Instruction manual
ADL14Y1-COXI_NO_(EN)_V01
01/2023

COXIELLA

Reference: ADL14Y1-100

Test for the detection and quantification of *Coxiella burnetii* by real time enzymatic amplification PCR Test – 100 reactions

For veterinary *in vitro* use only

Sample	Individual analysis	Pool of sample possible*, up to:
Swab (placental, vaginal…)	✓	3
Tissue (placenta, foetal tissues…)	✓	✗
Faeces	✓	✗
Amniotic fluid	✓	✗
Milk	✓	✗

* Depending on the epidemiological case and on the quality of samples

Kit composition

Content		ADL14Y1-100 Kit
		100 reactions
A6	Amplification solution	1 lyophilized vial with blank caps (To reconstitute)
Rehydration buffer	Rehydration solution	1 x 6 mL vial (Ready to use)
COX CTL+	*Coxiella burnetii* positive control	1 tube with purple cap (To reconstitute)
EPC-Ext	Exogenous extraction control for faeces or acellular samples	1 tube with yellow cap (To reconstitute)
NF-Water	Nuclease-Free Water	3 x 1000 µL tubes with white cap (Ready to use)

Revision history

Date	Version	Modifications
01/2023	V01	First version

Note: minor typographical, grammar and formatting changes are not included in the revision history.

A. Introduction

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.

B. Test principle

ADIALYO™ COXIELLA test is based on the amplification of specific *Coxiella burnetii* DNA (IS1111 gene). This test is intended to detect simultaneously, in one well:

- *Coxiella burnetii* (FAM labelled probe)
- GAPDH internal control of extraction and amplification specific from an endogenous nucleic acid (HEX labelled probe or its equivalent).
  or
  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 positive control COX CTL+, included in the kit, enables the quantification of *C. burnetii* (Equivalent Genome/PCR).

C. Storage conditions

- Store the kit at a temperature below +2/8 °C after reception.
- Store away from sunlight and keep dry.
- After reconstitution, prepare aliquots and store them at a temperature below -15 °C until the expiration date.
- Do not thaw more than 3 times.

D. Material required but not provided

- Real-time Thermal cycler and device.
- Instrument for homogenous mixing of tubes.
- Pipettes of 1 - 10 µL, 20 - 200 µL and 200 - 1000 µL.
- Nuclease-free filtered pipette tips.
- Nuclease-free microtubes of 1,5 mL and 2 mL.
- Powdered-free latex or nitrile gloves.
- Nuclease-free water.
- Kit for nucleic acids extraction.

Additional kits for method adoption and PCR

- **LD_{PCR} Positive Control – COX (Ref.: ADC14YLD) Confirmation of performances – LOD_{PCR} of kit.**

E. Warnings and precautions

- For veterinary *in vitro* use only.
- For animal use only.
- For professional use only.
- All instructions must be read before performing the test and strictly respected.
- Do not use reagents after the expiration date.
- Do not use reagents if the packaging is damaged.
- Do not open PCR wells or tubes after amplification.
- Do not mix reagents from different batches.
- Used material must be disposed of in compliance with the legislation in force regarding environmental protection and biological waste management.
- This kit contains products of animal origin. Certified knowledge of the origin and/or sanitary state of the animals does not totally guarantee the absence of transmissible pathogenic agents. It is therefore recommended that these products be treated as potentially infectious and handled observing the usual safety precautions (do not ingest or inhale).

F. Nucleic acids extraction

1. Extraction kits

Nucleic acids must be extracted from the samples before using the kit. The RNA/DNA purification kits listed below are recommended by Bio-X Diagnostics:

Product name	Extraction system	Number of tests and reference
ADIAMAG	Magnetic beads	200 tests: ref. NADI003 800 tests: ref. NADI003-XL

For the extraction, consult the user manual version, available on the website, indicated on the certificate of analysis included in the PCR kit of interest.

Extraction protocols are described in validation data. Other purification kits can be used if they have been validated by the user.

After extraction, nucleic acid extracts can be kept on ice or at +2/8 °C for a few hours or until use. For long term storage, they must be kept at a temperature below -15 °C or -65 °C.

2. Controls

Using controls allow to verify the reliability of the results. Controls can be included.

Control	Validation of	Usage
No Template Control (NTC)	Absence of amplification contamination	5 µL NF-Water in a well per run
COX CTL+ (Dilution pure to 1/10 000)	*C. burnetii* target amplification and standard range	5 µL CTL+ for each range point in a well per run
Negative extraction control	Absence of contamination for the extraction and amplification	1 extraction (water or lysis buffer) per run
Positive extraction control	Extraction and amplification	1 extraction (Positive sample between 1 et 100X LOD_{Method}) per run

G. Procedure

1. Amplification solution A6 preparation

- Add **1000 µL** of **« Rehydration buffer »** per A6 tube.
- Homogenize tube contents using a mixer, such as vortex, at least 20 seconds.
- After reconstitution, aliquot the solution and store at a temperature below -15 °C until the expiration date. Do not thaw more than 3 times.
- To use the A6, please refer to §« Amplification », Step 1.

2. Preparation of controls

a. Use of EPC-Ext

EPC-Ext must be added to each feaces or acellular sample.

- Add **1000 µL** of **« NF-Water »** per tube.
- Homogenize the tube contents using a shaker such as a vortex, > 20 seconds.
- After reconstitution, aliquot and store the solution at a temperature below -15 °C until the kit expiration date. Do not thaw more than 3 times.
- To use it, add **5 µL** of EPC-Ext in the first nucleic acids extraction lysis buffer.

b. Use of CTL+

- Add **200 µL** of **« NF-Water »** per tube.
- Homogenize the tubes using a mixer, such as vortex, > 20 seconds and until dissolution of the blue pellet.
- After reconstitution, aliquot and store the solution at a temperature below -15 °C until the kit expiration date. Do not thaw more than 3 times.

- If qualification is needed, use **5 µL** of CTL+ in one of the dedicated wells (see § « Amplification », Step 2).
- If quantification is needed, prepare the following ranges in Nuclease-free water :

Dilution	COX CTL+ concentration (EG/PCR)
Pure	20 000
1/10	2 000
1/100	200
1/1000	20
1/10000	2

- Use 5 **µL** of each dilution in the dedicated wells (see § "Amplification", Step 2).

3. Amplification

Warning:

- Before starting, rehydrate or thaw reagents at room temperature in the dark.
- Homogenize all reagents and samples before use.
- Store reagents at a temperature below -15 °C after use.

Step 1: Dispense **10 µL** of amplification solution (A6) per well.
Step 2: Dispense **5 µL** of nucleic acids extracts and **5 µL** of controls in each dedicated well.
Use NF-Water for the No Template Control (NTC).
Step 3: Cover the wells with an appropriate optical film or caps.
Step 4: Set up the amplification program.

The following program is defined for ABI Prism thermocyclers (like 7500, QuantStudio5, Step-one…) from Applied Biosystems, for Mx3000, Mx3005P and AriaMx from Agilent, for LightCycler from Roche Diagnostics, for Rotor-Gene Q from Qiagen, for CFX96 and Chromo 4 from Biorad and for MIC from BioMolecular System.

DNA/RNA Program	
10 min. 45 °C	
2 min. 95 °C	
5 sec. 95 °C	40 cycles
30 sec. 60 °C*	

*Reading and parameters for fluorescence acquisition:

Fluorochrome	Absorbance (nm)	Emission (nm)
FAM	494	520
HEX or equivalent	538	554
ROX	575	602

Note: The Quencher is non-fluorescent. The A6 solution contains a passive reference read in the same spectra as ROX for ABI machines.

For other thermal cycler instruments, please contact your sales representative or the customer relations department.

H. Reading and interpretation

1. Validation and interpretation of qualitative results

Display all curves and position the threshold line for each fluorochrome.

a. Test validation

Amplification is valid if the following results are obtained.
Expected Ct (Threshold Cycle) values for the CTL+ are indicated on the certificate of analysis of the kit.

Controls	Amplification		Validation of
	FAM	HEX or equivalent	
No Template Control (NTC)	No	No	Absence of amplification contamination
COX CTL+	Yes	No	Target amplification
Extraction negative control	No	No	Absence of extraction contamination
Extraction positive control	Yes	Yes/No	Extraction and amplification steps

b. Results interpretation

Nucleic acids extraction and amplification are **valid** for each sample if at least one typical amplification curve is observed in FAM and/or HEX or equivalent.

Amplification		Interpretation
FAM	HEX or equivalent	*C. burnetii*
No	Yes	Undetected
Yes	Yes	Detected
Yes	No	Detected
No	No	Undetermined

« Undetermined » : no characteristic amplification curve.
Possible causes:
Defective PCR due to inhibitors, set up error, absence of samples, degraded samples and/or issue with nucleic acids extraction (loss or destruction of nucleic acids).
Recommendations:
Set up a new PCR assay using pure nucleic acids extracts and 10x dilutions in Nuclease-free water;
If the assay is inconclusive, perform a new nucleic acids extraction.

2. Validation and interpretation of quantitative results

a. CTL+ range

COX CTL+ dilution	Concentration (EG/PCR)	FAM amplification	Validation of
Pure	20 000	Yes	*C. burnetii* target amplification and calibration curve set up
1/10	2 000	Yes	
1/100	200	Yes	
1/1000	20	Yes	
1/10000	2	Yes	

To interpret quantitative results, set up a calibration curve (number of cycles = f (Log concentration), determine the curve equation ($y = ax + b$) and check PCR efficiency ($Eff\% = \left(10^{\left(\frac{-1}{a}\right)} - 1\right) \times 100$).
The calibration curve is valid if:

- The 5 points of the range are amplified. However, one point of the range can be omitted if that point is not one of the extreme points.
- The coefficient of correlation R^2 is higher than 0,9.
- Efficiency between 75 et 125 %.
- Points of the range are spread homogenously.

b. Quantification interpretation

Quantification of a positive sample is only possible in the quantification domain of the method use (see validation data).

FAM amplification	Sample status for *C. burnetii*
No signal	Undetected Nucleic acid undetected
Signal < LQ_{METHOD}	Detected Nucleic acid detected with a quantity under the LQ_{METHOD}
LQ_{METHOD} < signal < LQ_{max}	Detected Quantifiable nucleic acid
Signal > LQ_{max}	Detected Nucleic acid detected with a quantity over the LQ_{MAX}

In the case of a "quantifiable" sample, *C. burnetii* concentration is determined using the calibration curve equation:

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

Where x : concentration (in EG/PCR if F is omitted)
y : Ct value in FAM for the sample to quantify
b : intercept
a : slope
F : multiplying coefficient (optional)

The multiplying coefficient is determined according to the sample matrix and extraction method.

Multiplying coefficient examples with ADIAMAG extraction kit according to the NEKF user manual:

Matrix	Multiplying coefficient (F)	Unit
Vaginal swab, placenta swab, milk, fœtal liquide	120	*C. burnetii* / mL
Faeces	600	*C. burnetii* / g
Tissue	600	*C. burnetii* / g

Bibliography

- 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
- EFSA Journal, Scientific Opinion on Q fever, 2010 ; 8(5) :1595 [114pp].

Symbols

Symbole	Signification
	Catalog number
	Manufacturer
	Temperature limitation
	Use by
	Batch code
	Consult Instructions for Use
	Contain sufficient for "n" tests
	For veterinary *in vitro* use only – For animal use only
	Keep away from sunlight
	Keep dry

Notes

1 Extract nucleic acids with

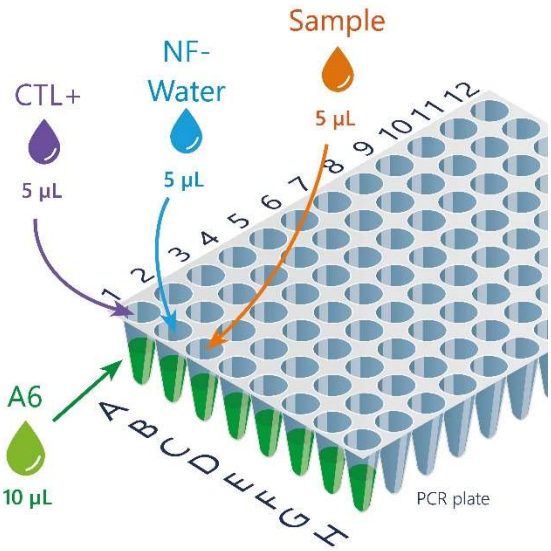
Adia X Mag

Scan me to discover Adiamag™

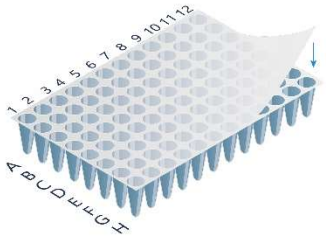
2 Add **1000 µL** of Rehydration buffer to the **A6** amplification solution

3 Distribute **10 µL** of **A6** amplification solution

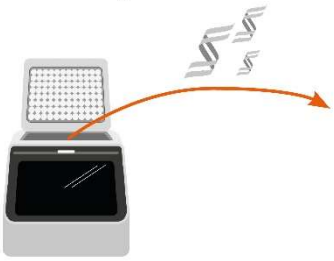
4 Distribute **5 µL** of nucleic acids, CTL+ and NF-Water

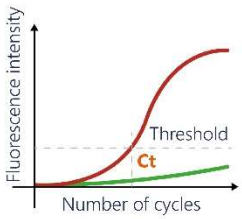


5 Seal the wells



6 Start PCR analysis





*The notes do not replace the instructions for use of which they are a summary.

Contact us
support.pcr@biox.com
www.biox.com +32 (0) 84 32 23 77
Bio-X Diagnostics
38, rue de la Calestienne
5580 Rochefort (Belgium)