according to the matrix

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; Qiagen – RNeasy® Mini Kit Cat N° 74104)

Veterinary use only

DOCUMENTS MANAGEMENT

The Bio-T kit® *Mycoplasma bovis* & *Histophilus somni* has two technical handbooks:

- The extraction handbook shared between all the Bio-T kit® of the RESPIRATORY line, displaying BioSellal's recommended extraction protocols for each type of sample.
- The Bio-T kit® *Mycoplasma bovis* & *Histophilus somni* qPCR handbook, presenting the instruction information to perform the qPCR.

The last versions in use for each handbook are indicated on the certificate of analysis (CA) provided with the Bio-T kit® *Mycoplasma bovis* & *Histophilus somni*.

Besides these two handbooks, a summary report of the validation file is 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 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

In order to correctly diagnose all valences of the RESPIRATORY line, BioSellal recommends on alive animals the analysis of TTA and ABW and lungs analysis on dead animals. For this last sample, it's important to collect both healthy area and adjacent injured area. DNS analysis on alive animals is possible but the results interpretation must take into account the vaccination context for BoRSV and PI3, and the presence of commensal bacteria (*Mannheimia haemolytica, Pasteurella multocida* and *Histophilus somni*) in the oropharyngeal sphere.

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

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.

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.

RESPIRATORY Line

Bovine respiratory disease (BRD) is the most common and costly disease affecting calves. These disorders occur every year during the cold and wet season, usually from December to March. Classical symptoms include coughing, severe breathing difficulty (dyspnoea), hyperthermia, depression with an impact on their growth.

The pathogenesis involves an initial infection (viral: bovine syncytial respiratory virus, BoRSV; Para-Influenza virus type 3: PI3; or bacterial: *Mycoplasma bovis*) that may alter the animal's defence mechanisms, allowing colonization of the lower respiratory tract by commensal germs of the oropharyngeal sphere (*Pasteurella multocida, Mannheimia haemolytica* or *Histophilus somni*) resulting in superinfections and more severe lesions of bronchopneumonia.
Another pathogen, the bovine respiratory coronavirus (BCoV), also appears to be a major viral actor in the aetiology of bronchopneumonia in young cattle since studies in the USA, Northern Europe and France show that its prevalence is comparable to BoRSV.
Recently, Influenza D virus has been clearly identified as a pathogen involved in BRD. A study carried out by the French National Veterinary School of Toulouse in collaboration with a French Veterinary Laboratory (LDA71) shows that its prevalence is of the order of 5% in France.

Due to the economic impact in terms of mortality, cost of treatment, vaccination, growth stunting and to limit the spread of infection in the herd, it is important to identify involved pathogens. Thus, the diagnosis must be rapid and reliable in order to establish the most appropriate methods of prophylaxis and treatment. Since BRD is multifactorial, it is important to achieve a simultaneous detection for all involved pathogens.

That's why, BioSellal has developed four real-time PCR kits (qPCR) targeting two pathogens and endogenous positive control (IPC). These kits, belonging to BioSellal RESPIRATORY line, allow, from a common nucleic acids (NA) extraction and PCR amplification program, to diagnose the 8 major BRD pathogens:

- *Mycoplasma bovis* / *Histophilus somni* / endogenous IPC
- *Mannheimia haemolytica* / *Pasteurella multocida* / endogenous IPC
- BoRSV / PI3 / endogenous IPC
- Bovine coronavirus / Influenza D / endogenous IPC.

The kits of the RESPIRATORY line share common extraction and qPCR protocols. They are also compatible with other BioSellal's kits except those from the PIG and AVIAN lines (information available via contact@biosellal.com).

In addition to the kits of the RESPIRATORY line, BioSellal offers real-time PCR or ELISA kits for the identification of other pathogens potentially involved in BRD such as BVDV or BoHV-1. For information on other available kits please contact us via contact@biosellal.com.

Description of
the Bio-T kit® *Mycoplasma bovis* & *Histophilus somni*

The **Bio-T kit® *Mycoplasma bovis* & *Histophilus somni*** (Cat. N° BIOTK052) contains a ready to use **PCR Master Mix** allowing the detection **in the same reaction well of**:

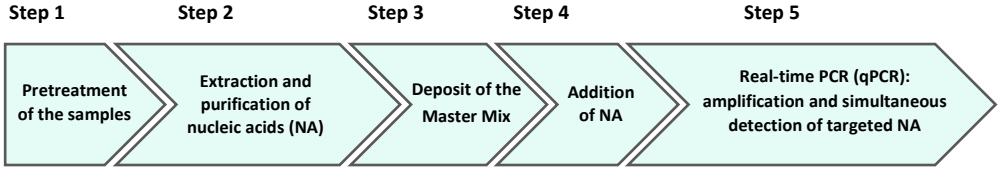
- ***Mycoplasma bovis* (*M. bovis*)** with a 6-FAM labelling;
- ***Histophilus somni* (*H. somni*)** with a VIC labelling;
- An **Endogenous internal positive control IPC (gapdh)**, with a Cy5 labelling, to assess the presence of sufficient amount of host cells, sample integrity, nucleic acids extraction quality and absence of PCR inhibitors.

This kit, based on qualitative detection (detected or non-detected) from trans-tracheal aspiration liquid, alveolar bronchial washing, deep nasopharyngeal swab, organs (lungs) samples was developed and validated according to the **French regulatory standard NF U47-600-2 edited by AFNOR** for the PCR part.

Extraction protocols recommended by BioSellal are described in the extraction handbook of the RESPIRATORY line.

Note: For easier work with our kits, BioSellal has showed that for all DNA detecting kits of RESPIRATORY line there is no impact on its performances of adding the reverse transcription step. So, all the kits of this line can be used on the same real time PCR program either DNA or RNA detecting kits.

Description of the whole process



RESPIRATORY line Extraction handbook		qPCR handbook of the Bio-T kit® *Mycoplasma bovis* & *Histophilus somni*		
Trans-tracheal aspiration liquid* Alveolar bronchial washing* Deap Nasopharyngeal swab* Organs (lungs)*	BioExtract® SuperBall® BioExtract® Column RNeasy® Mini Kit	Ready-to-use Master Mix MMMybHss-A	Samples NC/NCS Process positive Control EPC (EPCMybHss-A)	Dyes: FAM/VIC/Cy5 Passive reference: ROX Program: Classical program ± RT Standard ramping

* pretreatment mandatory

Kit contents and storage

Table 1. Description of the kit contents				
Description	**Reference**	**Volume/tube**	**Presentation**	**Storage**
Master Mix (MM) Ready to use	MMMybHss-A	750 µl	White cap tube Bag A	≤-16°C Protected from light, « MIX » Area
External Positive Control (EPC) Positive PCR control of *H. somni* and *M. bovis*	EPCMybHss-A	110µl	Orange cap tube Bag B	≤-16°C « Addition of Nucleic acids » Area
Water RNase/DNase free	Aqua-A	1 ml	Blue cap tube Bag B	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
BioExtract® Column	DNA/RNA column extraction kit (250)	BioSellal	BEC250
BioExtract® SuperBall®	DNA/RNA Magnetic beads extraction kit (4 x 96)	BioSellal	BES384
RNeasy® Mini Kit	RNA column extraction kit (50)	Qiagen	74104

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

List of reagents to confirm laboratory performances

Synthetic DNA of *M. bovis* and *H. somni* (titrated in number of copies/qPCR) used by BioSellal for the validation of the kit can be used to confirm the performance of your thermal cycler(s).
An internal reference material (MRI), for *M. bovis* and *H. somni*, is also available to confirm the performance of the complete method over the time (extraction + PCR).
BioSellal sells these reagents under the following references:

Table 3. Optional reagents*

Reagent	Description	Provider	Cat. N°
***M. bovis* DNA**	Quantified DNA of *M. bovis* (3×10^5 copies/qPCR)	BioSellal	cADN-Myb-001
***H. somni* DNA**	Quantified DNA of *H. somni* (3×10^5 copies/qPCR)	BioSellal	cADN-HSS-001
MybHSS MRI	*M. bovis* and *H. somni* MRI	BioSellal	MRI-MybHSS-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, qPCR plates preparation), "Nucleic acids Addition" (Nucleic Acids storage and addition of extracted nucleic acids and controls in the qPCR 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.
- Vortex and spin briefly (mini-centrifuge) all reagents before use.
- Avoid the repetition of freezing-thawing cycles for samples, lysates, extracted nucleic acids.
- **Pathogen's genome detected by the RESPIRATORY line's 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 *M. BOVIS* AND *H. SOMNI* BY qPCR WITH BIOTK052 KIT

Global Procedure

1) Establish qPCR 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 qPCR 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 *H. somni* and *M. bovis* (EPC)** : Synthetic DNA (tube **EPCMybHss-A**, **orange** cap), containing specific target of *M. bovis* and *H. somni*.
  This control is mandatory.

⚠ 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 qPCR plate.*

- If available, **a Process Positive Control (MRI),** a weak positive sample of trans-tracheal aspiration liquid, alveolar bronchial washing, organs (lungs) or deep nasopharyngeal swab, is extracted in parallel with tested samples. After qPCR, 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.

2) qPCR plate preparation

In the “MIX” dedicated area

1. After thawing, vortex and rapid centrifugation, **transfer 15 µl Master Mix MMMybHss-A (white** cap) in each well of interest (samples and controls).

In the “Nucleic Acids addition” dedicated area

2. **Add 5 µl of extracted nucleic acids (or NCS, water, MRI or EPC**: **EPCMybHss-A orange** 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.

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)

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 qPCR program. Approximate run time: 60 min.

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), but it is compatible with all thermal cyclers able to read 6-FAM, VIC and Cy5 channels in the same PCR well.For other thermal cyclers, contact our technical support.

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**

Table 5. Thermal cycler Settings

Target	Detectors		Final Volume / well
	Reporter	Quencher	
M. bovis	FAM	NFQ-MGB or None*	**20 µl** = 15 µl Master Mix + 5 µl extracted nucleic acids or controls†
H. somni	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 or extracted MRI.

Table 6. CLASSICAL Amplification program settings without RT†

Standard ramping		
Cycles	**Time**	**Temperature**
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.
† optional step, in case of simultaneous detection of RNA genomes. Achieving a reverse-transcription (RT) step prior to PCR for the amplification of RNA genomes has no impact on the effectiveness of the Bio-T kit® *Mycoplasma bovis* & *Histophilus somni* (see the summary of the validation file).
NB: This amplification program is compatible with all Bio-T kit® except for ones belonging to the PIG and AVIAN LINES.

For thermal cycler such as LightCycler®480 and LightCycler®96 (Roche Life Science), it is recommended to use the following program:

Table 7. PIG/AVIAN Amplification program settings without RT†		
Ramping by default		
Cycles	**Time**	**Temperature**
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.

† An optional step of 50°C for 20 min is possible in case of simultaneous detection of RNA genome.

RESULTS INTERPRETATION

To analyze and interpret the signals obtained by qPCR, 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 qPCR 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 8. PCR Controls results interpretation

	Targets			Interpretation
	M. bovis (FAM)	*H. somni* (VIC)	Endogenous IPC (Cy5)	
NCS Negative Control Sample **MANDATORY**	Neg	Neg	Neg	Valid
	At least one of the three targets **Pos**			Contamination with a positive/negative sample during extraction step or during qPCR plate preparation.
NC Negative PCR Control **OPTIONAL**	Neg	Neg	Neg	Valid
	At least one of the three targets **Pos**			Contamination with a positive/negative sample during extraction step or during qPCR plate preparation or Master Mix/water contamination.
EPC *M. bovis* and *H. somni* PCR external positive control **MANDATORY** *IN ABSENCE OF MRI*	Pos*	Pos*	Neg	Valid
	Neg	Neg	Neg	Problem during qPCR plate preparation: Master Mix error? EPC omission?
	Pos*	Pos*	Pos	Contamination with a sample during qPCR plate preparation?
Sample process positive Control MRI **RECOMMENDED** *IF AVAILABLE*	Pos†	Pos†	Pos¥	Valid
	Neg	Neg	Neg	Problem during qPCR plate preparation: Master Mix error? Nucleic acids extract omission or extract not in contact with Master Mix? Process drift: extraction and/or qPCR ? Degradation of the sample process positive control?

* The Ct value obtained must be conform with the value indicated on the Certificate of Analysis (CA).
† The Ct value must be included within control card limits.
¥ The obtained Ct value depends on the thermal cycler, the sample type and the used extraction protocol. Ct values for IPC using the recommended extraction protocols are available upon request. BioSellal recommends you to determine your own maximal IPC Ct value depending on your own extraction method and thermal cycler.

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 over than 35.

Samples Reading

- Lung, trans-tracheal aspiration liquid (TTA) or alveolar bronchial washing samples (ABW)

Recommended sample types for *Pasteurellaceae* (*Pasteurella multocida*, *Mannheimia haemolytica*, *Histophilus somni)* and *Mycoplasma bovis* identification, are either lung collected from dead animals or trans-tracheal aspiration liquid (TTA) or alveolar bronchial washing (ABW) samples harvested from live animals. Table 9 summarises the type of results that can be obtained.

Table 9. Different types of results obtained for the samples (lung, TTA, ABW)

Targets			
M. bovis **(FAM)**	***H. somni*** **(VIC)**	**Endogenous IPC (Cy5)**	**Interpretation**
Neg	**Neg**	**Pos***	**Negative or Undetected**
Pos	**Pos**		**Positive or Detected**
At least one of two targets **Pos**			**Positive or Detected for the positive target** **Negative or Undetected for the negative target**
Pos	**Pos**	**Neg or Ct>35**	**Positive or Detected** Lack of host cells? Presence of inhibitors ǂ? Competition with the main target?
At least one of the targets is **Neg**		**Neg or Ct>35**	**Positive or Detected for the positive target** **Uninterpretable for the negative target** **= Repeat the analysis.** Presence of inhibitors ǂ? Nucleic acids degradation in the sample? Sampling problem: lack of cells? Extraction problem?
Neg	**Neg**	**Neg or Ct>35**	**Uninterpretable= Repeat the analysis** Problem during qPCR plate preparation: Master Mix error? Nucleic acids extract omission or extract not in contact with Master Mix? Presence of inhibitors ǂ? Nucleic acids degradation in the sample? Sampling problem (concerns TTA and ABW): lack of cells? Extraction problem?

* The obtained Ct value depends on the thermal cycler, the sample type and the used extraction protocol. IPC Ct values obtained for the recommended extraction protocols are available upon request. BioSellal recommends you determine your own maximal IPC Ct value depending on your own extraction method and thermal cycler.

ǂ In case of inhibition suspicion, 1) Repeat the qPCR 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

- Deep nasopharyngeal swab samples (DNS)

Histophilus somni, Pasteurella multocida and *Mannheimia haemolytica* (cryptical) are naturally presented at the level of higher respiratory tract mucosa. Thus, the chance of positivity linked to natural contamination of the swab is strong and can limit the diagnostic value for this type of sample. So, it's necessary to compare the results obtained with the Bio-T kit® *Mycoplasma bovis & Histophilus somni* from the nasal swab samples with the level of bacteria naturally present in the sample.

In parallel, it is difficult to detect *Mycoplasma bovis* from nasal swabs due to its preferential localization in pulmonary macrophages. So negative result for this target will give a poor negative predictive value.

Table 10. Different types of results obtained for the samples (DNS)

Targets			
M. bovis **(FAM)**	***H. somni*** **(VIC)**	**Endogenous IPC (Cy5)**	**Interpretation**
Neg	**Neg**	**Pos***	**Negative or Undetected**
Pos	**Pos** **Ct<35**		**Positive or Detected** **Significant Positive result for both targets**
	Pos **Ct>35**		**Positive or Detected** **Significant Positive result for *M. bovis* and Non-significant Positive result for *H. somni*** **As indicated above for *H. somni* bacteria** a late Ct value (Ct>35) does not have a significant positive predictive value.
Neg	**Pos** **Ct>35**		**Positive or Detected for *H. somni*** **Non-significant Positive result for *H. somni*** **As indicated above for *H. somni* bacteria** a late Ct value (Ct>35) does not have a significant positive predictive value.
Pos	**Pos** **Ct<35**	**Neg or Ct>35**	**Positive or Detected** **Significant Positive result for both targets** Lack of host cells? Presence of inhibitors ǂ? Competition with the main target?
Neg	**Neg or Pos** **Ct>35**	**Neg or Ct>35**	**Uninterpretable** **= Repeat the analysis** Problem during qPCR plate preparation: Master Mix error? Nucleic acids extract omission or extract not in contact with Master Mix? Presence of inhibitors ǂ? Nucleic acids degradation in the sample? Sampling problem: lack of cells? Extraction problem?

* The obtained Ct value depends on the thermal cycler, the sample type and the used extraction protocol. Ct values for IPC using the recommended extraction protocols are available upon request. BioSellal recommends you determine your own maximal IPC Ct value depending on your own extraction method and thermal cycler.

ǂ In case of inhibition suspicion, 1) Repeat the qPCR 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.

Warning

Some thermal cyclers (eg: ABI PRISM® 7500, Light Cycler®, Thermo Fisher Scientific, QuantStudio® 5 Real-Time PCR System) use a common excitation source for all fluorophores that could lead to false positive result in VIC channel. The closeness of exictation and emission spectrum of FAM (*Mycoplasma bovis* target) and VIC (*Histophilus somni* target*)* fluorophores results in interferences between these two signals leading to the reading of a VIC signal in absence of the *Histophilus somni* target resulting from a strong FAM signal (*Mycoplasma bovis strong positive sample).*
This phenomenom depends on the thermal cycler type, its calibration and/or the used analysis software. In example, the figure below shows the observation of a non-specific VIC signal due to the presence of the *Mycoplasma bovis* (FAM) target at a high intensity level (7500 Fast, VIC detection, sample 1).

To exclude the possibility of false-positive results and to avoid result interpretation limited to Ct value data, BioSellal recommends pay attention to amplification curves aspect for the *Histophilus somni* target (VIC) in case of strong positivity for the *Mycoplasma bovis* (FAM) target

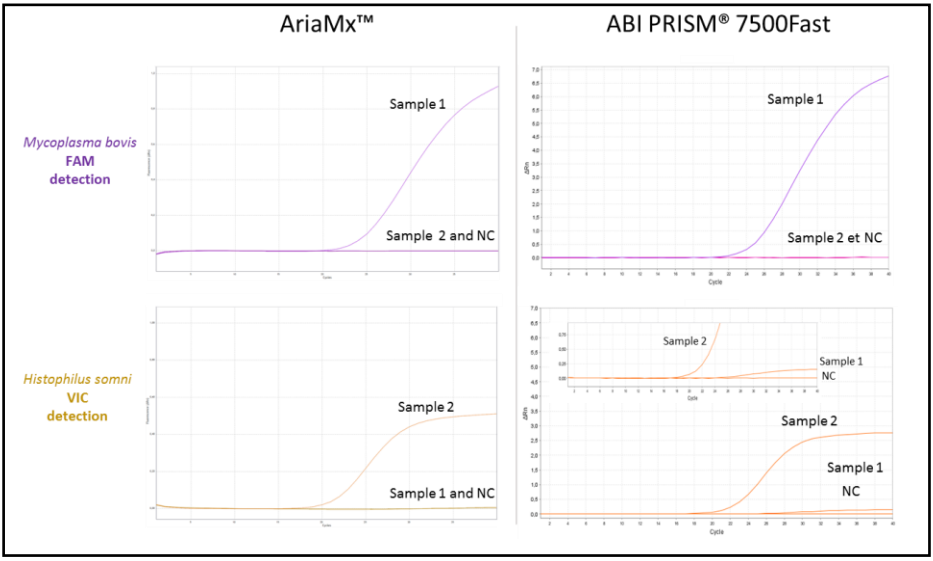


Figure 1: Example of amplification curves obtained for a strong positive *Mycoplasma bovis* (FAM) sample and negative for *Histophilus somni* (VIC) (Sample 1), a negative *Mycoplasma bovis* (FAM) sample strong positive for *Histophilus somni* (VIC) (Sample 2) and a negative control sample (NC), obtained using AriaMx™ thermal cycler (no FAM/VIC compensation issue) and using the 7500Fast thermal cycler (insufficient FAM/VIC compensation). Due to the inadequate FAM/VIC compensation of the 7500Fast thermal cycler, an *Histophilus somni* (VIC) false-positive signal is observed for Sample 1 (bottom right-hand curve, sample 1). Such signal is not observed using a thermal cycler not presenting FAM/VIC compensation issue (AriaMx™).

biosellal

www.biosellal.com

Technical Support

tech@biosellal.com +33 (0) 4 26 78 47 62

Information and orders

contact@biosellal.com +33 (0) 4 26 78 47 60