ADIAVET™ COXIELLA REAL TIME

TEST FOR THE DETECTION OF *COXIELLA BURNETII* BY REAL-TIME ENZYMATIC DNA AMPLIFICATION (PCR TEST)

References:
ADI143-100 (100 reactions)

VET

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English version
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I. Revision history

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 history.

Release Date	Part Number	Change type	Change summary
2013/11	NE143-07	Technical change	Addition of "Extraction and purification using DNA/RNA magnetic beads kit" paragraph, in page 12, § V-3.
2013/11	NE143-07	Technical change	Addition of "Definitions" paragraph, in page 14, § VII-1.
2013/11	NE143-07	Technical change	Addition of "Validation and interpretation of quantitative results" paragraph, in page 15, § VII-3.
2014/12	NE143-08	Technical change	Addition of "Index of symbols" section, in page 18.
2014/12	NE143-08	Technical change	Removal of reference ADI143-50 (50 reactions)
2016/07	NF143-09	Administrative	Changing logos
2016/07	NF143-09	Administrative	Biosearch legal mention
2016/07	NF143-09	Technical change	Addition of table "Analysis options according to the specimen" §I.3.
2018/05	NF143-10	Administrative	Modification of the address of ADIAGENE page 21, footer

II. General information

1. Purpose of the test

ADIAVET™ COXIELLA REAL TIME kit is intended to detect and to quantify *Coxiella burnetii* using real-time Polymerase Chain Reaction (PCR) technology from swab, tissue, faeces, amniotic fluid and milk specimens of bovine, ovine and caprine.

2. Pathogen

Coxiella burnetii is a strictly intracellular Gram-negative bacterium, agent of the Q fever (Burnet and Freeman, 1937; Cox, 1938). The disease is present worldwide. *C. burnetii* is able to infect a large range of hosts including humans. Q fever is a **zoonose** (pathology which can be transmitted to human) mainly transmitted by ovine, bovine and caprine. Q fever usually results from inhalation of contaminated aerosols originating mostly from dropping and body fluids of infected animals.

C. burnetii is present in reproductive apparatus, placenta and fluids produced during parturition or abortion. The bacterium is excreted in milk and faeces of infected animals presenting no clinical signs. Mostly infections are asymptomatic or subclinical by Human, but can be manifested as a flu-like disease, or as hepatitis. A neurological involvement is also possible. Pericarditis and myocarditis are rare. *C. burnetii* can induce abortions, causing economic losses in cattle. Prevention consists mostly in precautions with pregnant females and with fluids from parturition.

Detection of *C. burnetii* by culture is long and difficult. Detection of antibodies doesn't give information on sanitary status of the animal.

The real-time PCR kit, ADIAVET™ COXIELLA REAL TIME, allows the identification of the bacteria *C. burnetii*. It is applicable to a large number of samples and various biological matrixes.

3. Test performance

This PCR test was assessed against a panel of 130 issues from organisms preferencially found in the same ecologic niche and/or close phylogeneticaly, among which *Legionella*. No crossing reaction was observed.
The PCR detection limit is 1.5 *C. burnetii* /5µl PCR.
The PCR quantification limit is 2 *C. burnetii* /5µl PCR.

4. Description and purpose of the test

This test is based on enzymatic gene amplification or PCR technology.
Amplified products are detected in real-time thanks to a specific labelled hydrolysis probe (5'-exonulease technology).
The ADIAVET™ COXIELLA REAL TIME kit enables the simultaneous detection of:

- The target *Coxiella burnetii* (probe labelled in FAM), specific of the IS1111 sequence
- GAPDH, an internal control of extraction and amplification steps specific from an endogenous DNA (probe labelled with a fluorochrome read in the same spectra as VIC and HEX).

An exogen control EPC-Ext added during the extraction of acellular samples allows validating extraction and amplification steps (probe labelled with a fluorochrome read in the same spectra as VIC and HEX).
The COX CTL+ contains a known amount of *C. burnetii* DNA. It allows the quantification of the positives samples. The mesure unit is the equivalent genome (EG) number (or bacteria) per ml.
Each sample is analysed in a single well.

ADIAGENE validated the test using DNA purification kits (Qiagen, Macherey-Nagel). Other purification kits can be used if they have been validated by the user.

Analysis options according to the specimen:

Specimen	Individual analysis	Pool of sample is possible*, up to
Swab (placental, vaginal…)	☑	3
Tissue (placenta, foetal tissues…)	☑	☒
Faeces	☑	☒
Amniotic fluid	☑	☒
Milk	☑	☒

* It depends on the epidemiological case and on the quality of the specimen.

III. Material and reagents

1. Reagents provided with the kit

Designation	Reagent	ADI143-100
A5	Amplification solution	2 x 1000 µl green tubes
COX CTL+	Positive control *Coxiella burnetii*	1 purple tube
EPC-Ext	External control of extraction	2 x 300 µl yellow tubes

2. Validity and storage

On receipt, the whole kit should be stored at **<-15°C**.
It is recommended to make fractions of A5 solution if it should be defrosted more than 3 times.
Do not defrost reagents more than 3 times.
Realtime reagents are susceptible to light: store them in the darkness.
The A5 reagent is ready to use for PCR reaction.
Do not mix reagents of two different batches.

3. Use of controls

A. Use of EPC-Ext

Make fractions of EPC-Ext and store them at <-15°C. It should not be defrosted more than 3 times.
For each feaces or acellular sample, it is recommended to add 5 µl of EPC-Ext per sample.

B. Use of COX CTL+

Carefully use this CTL+. It contains a large amount of DNA that could be a source of contamination.

The tube COX CTL+ contains a titrated solution of *C. burnetii* DNA as EG/ml or as *C. burnetii*/ml.

Add **200 µl** of Nuclease-free water to the COX CTL+ tube included in the kit. Homogenize tube contents using a mixer such as vortex, at least 20 seconds. Aliquot this solution by 12 µl and store them to <-15°C.

This tube COX CTL+ should not be defrosted more than 3 times.

Realise a standard range with 10-fold serial dilutions of COX CTL+

A standard range	*DNA Concentration (C. burnetii/ml)*
pure	$4x10^6$
1/10	$4x10^5$
1/100	$4x10^4$
1/1000	$4x10^3$
1/10000	$4x10^2$

The dilutions are performed in Nuclease-free water.

If you want to make the qualitative PCR:
Five microlitres of the range 1/1000 will be used per analysis.

If you want to make the quantitative PCR:
Five microtitres of each range will be used per analysis.

4. Equipment required but not supplied

Material should be Nuclease-free (e.g. autoclaved 25 minutes twice at +120°C)

- Thermal cycler with consumables for real-time PCR: 0.2 ml PCR tubes or closed 96-wells PCR plates with optical quality.
- Class II Microbiological Safety Cabinet
- Centrifuge for microtubes, tubes of 5, 10 or 15 ml
- Etuve, heating baths or block heaters (+70°C)
- Instrument for homogenous mixing of tubes
- 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 5, 10 or 15 ml
- Powder-free Latex gloves
- 96-100% ethanol solution
- PBS buffer 1X pH 7.4
- Nuclease-free water

- Manual DNA extraction kit (individual columns)

- QIAamp® DNA Mini Kit (Qiagen, 50 tests: ref. 51304 or 250 tests: ref. 51306)
- NucleoSpin® Tissue (Macherey-Nagel, 50 tests: ref.740952.50 or 250 tests: ref. 740952.250)

or

- Automated DNA/RNA extraction kit (magnetic beads)

See the NEKF user manual available of the web site mentioned on the certificate of analysis included in the used ADIAVET™ kit.

IV. Recommandation before the analysis of samples

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

1. Precautions

Adiagène has elaborated this PCR test with the use of Qiagen, Macherey-Nagel and extraction kits. Other extraction kits can be used with a previous validation.

Follow the supplier's instructions for the storage, the preparation and the use of the extraction reagents.

Some kits include and/or need the use of toxic reagents. These reagents should be use with gloves and into chemical cabinet.

We strongly recommend that only appropriately trained personnel perform this test. The quality of the obtained results depends upon rigorous respect of good laboratory practices. Ensure the accuracy and precision of the micropipettes used.

The PCR generates large amount of amplified DNA. A few molecules of amplified products are sufficient to generate a positive result. It is important to reserve 2 rooms, one for manipulation of samples to be tested, and another one for amplified products analysis. Do not open the PCR tubes 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.**

We recommend using fractions of PBS 1X. Take a new fraction for each new manipulation to avoid contamination.

2. Samples receipt

Samples should be cleaned and disinfected before to be identified and stocked. Control the integrity of the sample.

3. Storage of samples and DNA extracts

Samples can be stored a couple of days at +2/8°C. After 2 days, we recommend to store them at <-15°C. Extracted DNAs are quite sensitive molecules. Crude extracts should be stored at the end of extraction at +2/8°C for 24 hours, then at <-15°C.

4. Samples preparation

A. Placentas

Warning!
Placentas can contain a large number of bacteria. They should be manipulated with extreme precaution using Class II Microbiological Safety Cabinet.

To remove surface contaminants, it is recommended to soak the cotyledons sampled in a bath of iodized alcohol (or 70% alcohol) for a few seconds and transfer them in a Petri dish, then cut with a scalpel. Rub within three cotyledons using three dry swabs.

Introduice the three swabs in a previously identified 5 ml sterile tube and cut if necessary the stem of the swab. Add **3 ml** of PBS 1X pH 7.4 to the swabs. Homogenize for 30 seconds. Transfer **200 µl** of the supernatant in a previously identified microtube.
See § IV for the extraction and purification of DNA.

B. Vaginal swabs.

The type of swab is a dry swab, cotton, with a flexible plastic stem.

For the individual analysis

Introduice the vaginal swab in a previously identified 2 ml-microtube and cut if necessary the stem of the swab. Add **1 ml** of PBS 1X pH 7.4 to the vaginal swab. Homogenize for 30 seconds. Transfer **200 µl** of the supernatant in a previously identified microtube.
See § IV for the extraction and purification of DNA.

For the pool analysis

Introduice three vaginal swabs in a 5 ml previously identified sterile tube and cut if necessary the stem of the swabs. Add **3 ml** of PBS 1X pH 7.4 to the vaginal swabs. Homogenize for 30 seconds. Transfer **200 µl** of the supernatant in a previously identified microtube.
See § IV for the extraction and purification of DNA.

C. Milk

Homogenize the milk. Transfer **200 µl** of individual milk or of bulk milk in a previously identified microtube.
See § IV for the extraction and purification of DNA.

D. Tissues

Place **20-30 mg** of minced tissues in a previously identified microtube.
See § IV for the extraction and purification of DNA.

E. Foetal liquid

Homogenize the foetal liquid. Transfer **200 µl** of foetal liquid in a previously identified microtube.
See § IV for the extraction and purification of DNA.

F. Faeces

Place **1 g** of faeces in a previously identified 10 ml or 15 ml sterile tube. Add **5 ml** of PBS 1X pH 7.4. Homogenize the tube for about 30 seconds. Centrifuge at 3 000 g for 2 minutes. Transfer **200 µl** of the supernatant in a previously identified microtube.
Add **5 µl** of **EPC-Ext** in the sample.
See § IV for the extraction and purification of DNA.

5. Controls to include

Several controls should be included per trial of analysis.
The internal endogenous control (GAPDH) naturally found in the samples allows verifying the extraction and amplification steps of each sample. The COX CTL+ allows validating the amplification of the *C. burnetii* target.
The mix of the different controls allows validating all the steps (extraction and amplification) of the analysis process for all the samples.
Other controls should or must be added.

- **Negative control of extraction (required)**

  To verify the absence of cross-contamination, at least one negative control must be included per trial (e.g. AFNOR NF U47-600 guidelines suggests to include a negative control per 24 columns centrifuged). The control is a negative sample, for example the buffer used for dilutions.

- **Positive control of extraction (recommended)**

  A positif control could be added in each trial. The control is a sample including *C. burnetii*.
  It could come from a positive sample available in the laboratory or from a negative sample spiked with a solution of *C. burnetii*. This positive control will be closed to the limit of detection of the method (e.g. 1 to 100 X LD_{METHOD}). It will inform about the fidelity of the obtained results between different trials.

V. Extraction and purification

1. Using QIAamp® DNA Mini kit

All the centrifugations are performed at room temperature.

Before extraction switch on incubators, block heaters and/or heating baths at +70°C or at +56°C.
Identify the columns and microtubes corresponding to the samples.

Sample	Take **200 µl** of **liquid sample** or **20/30 mg** of **minced tissues**, prepared as described before.
Lysis	Add **180 µl** of **buffer ATL** + **20 µl** of **proteinase K**. Homogenize byusing a mixer such as vortex, at least 5 seconds. Incubate **30 minutes** at **+70°C** (or a night at +56°C).
	Add **200 µl** of **buffer AL**. Homogenize by using a mixer such as vortex, at least 5 seconds. Incubate **10 minutes** at **+70°C**.
Binding preparation	Add **200 µl** of **ethanol 100%**. Homogenize by using a mixer such as vortex, at least 5 seconds.
Transfer to columns and binding to the membrane	Identify columns, apply the the **whole obtained solution** to the corresponding column. Centrifuge 1 minute at 10 000 g. *If the whole sample has not been loaded once, apply the residual volume onto the column and centrifuge 1 minute at 10 000 g.*
1st wash	Change the collection tube and add **500 µl** of **buffer AW1** to the column. Centrifuge 1 minute at 10 000 g.
2nd wash	Change the collection tube and add **500 µl** of **buffer AW2** to the column. Centrifuge 1 minute at 10 000 g.
Column dry step	Change the collection tube. Centrifuge 3 minutes at 10 000 g.
Elution	Transfer the column to a microtube. Add **200 µl** of **buffer AE**. Incubate ~1 minute at room temperature and centrifuge 1 minute at 10 000 g.
Storage	Close the tubes, identify and store at +2/8°C for 24 hours, then at <-15°C.

2. Using NucleoSpin® Tissue

All the centrifugations are performed at room temperature.

Before extraction switch on incubators, block heaters and/or heating baths at +70°C or at +56°C.
Identify the columns and microtubes corresponding to the samples.

Sample	Take **200 µl** of **liquid sample** or **20/30 mg** of **minced tissues**, prepared as described before.
Lysis	Add **180 µl** of **buffer T1** + **25 µl** of **proteinase K**. Homogenize by using a mixer such as vortex, at least 5 seconds. Incubate **30 minutes** at **+70°C** (or a night at +56°C).
	Add **200 µl** of **buffer B3**. Homogenize by using a mixer such as vortex, at least 5 seconds. Incubate **10 minutes** at **+70°C**.
Binding preparation	Add **200 µl** of **ethanol 100%**. Homogenize by using a mixer such as vortex, at least 5 seconds.
Transfer to columns and binding to the membrane	Identify columns, apply the the **whole obtained solution** to the corresponding column. Centrifuge 1 minute at 10 000 g. *If the whole sample has not been loaded once, apply the residual volume onto the column and centrifuge 1 minute at 10 000 g.*
1st wash	Change the collection tube and add **500 µl** of **buffer BW** to the column. Centrifuge 1 minute at 10 000 g.
2nd wash	Change the collection tube and add **600 µl** of **buffer B5** to the column. Centrifuge 1 minute at 10 000 g.
Column dry step	Change the collection tube. Centrifuge 3 minutes at 10 000 g.
Elution	Transfer the column to a microtube. Add **200 µl** of **buffer BE**. Incubate ~1 minute at room temperature and centrifuge 1 minute at 10 000 g.
Storage	Close the tubes, identify and store at +2/8°C for 24 hours, then at -15°C.

3. Using RNA/DNA magnetic beads

See the NEKF user manual available of the web site mentioned on the certificate of analysis included in the used ADIAVET™ kit.

VI. Amplification

a - Determine the number samples to analyze, including the following controls:

Control	*Qualitative analysis*	*Quantitative analysis*
COX CTL+ pure	No	**Yes**
COX CTL+ 1/10	No	**Yes**
COX CTL+ 1/100	No	**Yes**
COX CTL+ 1/1000	**Yes**	**Yes**
COX CTL+ 1/10000	No	**Yes**
Negative control of amplification	**Yes**	**Yes**
Negative control of extraction	**Yes**	**Yes**
Positive control of extraction	Recommended	Recommended
Whole number of controls	**3 (or 4)**	**7 (or 8)**

b – Defrost the A5 solution at room temperature. Homogenize. Dispense **20 µl** of A5 solution in each PCR tubes or PCR plate wells with a micropipette with a Nuclease-free tip. **Immediately replace the A5 solution tube at <-15°C and in darkness.**

c- For each test, add **5 µl** of purified DNA or 5 µl of controls to the 20 µl of A5 solution. For the negative control of amplification (NTC), nothing is added to the A5 solution.
Immediately replace the purified DNA extracts at +2/8°C or at <-15°C. Take care to have no bubbles in the bottom of the wells.

d- Once all the tubes have been prepared, run real-time PCR amplification.

The *Coxiella burnetii* target is read in FAM. The Internal Control is read in VIC or HEX. The Quencher is non-fluorescent. The solution contains a passive reference ROX for the ABI machines. Fluorescence is read during the elongation step (1 minute at 60°C).

The following program is defined for **ABI Prism** thermalcyclers (like 7500, StepOne...) from **Applied Biosystems** (check the "emulation 9600" option if available), for the **Rotorgene** from **Qiagen** and for the **Chromo 4** from **Biorad**:
2 minutes 50°C
10 minutes 95°C
15 seconds at 95°C and 1 minute at 60°C during 45 cycles

This program is concerning the **MX3000P** and **MX3005P** of **Stratagene**:
2 minutes 50°C
10 minutes 95°C
30 seconds at 95°C and 1 minute at 60°C during 45 cycles

Roche diagnostic: LightCycler 2*, LightCycler 480*
**** NOTE:*** *The use of LightCycler thermalcyclers requires a calibration manipulation. Adiagene will furnish process chart and reagents required for this calibration.*

Contact us if you wish to use other thermalcyclers.

VII. Interpretation of results

1. Definitions

The **« base line »** corresponds to the background of fluorescence and qualifies the non-characteristic part of the curve observed during the first cycles.

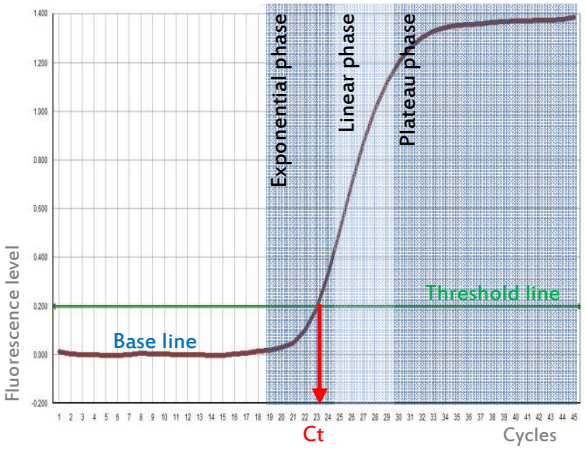
The **« Characteristic amplification curve »** qualifies a fluorescence curve with an exponential phase, a linear phase and a plateau phase.

The **« threshold line »** has to be placed over the background in the exponential phase of a characteristic amplification curve or a group of characteristic amplification curves.

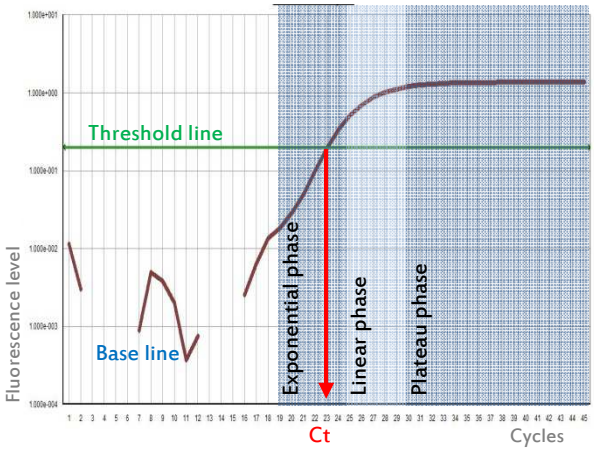
The **« threshold cycle » (Ct)** of a well corresponds, for each detected fluorophore, at the crossing point of the threshold line with the fluorescent curve. The Ct value expressed by the machine for each well depends on the threshold position and on the quantity of target sequences initially present in the PCR well.

Example of characteristic amplification curve

Arithmetic scale of Y axis



Logarithmic scale of Y axis



2. Reading and validation of qualitative results

Display the FAM curves from the plate and set the threshold value as indicated above.
Proceed in the same mean for the VIC or HEX curves.

A. Validation of the run

The PCR test is considered to be **valid** if results are as described in the table.

Controls	Qualitative analysis	
	FAM Amplification	VIC or HEX Amplification
COX CTL+ 1/1000	Yes	No
Negative control of amplification	No	No
Negative control of extraction in Matrix/PBS	No	Yes/No
Positive control of extraction in Matrix/PBS	Yes	Yes/No

The indicative Ct values expected in FAM and VIC or HEX for the positive control is noted on the certificate of analysis of the ADIAVET™ COXIELLA REAL TIME kit. If the results of the controls are corresponding to the above table, samples analysis is allowed.

B. Result interpretation

DNA extraction and amplification for each sample are considered to be **valid** if at least a characteristic amplification curve is observed for *C. burnetii* (FAM) or for the internal control (VIC or HEX).

Example	A	B	C	D
FAM amplification	no	yes	yes	no
VIC/HEX amplification	yes	no	yes	no
Result	negative	positive	positive	undetermined

The sample is considered as **negative** if a characteristic amplification curve is observed in VIC or HEX without any amplification in FAM (example A).

The sample is considered as **positive** if a characteristic amplification curve is observed in FAM (example B). Internal control can be co-amplified (example C).

A total absence of characteristic amplification curve for a sample (example D) shows a defective DNA extraction (lost or destruction of DNA) or a deficient real-time PCR (inhibitors in the sample, program error or no template added). In this case, we recommend first to repeat the test with pure and tenfold diluted DNA in Nuclease-free water. Then, if the test is still not valid, a new extraction is recommended.

3. Validation and interpretation of quantitative results

Display the FAM curves from the plate and set the threshold value as indicated above.
Proceed in the same mean for the VIC or HEX curves.

Expected results for the *Coxiella* range:

Controls	DNA Concentration (*C. burnetii*/ml)	FAM Amplification	VIC or HEX Amplification
COX CTL+ pure	$4x10^6$	Yes	No
COX CTL+ 1/10	$4x10^5$	Yes	No
COX CTL+ 1/100	$4x10^4$	Yes	No
COX CTL+ 1/1000	$4x10^3$	Yes	No
COX CTL+ 1/10000 (LQ_{PCR})	$4x10^2$	Yes	No
Negative control of amplification	0	No	No
Negative control of extraction	0	No	No
Positive control of extraction	1 to 100 X LD_{METHOD}	Yes	Yes/No

The indicative Ct values expected in FAM and VIC or HEX for the positive controls are noted on the certificate of analysis of the ADIAVET™ COXIELLA REAL TIME kit. If the results of the controls are corresponding to the above table, samples analysis is allowed

For the quantitative interpretation, check the PCR efficiency. The values of the obtained Ct should increase in proportion with the DNA dilution (about 3.33 Ct for 1/10 dilution). In general the software supplied with the thermalcyclers establishs a calibration line (cycles number = f (log concentration)), calculate the standard curve ($y = ax + b$) and PCR efficiency ($Eff\% = \left(10^{\left(\frac{-1}{a}\right)} - 1\right) \times 100$

Before the results analysis, the standard curve should be validated:

- the 5 points of the standard range should be amplified; however, one point of the range may be remove if this point is not one the two extremes
- $R^2>0.9$
- 85%<Efficacity<115%
- homogenous distribution of the points

The quantification of a positive sample is only possible in the field of quantification of the method used (Cf Registration data).

Interpretation of the results

The interpretation of the result of a sample is represented in the below table:

	FAM Amplification			
	No signal	***Signal <$LQ_{METHODE}$***	***Signal between LQ_{METHOD} and LQmax***	***Signal >LQmax***
Interpretation of the résultat	*Negative* DNA no detected	*Positive:* DNA detected in a quantity less than the LQmethod	*Positive :* DNA quantifiable	*Positive:* DNA detected in a quantity greater than the LQmax

The sample is considered as **positive** for *C. burnetii* if a characteristic amplification curve is observed in FAM. The internal control can be co-amplified in VIC or HEX.

The sample is considered to be **negative** if a characteristic amplification curve is observed in VIC or HEX without any amplification curve in FAM.

A total absence of characteristic amplification curve for a sample shows a deficient PCR (inhibitors in the sample, program error). In this case, we recommend repeating the test with DNA pure and diluted tenfold in Nuclease-free water. Then, if the test is still not valid, a new extraction is recommended.

The direct quantification of positive samples is possible with the aid of the standard range. The equation of the PCR standard range allows determining concentration in *C. burnetii*:

$$x = 10^{\left(\frac{y-b}{a}\right)} \times F$$

With: x: Concentration of *C. burnetii*
y: Ct value FAM of positif sample
b: Intercept
a: Slope
F: multiplying coefficient

The multiplying coefficient is determined according to the type of matrix and extraction method:

Matrix	**Multiplying coefficient *(F)* according to extraction method**		**Units**
	QIAamp® DNA Mini Kit or NucleoSpin® Tissue	**RNA/DNA magnetic beads**	
Vaginal swab, placenta swab, milk, fœtal liquide	1	0,6	*C. burnetii* / ml
Faeces	5	3	*C. burnetii* / g
Tissue	10	3	*C. burnetii* / g

VIII. References

Burnet, F. M., and M. Freeman (1937). Experimental studies on the virus of Q fever. Med. J. Aust. 2:299-302

Cox, H. R. (1938). A filter-passing infectious agent isolated from ticks. III. Description of organism and cultivation experiments. Public Health Rep. 53:2270-2276

Norme AFNOR NF U47-600-1 (Février 2015), Méthodes d'analyse en santé animale – PCR (réaction de polymérisation en chaîne - Partie 1 : exigences et recommandations pour la mise en œuvre de la PCR en santé animale.

Norme AFNOR NF U47-600-2 (Fèvrier 2015), Méthodes d'analyse en santé animale – PCR (réaction de polymérisation en chaîne - Partie 2 : exigences et recommandations pour le développement et la validation de la PCR en santé animale.

IX. Index of symbols

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 – For animal use only

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