Certified management system

ID Gene™ Q Fever-Chlamydophila spp Triplex

Ref: IDQFCH-50 / IDQFCH-100

50 / 100 tests

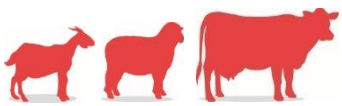
Real-time PCR assay for the **qualitative** or **quantitative** detection of *Coxiella burnetti* and *Chlamydophila spp*

Suitable samples: Ruminant swab supernatant (placental, cervical, vaginal) pools of up to 3, organ (spleen), milk, gastric juice, faeces.

***In-vitro* Use**

May 2018 :

- **Kit with lyophilized Target Positive Control (TPC-QFCH)**
- **Possibility of testing pools of up to 3 swabs for *Coxiella burnetii* detection**
- **Addition of faeces matrix for *Coxiella burnetii* detection**



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General information

▪ Characteristics

ID Gene™ Q Fever-Chlamydophila spp Triplex (IDQFCH) is a real-time PCR kit that amplifies a target sequence in *Coxiella burnetti* and in *Chlamydophila spp* genomes, causative agent of Q fever and Chlamydiosis.

This kit is a relative qualitative or quantitative (Q Fever only) triplex test. It simultaneously amplifies target DNA of *Coxiella burnetti*, of *Chlamydophila spp* and an endogenous non-target positive control.

The kit contains the target positive control (TPC-QFCH) which is to be extracted in the same manner as the samples to evaluate extraction and amplification steps.

This kit can be used to test ruminant swab supernatant (placental, cervical or vaginal), organ homogenate (spleen), milk or gastric juice and faeces.

It is possible to perform tests on pools of up to 3 samples (swab matrix only) for detection of *Coxiella burnetii.*

▪ Kit composition and storage conditions

The IDQFCH kit contains the reagents shown below:

Reference	*Component*	*Volume*	*Description*
PLS-QF	Plasmid	50 µl 1 tube (blue cap)	Calibrated synthetic DNA target of *Coxiella burnetti*.
TPC-QFCH	Target Positive control	550 µl 1 vial	Inactivated *Coxiella burnetii* bacteria, and inactivated *Chlamydophila abortus* vaccinal strain, diluted in a negative endo-cervical swabs matrix, freeze-dried and calibrated at between 10 and 100 times the detection limit of the method (MDL). *Coxiella burnetii* target is calibrated at 1 x 10^4 GE*/ml. This is the clinical decision threshold for individual analysis for France. Freeze-dried pellet to be reconstituted in **550 µl** distilled or Nuclease-free water.
ARM-QFCH	Amplification Reaction Mix	400 µl 1 or 2 tubes (white cap)	Ready-to-use reaction mixture containing Taq polymerase and oligonucleotides for detection of *Coxiella burnetti*, of *Chlamydophila spp* and of the endogenous non-target positive control.

All components should be stored at ≤ -16°C. It is recommended to prepare aliquots (minimum 100 µl) in order to avoid multiple freeze/thaw cycles (> 3 not recommended).

*GE : Genome equivalent

▪ Required equipment, not provided in the kit

All material used should be of suitable quality for molecular biology.

Amplification Instrument:

Real-time thermal cycler with channels capable of reading the following fluorophores: FAM, HEX or VIC and Cy5.

Examples of compatible thermal cyclers: CFX96™, Chromo4™ (Biorad), LC®480 I, LC®480 II, LC®96 Roche, AB® 7500 and Rotor-Gene Q Qiagen. Please contact **support.genetics@id-vet.com** regarding suitability with other thermal cyclers.

Consumables:

- Precision pipettes capable of delivering volumes of between 1 µl and 1000 µl
- Nuclease-free filtered tips
- 1.5 ml tubes
- 96-well PCR plates, strips or PCR micro-tubes (with optical quality compatible with the thermal cycler) and appropriate adhesive films or caps

Reagents:

- Distilled or Nuclease-free water (recommended)
- 1X TE (10mM Tris- 1mM EDTA) buffer for Plasmid dilution

Remarks and precautions

The material used contains less than 0,1% hazardous or carcinogenic substances, thus MSDS sheets are not required. However, it is recommended to take appropriate precautions, as with any biochemical product, and to wear appropriate clothing.

Extraction and amplification controls

The number of controls to be analyzed according to the type of PCR to be used, for the detection of *Coxiella bunetii* is described in the table below.

		Type of analysis to be used	
		Qualitative	Quantitative
Amplification controls	*PLS-QF to be diluted for quantification*		x
	NAC (Negative Amplification Control)	x	x
Extraction controls	*TPC-QFCH (Target Positive Control)*	x	x
	NEC (Negative Extraction Control)	x	x

▪ Positive controls

The IDQFCH kit contains the following positive controls:

- Endogenous Non-Target Positive Control (NTPCen):

This control is constitutively expressed in the cells of sample to be tested. It validates cell lysis and amplification of a non-target gene. It also confirms the presence of cells, and gives an indication as to quality of the sample.

- Target Positive Control (TPC-QFCH):
Inactivated *Coxiella burnetii* bacteria, and inactivated *Chlamydophila abortus* vaccinal strain, diluted in a negative endo-cervical swabs matrix, freeze-dried and calibrated at between 10 and 100 times the detection limit of the method (MDL). *Coxiella burnetii* target is calibrated at 1 x 10^4 GE/ml. This is the clinical decision threshold for individual analysis for France.

This control is prepared and extracted in the same way as samples.

- Q Fever Plasmid (PLS-QF):
This control consists of a synthetic target DNA calibrated in GE/ml (genome equivalent/ml) of sample (refer to the Quality Control Certificate to get the value in GE/ml). This control allows quantitative interpretation of the results. It is be used to prepare by serial dilutions a standard curve of quantification (standard curve method).
The preparation of a standard curve from this synthetic DNA and then its amplification by the real-time PCR allows the quantification of the samples. This allows a quantitative analysis of a sample (number of bacteria /ml).

▪ Negative controls

It is recommended to include the following negative controls in each run:

- Negative extraction control (NEC)

It is recommended to include a negative control for extraction (**NEC)** in each run.
This control should be prepared and extracted in the same way as samples, but should not contain target DNA from *Coxiella burnetii* nor *Chlamydophila spp*. The volume occupied by the sample is replaced by a negative matrix or Nuclease-free water.

- Negative control for amplification (NAC)
This control contains 8 µl of reaction mix (ARM-QFCH) and 5 µl of Nuclease-free water. It is included in each analysis cycle to control for the presence of aerosol contaminants.

Amplification protocol

▪ Extraction of DNA

The DNA of *Coxiella burnetti* and *Chlamydophila spp* must be extracted from the sample before being amplified by PCR.
For this, IDvet Genetics offers a range of extraction kits that conform to the French NF U47-600 standard:

Description	***Product name***	***Product code***
Magnetic bead extraction system	ID Gene™ Mag Universal Extraction Kit	MAG192/MAG384
	ID Gene™ Mag Fast Extraction Kit	MAGFAST384
Column extraction system	ID Gene™ Spin Universal Extraction Kit	SPIN50/SPIN250

*Contact **support.genetics@id-vet.com** for more information.*

▪ Extraction of the control

The volumes of the control to extract is described in the table below:

Important:
- The volumes indicated are valid regardless of the extraction system.
- The controls must be extracted at the same time as the samples.

Control	***Volume***
TPC-QFCH	**50 µl**

*Note: If the **NEC** is prepared with a negative matrix sample, refer to the extraction kit protocol for the corresponding matrix.*

▪ Preparation of the real-time PCR amplification reaction

1. Prepare an experimental plan for the analysis of the samples and controls, being sure to distance the positive control (PLS-QF and TPC-QFCH) from the other samples.
2. Thaw the IDQFCH kit, ideally at 5°C (± 3°C) in a refrigerated rack. Thaw at room temperature 21°C (± 5°C) only if the mix is to be used immediately after thawing.
3. For **quantitative analysis**, dilute extemporaneously the PLS-QF by ten-fold serial dilutions in 1X TE buffer.

   Note: Ideally test 5 points to evaluate the linearity and efficiency of the calibration range.
4. Homogenise the contents of the **ARM-QFCH** tube by vortexing. Centrifuge down briefly.
5. Distribute **8 µl of ARM-QFCH** per well. Use PCR strips, or microplates adapted to the thermal cycler in use.
6. Add the following to the reaction mix :
   - 5 µl of DNA extracted from each sample to be analyzed
   - 5 µl of TPC-QFCH
   - 5 µl of each PLS-QF dilutions
   - 5 µl of extracted NEC
   - 5 µl of Nuclease-free water (NAC)
7. Cover the plate or strips with appropriate adhesive film or caps.

▪ Programming the amplification phase

1. Program the thermal cycler detectors to read the following wavelengths for each well:

Target	*Channel capable of reading*	*Quencher*
Coxiella burnetti specific sequence	FAM	non fluorescent*
Chlamydophila spp specific sequence	VIC/HEX	non fluorescent* (compatible VIC/HEX)
Cell-specific sequence (NTPCen)	Cy5	non fluorescent*

Note: For devices requiring an internal reference, the amplification mix ARM-QFCH contains ROX.
**Using a TAMRA™ quencher can improve the data analysis with some instruments.*

2. Choose between the two 2 different amplification programs validated by IDvet Genetics:
   - Standard program (allows for PCR kits from different vendors to be used in a single run)
     or
   - Rapid program

Step	*Standard program*	*Rapid program*	*Number of cycles*
(1) Polymerase activation	10 min at 95°C	2 min at 95°C	1
(2) DNA denaturation/elongation	15 sec at 95°C 60 sec at 60°C	10 sec at 95°C 30 sec at 60°C	40

Note: The fluorescence reading is read at the end of the elongation phase at 60°C.

3. Enter one or these programs in the thermal cycler and select a final volume of **13 µl per PCR**. If different volumes are combined in a single run, enter the largest volume on the plate.
4. Place the PCR plate, PCR strips or capillaries in the thermal cycler and start the program.

Validation and interpretation of results

▪ Assay validation

The analysis of results is based on the Cq (Quantification cycle) value of each sample that is obtained by each detector. The Cq is also known as the Ct value (Threshold cycle).
The test is validated according to criteria outlined in the table below. **Results should not be interpreted if any of these criteria are not met**.

Control	*Expected result*	*Acceptability criteria*
TPC-QFCH	**Detected** in FAM, VIC/HEX and Cy5	Presence of a characteristic curve Refer to the Cq value indicated in the quality control certificate of the corresponding batch
NEC	if water used : No detection	Absence of a characteristic curve
	if negative matrix used : **Detected i**n Cy5	Presence of a characteristic curve
NAC	No detection	Absence of a characteristic curve
PLS-QF *Quantitative analysis*	Minimum 4 dilutions of the range detected in FAM*	Efficiency value between 85 and 115% R^2 higher than 0.980

** Please refer to the different values of the standard curve indicated on the quality control certificate of the corresponding batch.*

Note: The TPC-QFCH may be used to monitor variations in analytical sensitivity as it is calibrated at between 10 and 100 times the MDL.

- **Suggested Qualitative interpretation of results**

For each sample, results may be interpreted according to the following criteria:

Analysis	*Sample*	*QF Signal*	*CHLM Signal*	*NTPCen Signal*	*Interpretation*
Qualitative	**Individual or pool** *(Pool testing for QF only)*	**Detected**	**Detected**	Detected or Not Detected	The sample or the pool of samples are **Detected** as positive for Q Fever and Chlamydia
		Not Detected	**Detected**	Detected or Not Detected	The sample is **Detected as positive** for Chlamydia
		Detected	Not Detected	Detected or Not Detected	The sample or the pool of samples are **Detected** as positive for Q Fever
		Not Detected	Not Detected	**Detected**	**Not detected** as positive for Q Fever nor Chlamydia
		Not Detected	Not Detected	Not Detected	PCR reaction was inhibited

- **Suggested Quantitative interpretation of results (only for *Coxiella burnetii*)**

For each sample, results may be interpreted according to the following criteria:

Analysis	*Sample*	*QF Signal*	*NTPCen Signal*	*Interpretation*
Quantitative	**Individual or pool**	**Detection** of x bacteria / ml of sample	Detected or Not detected	The sample or the pool of samples are **Detected as positive** at x bacteria / ml of sample
		Detected unquantifiable	Detected or Not detected	The sample or the pool of samples are **Detected as positive** in quantity below the limit of quantification of the method (LQm*)
		Not detected	Detected	**Not detected** as positive for Q Fever
		Not detected	Detected or Not detected	PCR reaction was inhibited

* LQm = 5 x10^2 GE/ml = value of the last dilution of the standard curve indicated on the quality control certificate.

Quantitative analysis

The amount of the *Coxiella burnetii.* target present in each extract of nucleic acid detected as positive is obtained by using the real-time PCR software and the values of the standard curve.

For liquid samples: The NL number of target per ml of initial matrix is determined as follows:

NL = Nr x (Ve/Vp)

NL = Number of genome equivalent per matrix volume (GE/ml)
Nr = Number of GE / ml in the PCR reaction (according to standard curve method)
Ve = Volume of elution after extraction (µl)
Vp = Volume of sample used before extraction (µl)

For solid samples: The NS number of target per mg of initial matrix is determined as follows:

NS = NL x (V_{PBS} / m)

NS = Number of genome equivalent per tissue mass (GE / mg)
NL = Number of genome equivalent per matrix volume (GE / ml organ supernatant) determined above
V_{PBS} = Volume of buffer used for grinding
m = Quantity of the sampling in mg

Non-validated samples:

- **If the NTPCen is not detected**, a problem occurred during sample distribution or during the extraction process. In this case, the sample should be extracted again.

Procedure to follow if the PCR reaction was inhibited:

1. Dilute the extracted DNA 10 times in Nuclease-free water.
2. Perform a new amplification reaction on 5 µl of this dilution. Apply the dilution factor 10 in the new quantification (standard curve) using the PLS-QF.
3. If the NTPCen is detected, interpret the sample according to the table above.
4. If the NTPCen is not detected, re-extract the sample or consider it uninterpretable.

Documentation and support

For questions or technical support, please contact: **support.genetics@id-vet.com**

For additional information, visit **www.id-vet.com**