Instruction manual
ADI122-SALM_NO_(EN)_V01
02/2023

ADIAVET™ SALMO FAST TIME

Reference: ADI122-100

Test for the detection of *Salmonella enterica spp* 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, tissue, fetal fluid from abortion	✓	✗

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

Kit composition

Content		ADI122-100
		100 reactions
A5	Amplification solution	2 x 500 µL tubes with green cap
SALMO CTL+	Salmonella enterica spp positive control	1 tube with purple cap
EPC-Ext	Exogenous extraction control	2 x 300 µL tubes with yellow cap
EPC-Amp	Exogenous internal control of amplification	1 x 150 µL tubes with colorless cap
NF-Water	Nuclease-Free Water	1 x 1000 µL tube with white cap

Revision history

Date	Version	Modifications
06/2022	NE122-03	First version
02/2023	ADI122-SALM_NO_(EN)_V01	Change to simplified format

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

A. Introduction

Salmonella is a gram-negative bacillus belonging to the Enterobacteriaceae family. More than 2,500 different serotypes or serovars have been identified so far within 2 species of this genus: *Salmonella bongori* and *Salmonella enterica*. Almost all of them are pathogenic to ruminants. *Salmonella enterica* subspps *enterica* serovars *Typhimurium*, *Dublin* and *Montevideo* are among the most frequently encountered in cattle.

The most typical symptoms of salmonellosis are hemorrhagic diarrhea, fever and abortions. The disease affects isolated animals but can sometimes take an epidemic form. Several *Salmonella* serovars can lead to abortions in ruminants with *Salmonella enterica* subspps *enterica* serovars *Dublin* being the most commonly involved in bovine abortions and *Salmonella Abortus-Ovis* the most commonly involved in sheep abortions.

Abortions due to salmonellosis do not present any typical characteristics and can hence only be diagnosed through laboratory analyses such as bacteriology or PCR. While bacteriology consists of culturing and can take from 4 to 7 days until a possible identification of the serovar, PCR can demonstrate the presence of *Salmonella* within hours. The presence of Salmonella can be verified on stomach fluid, fetal organ (including liver and spleen), placental cotyledon taken intrauterine, or vaginal swab.

B. Test principle

ADIAVET™ SALMO FAST TIME test is based on the amplification of specific *Salmonella enterica spp*. This test is intended to detect simultaneously, in one well:

- *Salmonella enterica spp* (FAM labelled probe)
- Exogenous internal control (HEX labelled probe or its equivalent)
  - Either extraction and amplification control if the EPC-Ext is added to each specimen during nucleic acids extraction steps
  - Or amplification control if the EPC-Amp is added to A5 solution.

C. Storage conditions

After reception, the kit should be stored at <-15 °C until the expiration date.

It is recommended to make aliquots of A5 solution if it should be defrosted more than 3 times.

Do not thaw more than 3 times.

Store away from sunlight.

Do not mix reagents from two different batches.

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

- **Extraction Positive Control SALMO (Ref.: ADC12EPC).** Supplier reference material for method adoption that can also be used as a sentinel (Calibrated between 1 and 100xLOD_{Method}).
- **LD_{PCR} Positive Control – SALMO (Ref.: ADC12LD)** 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.

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 by series of analysis according to the recommendations defined by the standards in force (Cf. AFNOR U47-600...).

Controls	Validation of	How to proceed
No Template Control (NTC)	Absence of amplification contamination	5 µL NF-Water in a well per run
SALMO CTL+	SALMO target amplification	5 µL CTL+ 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. Use of EPC-Ext

EPC-Ext must be added to each sample and extraction controls.

- Aliquot and store the solution at a temperature below -15 °C according to the size of extraction series. Do not thaw more than 3 times.
- Add **5 µL** of EPC-Ext in the first nucleic acids extraction lysis buffer.

2. Use of EPC-Amp

The EPC-Amp is used when the EPC-Ext has not been used for nucleic acid extraction.

- Aliquot and store the solution at a temperature below -15 °C according to the size of extraction series. Do not thaw more than 3 times.
- For each PCR well, add **0.5 µL** of EPC-Amp to the amplification solution. (see § « Amplification », Step 1).

3. 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.

For each assay, use **5 µL** of CTL+ in one of the dedicated wells (see § « Amplification », Step 2).

4. 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:

If use of EPC-Ext:

Dispense **10 µL** of amplification solution (A5) in each well.

If no use of EPC-Ext:

Place (n*+1) x 10 µL of amplification solution (A5) in a microtube and add (n*+1) x 0.5 µL of EPC-Amp. Dispense **10 µL** of the mixture into each well.

*n: total number of PCR to run

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: Start the PCR analysis.

The following programs are 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 standard program		DNA FAST program	
2 min. 50 °C		2 min. 95 °C	
10 min. 95 °C			
15 sec. 95 °C**	45 cycles	5 sec. 95 °C	45 cycles
60 sec. 60 °C*		30 sec. 60 °C*	

*30 sec. 95°C for MX3000 and MX3005P

** 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 A5 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. Interpretation of results

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

1. 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	Yes*/No	Absence of amplification contamination
SALMO CTL+	Yes	Yes*/No	Target amplification
Extraction negative control	No	Yes	Absence of extraction contamination
Extraction positive control	Yes	Yes	Extraction and amplification steps

* "Yes" if addition of EPC-Amp before amplification.

2. 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	*Salmonella enterica spp*
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.

Symbols

Symbols	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

Notes

1 Extract nucleic acids with

Adia X Mag

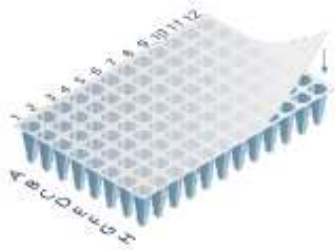
Scan me to discover Adiamag™

If using the EPC-Ext:

2 Distribute 10 µL of A5 amplification solution

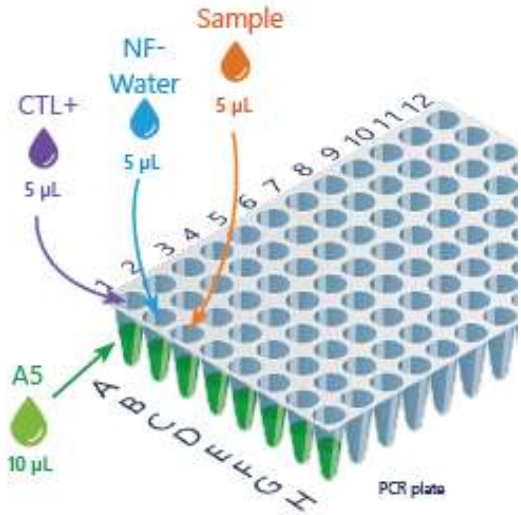
3 Distribute **5 µL** of nucleic acids, CTL+ and NF-Water

4 Seal the wells

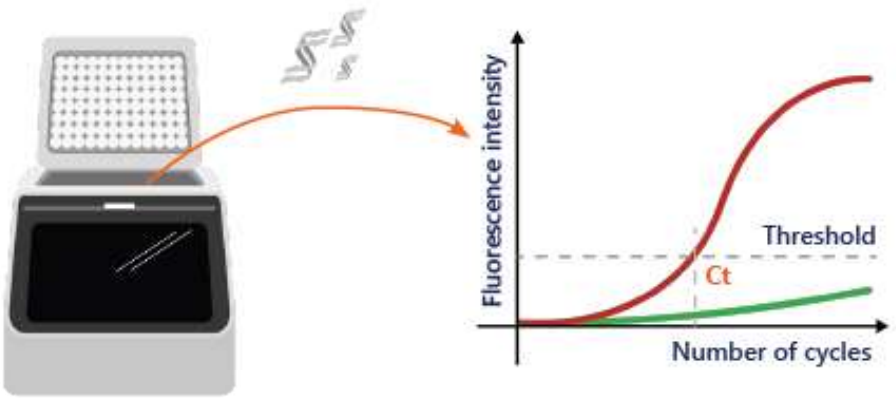


If not using the EPC-Ext:

2 Prepare a premix of 10 µL of A5 amplification solution + 0,5 µL of EPC-Amp
Dispense 10 µL of the premix



5 Start PCR analysis



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

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