ADIAVET™ ABORTION

VET

INTENDED USE

ADIAVET™ ABORTION kit is intended to detect 4 abortive pathogens from ruminant (*Coxiella burnetii, Chlamydia abortus, Toxoplasma gondii* and *Anaplasma phagocytophilum*) using real-time Polymerase Chain Reaction (PCR) technology on swabs. The ADIAVET™ ABORTION kit contains the reagents of 4 ADIAVET™ references, specific of these pathogens (ref. ADI143-100, 418027, 418025, 418028 respectively).

PRINCIPLE

ADIAVET™ ABORTION test contains 4 amplification solutions, each being specific of a pathogen, and internal controls of extraction and amplification specific of an endogenous nucleic acid.

	A5 COX	A5 CHLAM.A	A5 TOXO	A5 ANA PHA
FAM labeled probe	*Coxiella burnetii*	*Chlamydia abortus*	*Toxoplasma gondii*	*Anaplasma phagocytophilum*
HEX labeled probe or its equivalent	GAPDH	GAPDH	GAPDH	GAPDH

Included in the kit, the positive control COX CTL+ enables the quantification of *C. burnetii*.

CONTENT OF THE KIT

Kit	
REF **ADI661-4x50**	Pack of 4*50 tests

COMPOSITION

REF **ADI661-4x50**

A5 COX	*Coxiella burnetii* amplification solution	1 x 1000 µl with green cap (Ready to use)
A5 CHLAM.A	*Chlamydia abortus* amplification solution	1 x 1000 µl with yellow cap (Ready to use)
A5 TOXO	Toxoplasma gondii amplification solution	1 x 1000 µl with blue cap (Ready to use)
A5 ANA. PHA	*Anaplasma phagocytophilum* amplification solution	1 x 1000 µl with orange cap (Ready to use)
COX CTL+	*Coxiella burnetii* positive control	1 tube with stripped green cap (to reconstitute)
CHLAM.A CTL+	*Chlamydia abortus* positive control	1 tube with stripped yellow cap (to reconstitute)
TOXO CTL+	*Toxoplasma gondii* positive control	1 tube with stripped blue cap (to reconstitute)
ANA. PHA CTL+	*Anaplasma phagocytophilum* positive control	1 tube with stripped orange cap (to reconstitute)
NF-Water	nuclease-free Water	1 x 1000 µl tube with blank caps (Ready to use)

STORAGE CONDITIONS

- After reception, the whole kit should be stored at <-15°C.
- Prepare aliquots and store them at a temperature below -15°C until the expiration date.
- Store them in the dark.
- Do not thaw more than 3 times.

MATERIAL REQUIRED BUT NOT PROVIDED

- Real-time PCR Thermal cycler and its disposable
- 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
- Powder-free latex or nitrile gloves
- Nuclease-free water
- Kit for nucleic acids extraction

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.
- 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).

NUCLEIC ACIDS EXTRACTION

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

Product Name	Supplier	Extraction system	Number of test & Reference
ADIAMAG	Bio-X Diagnostics	Magnetic beads	200 tests: ref. NADI003 800 tests: ref. NADI003-XL

Analysis options according to the specimen:

Specimen	Individual analysis	Pool of samples is possible*, up to
Swab	☑	3

* Depending on the quality of the specimen and of the epidemiological case.

Other purification kits can be used if they have been validated by the user and according to the user manuals of ADIAVET kits, specific to the 4 pathogens (ref. ADI143-100, 418027, 418025, 418028 respectively). Contact support service at biox@biox.com.

SAMPLE PREPARATION

1. PREPARATION OF SWABS

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

For individual analysis

Introduce the vaginal swab in a previously identified 2 ml-microtube and cut the stem, if necessary. Add **1 ml** of PBS 1X pH 7.4 to the vaginal swab. Homogenize for 30 seconds. Transfer the supernatant in a previously identified microtube.

For pool analysis

Introduce three vaginal swabs in a previously identified sterile 5 ml-tube and cut the stems, if necessary, . Add **3 ml** of PBS 1X pH 7.4 to the vaginal swabs. Homogenize for 30 seconds. Transfer the supernatant in a previously identified microtube.

2. PREPARATION OF NEGATIVE CONTROL OF EXTRACTION (REQUIRED)

To verify the absence of cross-contamination, at least one negative control must be included per trial. The control is a negative sample, for example the buffer used for dilutions.

3. PREPARATION OF POSITIVE CONTROL OF EXTRACTION (RECOMMENDED)

A positive control can be added in each trial. The control is a positive sample. This positive control should be close to the limit of detection of the method. It will inform about the fidelity of the obtained results between different trials.

PROCEDURE

1. PREPARATION OF CONTROLS (CTL+)

1. Add 200 µl of “NF-water” to each CTL+ tube.
2. Homogenize tube contents using a mixer such as vortex, at least 20 seconds, until complete dissolution of the blue pellet.
3. After reconstitution, aliquot the solution. Store the obtained solution at a temperature below -15°C until the expiration date. Do not thaw more than 3 times.
4. To use the CTL+, please refer to the “AMPLIFICATION” section.
5. To use the COX CTL+ for quantification, prepare a standard range with Nuclease-free water, just before experiment.

Dilution	Concentration (*C. burnetii/ml*) of COX CTL+
Pure	$4x10^6$
1/10	$4x10^5$
1/100	$4x10^4$
1/1000	$4x10^3$
1/10000 (LQPCR)	$4x10^2$

For a quantitative PCR analysis, **5 µl** of each COX CTL+ dilutions are used per assay.

2. AMPLIFICATION

Warning:

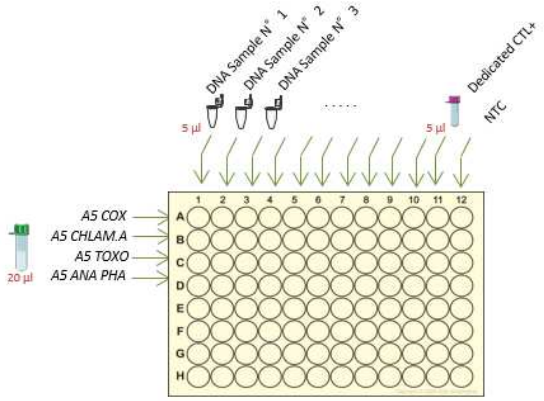
- Before starting, rehydrate or thaw reagents at room temperature in the dark.
- Homogenize all reagents and samples before use.
- Store reagents at a temperature below -15°C after use.
- Keep DNA extracts at +2-8°C for 24 hours and then store at a temperature below -15°C.

STEP 1: DISPENSE 20 µL OF AMPLIFICATION SOLUTIONS A5 PER WELL

Dispense the 4 A5 amplification solution according to the number of samples and controls.

STEP 2: DISPENSE 5 µL OF NUCLEIC ACIDS EXTRACTS AND CONTROLS

Dispense nucleic acids extracted from samples and controls in each dedicated well.
Nothing is added to the Negative Amplification Control (NTC).



STEP 3: COVER THE WELLS WITH APPROPRIATE OPTICAL FILM OR CAPS

STEP 4: SET UP THE APPROPRIATE AMPLIFICATION PROGRAM

The following program is defined for ABI Prism thermocyclers (like 7500, StepOne, QS5…) from Applied Biosystems (check the “emulation 9600” option if available), for MX3005P and ARIAMX from Agilent and for CFX96 from BioRad.

DNA Program	
2 min. +50°C	
10 min. +95°C	
15 sec. +95°C*	45 cycles
1 min. +60°C**	

*Put 30 sec. +95°C for the MX3005 and MX3005P thermocyclers.
**Reading and parameters for fluorescence acquisition:

Dyes	Absorbance (nm)	Emission (nm)
FAM	494	520
HEX (or equivalent)	530	549
Cy5	646	662
ROX	575	602

Note: The Quencher is non-fluorescent. Each A5 solution contains a passive reference ROX for the ABI machines
For other thermal cycler instruments**,** please contact your sales representative or the customer relations department.

READING AND INTERPRETATION

Set Threshold line separately for each dye.

1. VALIDATION OF TEST

The real-time PCR run is validated if the following results are met.
The indicative Ct (Threshold Cycle) values expected for CTL+ are notified on the kit's analysis certificate.

Qualitative method

	Amplification		
Controls	**FAM**	**HEX or equivalent**	**Validation**
Negative amplification control - NTC	No	No	No amplification contamination
Negative extraction control	No	No	No extraction and amplification contamination
COX CTL+	Yes	Yes/No	Amplification of the *Coxiella burnetii* target
CHLAM.A. CTL+	Yes	Yes/No	Amplification of the *Chlamydia abortus* target
TOXO CTL+	Yes	Yes/No	Amplification of the *Toxoplasma gondii* target
ANA PHA CTL+	Yes	Yes/No	Amplification of the *Anaplasma phagocytophilum* target

Qualitative method for *Coxiella burnetii*

Control		Amplification		
Dilution COX CTL+	**Concentration of *C. burnetii*/ml**	**FAM**	**HEX or equivalent**	**Validation**
pure	$4x10^6$	Yes	No	Amplification of the *C. burnetii* target and of the standard range
1/10	$4x10^5$	Yes	No	
1/100	$4x10^4$	Yes	No	
1/1000	$4x10^3$	Yes	No	
1/10000 (LQPCR)	$4x10^2$	Yes	No	

In order to interpret quantitative results, check PCR efficiency using, for instance, the software related to the thermal cycler instrument.
The standard curve is validated, if:

- The five points of the standard range are detected (one of the intermediate points can be omitted)
- The correlation coefficient (R^2) is over 0.9.
- The efficiency is comprised between 85% and 115%.
- The points distribution is homogenous.

2. RESULTS INTERPRETATION

Nucleic acids extraction and amplification are valid if at least one characteristic amplification curve is observed per sample in FAM and/or HEX or its equivalent.

Qualitative method

	Amplification		Status of sample
A5 amplification	**FAM**	**HEX or equivalent**	***Pathogen***
A5 COX	No	Yes	*C. burnetii* not detected
	Yes	Yes / No	*C. burnetii* detected
	No	No	Undetermined
A5 CHLAM.A	No	Yes	*C. abortus* not detected
	Yes	Yes / No	*C. abortus* detected
	No	No	Undetermined
A5 TOXO	No	Yes	*T. gondii* not detected
	Yes	Yes / No	*T. gondii* detected
	No	No	Undetermined
A5 ANA PHA	No	Yes	*A. phagocytophilum* not detected
	Yes	Yes / No	*A. phagocytophilum* detected
	No	No	Undetermined

«**Undetermined**»: no characteristic amplification curve.
Possible causes:

- Defective PCR due to inhibitors, set up error, absence of sample or degraded sample.
  and/or
- Issue with nucleic acids extraction (loss or destruction of nucleic acids).

Recommendations:

1. Set up a new PCR assay using pure nucleic acids extracts and 10x dilution in Nuclease-free water.
2. If assay is inconclusive, perform a new nucleic acids extraction.

Quantitative method for *Coxiella burnetii*
The quantification of a positive sample is possible in the quantification field of the method used only (*cf*. Validation report of ADI143 reference).

FAM Amplification	*C. burnetii* Sample
No signal	Negative Nucleic acids undetected
Signal < LQ_{METHOD}	Positive Nucleic acids quantity **<** $LQ_{METHODE}$
LQ_{METHOD} < signal < LQ_{max}	Positive Quantifiable nucleic acids
Signal > LQ_{max}	Positive Nucleic acids quantity **>** LQ_{max}

For positive samples, the direct quantification of *C. burnetii* is possible using an equation derived from the standard range:

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

With: x: Concentration of *C. burnetii*
y: Ct value FAM of positive sample
b: Intercept
a: Slope
F: Coefficient

The coefficient *F* varies with matrix and extraction method. For DNA extracted from swab using the ADIAMAG extraction kit, F has been determined at 0.6.

QUALITY CONTROL

ADIAVET™ ABORTION kit is planned and designed to ensure that the toughest quality requirements are met. The certificate of analysis is supplied with the kit.

WASTE DISPOSAL

Unused reagents may be considered as non-hazardous waste and disposed of accordingly. Dispose of all used reagents as well as any other contaminated disposable materials following procedures for infectious or potentially infectious products.
It is the responsibility of each laboratory to handle waste and effluents produced according to their type and degree of hazardousness and to treat and dispose of them (or have them treated and disposed of) in accordance with any applicable regulations.

INDEX OF SYMBOLS

Symbol	Meaning
REF	GB: Catalogue number US: Catalog number
	Manufacturer
	Temperature limitation
	Use by
LOT	Batch code
	Consult Instructions for Use
	Contains sufficient for *n* tests
VET	For veterinary *in vitro* use only – For animal use only
	Keep away from sunlight

REVISION HISTORY

Changes type categories:

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
2021/06	NE661-01	N/A	First publication

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