Instruction manual
ADC04SQ01-PTUB_NO_(EN)_V03
03/2024

Quantified Extraction Positive Control Paratb Faeces

Reference: ADC04SQ01

Faeces positive quantified of *Mycobacterium avium* subsp. *paratuberculosis*

For veterinary *in vitro* use only

Kit composition

Content		ADC04SQ01
EPCQ Paratb Faeces	Quantified *Mycobacterium avium* subsp. *paratuberculosis* positive faeces	1 freeze-dried vial (To reconstitute)
NF-Water	Nuclease-Free Water	1 x 2000 µL tube with white cap (Ready to use)

Related PCR kit(s)

Related PCR kit(s)	Reference(s)
ADIAVET™ PARATB REAL TIME	ADI045-100
ADIALYO™ PARATB	ADL04Y1-100

Revision history

Date	Version	Modifications
02/2023	V01	First version
11/2023	V02	Change of quantity for the dosage of the EPCQ from 50 µL to 200 µL
03/2024	V03	Added ADIALYO™ PARATB as related PCR kit

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

A. Introduction

Mycobacterium avium subsp. *paratuberculosis*, or Johne's bacillus, colonizes the jejuno-ileal region. The level of faecal matter contamination changes according to the stage of the disease, from a few bacteria per gram of faeces at the start of chronic expression, up to 10^4 to 10^{10} in the clinical phase (Collins *et al.*, 1993). This bacterium can then be disseminated in the body of the animal via macrophages; contamination by colostrum, milk and sperm is then possible.

The quantification of the level of contamination is of interest in order to classify the animals, and thus to isolate the most excretory animals. The ADIAVET PARATB REAL TIME PCR kit allows the detection and quantification of mycobacteria in faeces using relative PCR with EPCQ Paratb Faeces quantified at 10,000 genome equivalents (GE)/g of faeces (Kralik *et al.*, 2014; Navarro Gonzalez *et al.*, 2019).

B. Test principle

The EPCQ Paratb Faeces Control is quantified to 10 000 GE/g of faeces after resuspension.

The positive control is included in each extraction series and allows to semi-quantify the *Mycobacterium avium* subsp. *Paratuberculosis* target in faeces samples, with the related PCR kit(s).

Two extraction methods are validated in relative PCR using EPCQ ParaTb Faeces: ADIAMAG™ with ADIAFILTER™ and ADIAMAG™ with ADIAPREP™. (*cf.* validation data of PCR kit).

C. Storage conditions

Before reconstitution, store the EPCQ Paratb Faeces, at a temperature below +2/8 °C.
After reconstitution, prepare aliquots and store them at a temperature below -15 °C. Do not defrost 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.
- Negative Paratb faeces.
- Kit for nucleic acids extraction ADIAMAG (Bio-X Diagnostics ref.: 200 tests: NADI003; 800 tests: NADI003-XL).
- Fecal preparation system:
  - ADIAPREP™ (Bio-X Diagnostics ref.: 200 tests, ADPREP-200).
  - ADIAFILTER™ (Bio-X Diagnostics ref.: 100 tests ADIFIL100).
- Grinding Beads:
  For the Mixer Mill or similar:
  - ADIAPURE™ ALIQUOTED GLASS BEADS (Bio-X Diagnostics ref.: 480 tests, ADIADPBIA-480).
  - ADIAPURE™ GLASS BEADS RACKS 4x96 (Bio-X Diagnostics ref.: 384 tests, ADPBIAR-4x96).

  For the Ribolyser or similar:
  - Lysing Matrix B (MP biomedicals, 100 tubes, ref. 116911100).
- Related PCR 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. Reconstitution and use of the EPCQ Paratb Faeces

- Add **2 mL** of **« NF-Water »** per vial.
- Homogenize the tubes using a mixer, such as vortex, > 20 seconds and until dissolution of the pellet.
- After reconstitution, aliquot and store the solution at a temperature below -15 °C. Do not thaw more than 3 times.

This control is to be integrated into each extraction series of analysis during relative PCR.

It is recommended to perform at least one negative extraction control per session to verify the absence of contamination.

1. Extraction with ADIAPREP (ref. ADPREP-200)

- Collect 1 spoon of negative faeces and transfer to ADIAPREP.
- Add **200 µL of EPCQ Paratb Faeces.**
- Vortex until a homogeneous suspension is obtained.
- Transfer 1 mL to a microtube or tray, centrifuge for 5 minutes at 3000 g and discard the supernatant.
- Add 300 mg grinding beads and 500 µL sterile deionized water to the pellet.
- Grind for 5 minutes at 30 Hz on the Mixer Mill or 3 x 45 seconds on the Fast Prep/Ribolyser and centrifuge for 5 minutes at 3000 g.
- Extract 100 µL of supernatant with the ADIAMAG extraction kit *(refer to the version of the instructions available on the website, indicated on the certificate of analysis included in the extraction kit used).*

2. Extraction with ADIAFILTER (ref. ADIFIL100)

- Collect 3 g (+/- 0.2) of faeces into 20 mL of sterile deionized water (or same weight/volume ratio).
- Add **200 µL of EPCQ Paratb Faeces**.
- Vortex until a homogeneous suspension is obtained.
- Allow to settle for 10-20 minutes until sedimentation.
- Place 10 mL of supernatant to the ADIAFILTER, centrifuge for 5 minutes at 3000 g and discard the supernatant and ADIAFILTER.
- Add 500 µL sterile deionized water to the pellet, vortex, and transfer to a microtube containing 300 mg grinding beads.
- Grind for 10 minutes 30 Hz on Mixer Mill or 3 x 45 seconds on Fast Prep/Ribolyser and centrifuge for 5 minutes 15 000 g.
- Extract 100 µL of supernatant with the ADIAMAG extraction kit *(refer to the version of the instructions available on the website, indicated on the certificate of analysis included in the extraction kit used).*

3. Amplification

The extracted nucleic acids are amplified with the associated Bio-X Diagnostics PCR kit and according to its instructions for use.

G. Reading and interpretation

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

1. Validation of the test

The amplification is valid if the following results are obtained. The indicative values of Ct (Threshold Cycle) expected for the EPCQ Paratb Faeces are indicated on the certificate of analysis (Acceptance limits are 3 Ct).

Indicative Ct (Threshold Cycle) values expected for CTL+ are listed on the certificate of analysis for the associated PCR kit.

Control	Amplification		Validation of
	FAM	HEX or similar	
EPCQ Paratb Faeces	Yes	Yes	Extraction and amplification step
No Template Control (NTC)	No	No	Absence of contamination for amplification
PARATB CTL+	Yes	No	Amplification of target and internal control
Negative Extraction Control	No	Yes	Absence of contamination for extraction

2. Interpretation of relative PCR results

Nucleic acid extraction and amplification are valid for each sample if at least one characteristic amplification curve is observed in FAM and HEX or equivalent.

Amplification		Interpretation
FAM	HEX or similar	*Mycobacterium avium subsp. paratuberculosis*
No	Yes	Not detected
Yes $Ct_{sample} < Ct_{EPCQ-1Ct}$	Yes/No	Detected in greater quantities than the EPCQ Paratb Faeces
$Ct_{sample} = Ct_{EPCQ+/-1Ct}$	Yes/No	Detected in quantities close to the EPCQ Paratb Faeces
$Ct_{sample} > Ct_{EPCQ+1Ct}$	Yes/No	Detected in lower quantities than the EPCQ Paratb Faeces
No	No	Not detected

"Not determined": absence of characteristic amplification curve.
Possible causes:
Faulty PCR (presence of inhibitors, program error, absence of sample or sample too degraded) and/or
Deficiency in nucleic acid extraction (loss or destruction of nucleic acids).
Suggested actions: Redo the PCR with the pure nucleic acid extract and diluted to 1/10 in Nuclease-free water; Redo the nucleic acid extraction if the test is still not valid or request another sample.

Bibliography

- Collins J. D. *et al.* (1993), Comparison of polymerase chain reaction tests and faecal culture for detecting *Mycobacterium paratuberculosis* in bovine faeces. Vet. Microbiol. 36: 289-299.
- Kralik P., *et al.* (2014), Evidence of passive faecal shedding of Mycobacterium avium subsp. Paratuberculosis in a Limousin cattle herd. Vet J Jul ;201(1):91-4.
- Navarro-Gonzalez N. *et al.* (2019), Longitudinal study of Mycobacterium avium ssp. paratuberculosis faecal shedding patterns and concurrent serological patterns in naturally infected dairy cattle. J Dairy Sci Oct;102(10):9117-9137.

Symbols

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

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