IDvet GENETICS

Certified management system

ID Gene™ Q Fever Triplex

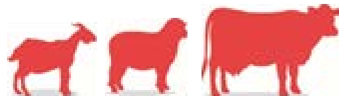
Product code : IDQF-50 / IDQF-100

50 tests / 100 tests

Real-time PCR for the relative qualitative or quantitative detection of *Coxiella burnetii*

Suitable samples: swab supernatant (placental, vaginal, cervical), milk, spleen, gastric juice, faeces

***In-vitro* Use**



September 2017 :
- Addition of a new sample type: Faeces

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General information

▪ Characteristics

ID Gene™ Q Fever Triplex (IDQF) kit is a real-time PCR kit that amplifies a target sequence in *Coxiella burnetii* genome, that allows for the diagnosis of Q Fever.

This kit is a **relative qualitative or quantitative triplex test**. It simultaneously amplifies DNA target of *Coxiella burnetii*, endogenous and exogenous non-target positive controls.

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

▪ Kit composition and storage conditions

The IDQF kit contains the reagents shown below:

Reference	*Component*	*Volume*	*Description*
PLS-QF	Plasmid	50 µl 1 tube (blue cap)	Calibrated synthetic nucleic acid for quantitative testing.
NTPC-QF	Non-Target Positive Control	2200 µl 1 vial	Non-pathogenic bacteria to be used as mime of the target. Freeze-dried pellet to be reconstituted in **2200 µl** distilled or Nuclease-free water.
ARM-QF	Amplification Reaction Mix	400 µl 1 or 2 tubes (white cap)	Ready-to-use reaction mix containing the Taq polymerase and oligonucleotides for amplification and detection of *Coxiella burnetii*, of the endogenous and exogenous non-target positive controls.

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).

▪ Required equipment, not provided in the kit

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

Amplification Instrument:

Real-time thermal cycler capable of reading the following wavelengths: 525 nm (FAM), 548 nm (Yakima Yellow, equivalent to VIC) and 650 nm (Cy5).

Examples of compatible thermal cyclers: CFX96, Chromo4 Biorad, LC480 I, LC480 II, LC96 Roche, 7500 AB, and Rotor-Gene Q Qiagen. Please contact us for use 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 (that have an optical quality compatible with the thermal cycler) and appropriate adapted adhesive film or caps

Reagents:

- Distilled or Nuclease-free water
- 1X TE (Tris-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

▪ Positive controls

The IDQF kit contains the following positive controls:

- Exogenous Non-Target Positive Control (NTPC-QF)

The exogenous positive control is a non-pathogenic bacteria using as mime of the target.
This control allows to evaluate the efficiency of the extraction process and to detect the presence of inhibitors in the amplification step.
This control is to be added to every sample as well as to the other control (NEC) before extraction.

- Endogenous Non-Target Positive Control (NTPCen):

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

- 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 methode).
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:

- Negative extraction control (NEC)

It is recommended to include a negative control for extraction (**NEC)** in each run.
This control is to be prepared and extracted in the same way as samples, but does not contain any target DNA. The volume occupied by the sample is replaced by a non-reactive matrix or Nuclease-free water.

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

Amplification protocol

▪ Extraction of bacterial DNA

The bacterial DNA of *Coxiella burnetii* must be extracted from the sample before being amplified by RT-PCR.
For this, IDvet Genetics offers a range of extraction kits that is compliant with the French NF U47-600 standard:

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

▪ Extraction of the controls

The volumes of the control to extract are 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*
NTPC-QF	**20 µl** to add to NEC and to each sample

*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, making sure to distance the positive control (PLS-QF) from the other samples.
2. Thaw the IDQF 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 standard curve method.*
4. Homogenise the **ARM-QF** tube by vortexing. Centrifuge down briefly.
5. Distribute **8 µl of ARM-QF** per well. Use PCR strips, or microplates adapted to the thermal cycler in use.
6. Add :
   - 5 µl of DNA extracted from each sample to be analyzed
   - 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 step

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

Target	*Fluorophore*	*λ (nm)*	*Quencher*
QF	FAM	495-525	non fluorescent
NTPCen	VIC / Yakima Yellow	426-548	non fluorescent (compatible VIC/HEX)
NTPC-QF	Cy5	649	non fluorescent

Note: For devices requiring an internal normalization of the signal , the amplification mix IDQF contains ROX.

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 session) 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 taken at the end of the elongation phase at 60°C.

3. Enter one of 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 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
NTPC-QF	Detected in Cy5 in each sample	+ 3 Cq compared to NEC Cq's
NTPCen	Detected in VIC in each sample	Presence of a characteristic curve
NEC	Detected in Cy5 if water used	+ 3 Cq compared to the Cq value indicated in the quality control certificate
	Detected in VIC and Cy5 if negative matrix used	Presence of a characteristic curve
NAC	No detection	Complete absence of a characteristic curve
PLS-QF Quantitative analysis	Minimum of 4 dilutions of the range detected in FAM	Efficiency value between 85 and 115% R^2 higher than 0.980

Suggested interpretation of results

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

Analysis	QF signal	NTPC-QF signal	Interpretation
Relative qualitative	Detected	Detected or Not detected	Detected as positive for Q Fever
	Not detected	Not detected or $Cq_{NTPC-QF} > Cq_{NEC} + 3$	PCR reaction was inhibited
	Not detected	detected	Not detected as positive for Q Fever
Quantitative*	Detection of x bacteria / ml of sample	Detected or Not detected	Detected as positive at x bacteria / ml of sample

*** 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 NTPC-QF is not detected but the sample is detected positive for QF, consider the sample as positive.**
- **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.
- **If $Cq_{NTPC\text{-}QF} > Cq_{NEC} + 3$ and no signal is detected for QF**, the reaction is inhibited. In this case, perform a new amplification run following the procedure below.

Procedure to follow if the PCR reaction was inhibited:

1. Dilute the extracted DNA 10 times in Nuclease-free water.
2. Perform a new real time amplification on 5 µl of this dilution.
3. If the NTPC is detected, interpret the sample according to the table in page 4.
4. If the NTPC is not detected, re-extract the sample or consider it uninterpretable.

Documentation and support

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

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