ADIAVET™ SALMO FAST TIME

TEST FOR DETECTION OF *SALMONELLA ENTERICA spp* BY REAL-TIME ENZYMATIC AMPLIFICATION (PCR TEST)

Reference:
ADI122-100 (100 reactions)

VET

English version
NE122-02
2021/09

ADIAVET™ SALMO FAST TIME

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
2021-09	NE122-02	N/A	creation

I. General information

1. Purpose of the test

The ADIAVET™ SALMO FAST TIME kit is used to detect *Salmonella enterica spp* by enzymatic amplification in real time (PCR).
This PCR kit has been developed and must be used on DNA from abortion (bovine swab, tissue and foetal fluid).

2. Salmonella

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 analyzes such as bacteriology or PCR. While bacteriology consists of culturing and can take from 4 to 7 days until a possible identification of the servoar, 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.

3. Description and purpose of the test

This test is based on enzymatic gene amplification or PCR technology.
Amplified products are detected in real-time using a specific labelled hydrolysis probe (5'-exonuclease technology).

The ADIAVET™ SALMO FAST TIME kit enables the simultaneous detection of:

- *Salmonella spp* (probe marked with FAM)
- an exogen control "EPC-Amp" added in the amplification reagent or "EPC-Ext" added during the extraction and that allows validation of the extraction and amplification steps (probe labelled with a fluorochrome read in the same spectra as VIC and HEX).

ADIAGENE has validated this test with different DNA purification kits (Bio-X Diagnostics, Qiagen). Other purification kits can be used after validation by the user.

Analysis options according to the specimen:

Specimen	Individual analysis
From abortion: Swab, tissue, Fetal gastric fluid	Yes

II. Material and reagents

1. Reagents provided with the kit

REF ADI122-100		
A5	amplification solution	2 x 500 µL tube with green cap (a ready-to-use reagent)
SALMO CTL+	Salmonella enterica spp. positive control	1 tube with purple cap (to reconstitute)
EPC-Amp	exogenous internal control of amplification	1 x 150 µL tube with colorless cape (Ready to use reagent)
EPC-Ext	Exogenous extraction control	2 x 300 µL tube with yellow cap (Ready-to-use reagent)
NF-Water	Nuclease-Free Water	1 x 1000 µL tube with white cap (Ready-to-use reagent)

2. Validity and storage

After reception, the kit should be stored at <**-15 °C**.
It is recommended to make aliquots of the A5 solution to prevent it from being defrosted more than 3 times. **Do not defrost reagents more than 3 times.**
Real-time reagents are sensitive to light: they should be stored in the dark.
The A5 reagent is ready to use for PCR reaction.
Do not mix reagents from two different batches.

3. Use of controls

A. « SALMO CTL+ »

« SALMO CTL+» is an amplification positive control.
Add **200 µL** of **NF-Water** to the **SALMO CTL+** tube. Homogenize with a vortex for 20 seconds until complete dissolution of the blue pellet. Divide this solution by 6 or 12 µL aliquots and store at <-15 °C.
For each analysis, we recommend using **5 µL** of **SALMO CTL+** in one of the wells.

B. « EPC-Amp »

"EPC-Amp" is an exogenous amplification internal control.
Aliquot and store this solution at <-15 °C according to the size of extraction series. Do not defrost reagent more than three times.
In the cas of amplification of DNA free of EPC-Ext, we recommend to add **0.5 µL** of **EPC-Amp** in each well.

C. « EPC-Ext »

EPC-Ext is a no-target extraction control.
Use of EPC-Ext allows the validation of the extraction, purification and amplification steps.
On first use, aliquot the solution according to the size of the extraction series and store at <-15 °C.
Add **5 µL** of **EPC-Ext** per sample extracted.

4. Equipment required but not supplied in the kit

Material should be Nuclease-Free (e.g. autoclaved 25 minutes twice at +121 °C or once 60 minutes at +121 °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
- A centrifuge for microtubes, 50 mL tubes or 96-wells plates
- Etuve, heating baths or block heaters
- Vortex
- 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
- Nuclease-Free water

- Extraction kits for RNA/DNA:
  - **Material needed for individual column extraction**
    - 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)
  - **Automated DNA/RNA extraction kit (magnetic beads)**
    - ADIAMAG (Bio-X Diagnostics; 200 extractions: ref. NADI003; 800 extractions: ref. NADI003-XL).

III. Recommendation before the analysis of samples

Before starting the test, read the entire protocol and follow it carefully.

1. Precautions

Adiagène has validated this PCR test with Bio-X Diagnostics, Qiagen and Macherey-Nagel extraction kits. Other extraction kits can be used after validation by the user.

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

Some kits include and/or need toxic reagents. These reagents should be manipulated with gloves and in a chemical hood.

Only appropriately trained personnel should perform this test. Ensure the accuracy and precision of the micropipettes used. The quality of the obtained results depends on rigorous respect of good laboratory practices.

The PCR generates large amount of DNA. A few molecules of amplified products are sufficient to generate a positive result. Hence, it is important to reserve 2 rooms, one for the manipulation of the samples to be tested, and the other one for amplified products analysis. PCR tubes should not be opened 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 aliquots of demineralised and saline water and autoclaving 25 minutes at +121 °C twice or 60 minutes at +121 °C. To avoid contamination, use a new aliquot for any new experiment.

2. Storage of samples and DNA extracts

Samples can be stored up to 2 days at +2/8 °C. After 2 days, we recommend to store them at <-15°C.
Extracted DNAs are sensitive molecules and can be stored, after extraction, on melting ice or at +2/8 °C for up to 24 hours, and must be stored long term at <-15 °C.

3. Controls to include

Controls allow monitoring of results consistency.
They must be included on each PCR run performed.

Each step of the analytical process (extraction and amplification), no matter the matrix, is validated thanks to the use of controls included in the kit

- The non-target EPC-Ext, added during extraction, allows the validation of the extraction process and of the amplification for each sample.
- « SALMO CTL+ » allow validation of the target amplification.

Other control could be included according to the process of laboratory:

- **Extraction Negative control**

To verify the absence of cross-contamination, a negative control can be included per trial. This control is a negative sample, for example the buffer used for dilutions.

- **Extraction Positive control**

A positive control, containing *Salmonella enterica spp.*, can be added in each trial. This control can be a known positive sample available in the laboratory or a negative sample spiked with a *Salmonella enterica spp.* solution. It will be close to the limit of detection of the method (1 to 100x LOD_{method}) and will be informative regarding the fidelity of the obtained results between different trials.

IV. Extraction and purification

1. Extraction using QIAamp® DNA Mini kit

All the centrifugations are performed at room temperature.

Particular case of placentas:
May contain a large number of microorganisms, manipulate them with extreme precaution.
1st method
Cut the cotyledon with a scalpel, then rub inside with a swab.
Perform analysis according to swab protocol.
2nd method
Perform analysis according to tissue protocol.

	Swab	**Tissue**	**Fetal gastric fluid**
Sample preparation	Mix the **swab** with **1 ml** of **1X PBS buffer.** Transfer **200 µL** in a microtube.	Put **20-30 mg** of **tissue** in a microtube.	Transfer **200 µL** in a microtube*.
Lysis	Add **180 µL** of **ATL buffer**, **20 µL** of **proteinase K (+ 5 µL EPC-Ext)****. Vortex. Incubate **30 minutes** at **+70 °C** (or **a night** at **+56 °C**).		
	Add **200 µL** of **AL buffer**. Vortex. Incubate **10 minutes** at **+70 °C**.		
Binding preparation	Add **200 µL** of **ethanol 100%**. Homogenize the mixture by pipeting (~10 times) or by vortex (~15 secondes).		
Transfer to columns and bing to the membrane	Identify columns, apply 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.*		
1er wash	Change the collection tube and add **500 µL** of **AW1 buffer** to the column. Centrifuge 1 minute at 10 000 g.		
2ème wash	Change the collection tube and add **500 µL** of **AW2 buffer** 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 **AE buffer**. 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.		

* if too viscuous, dip a swab in the matrix and process with the “swab” protocol
** optional

2. Extraction with NucleoSpin® Tissue kit

All the centrifugations are performed at room temperature.

Particular case of placentas:
May contain a large number of microorganisms, manipulate them with extreme precaution.
1st method
Cut the cotyledon with a scalpel, then rub inside with a swab.
Perform analysis according to swab protocol.
2nd method
Perform analysis according to tissue protocol

	Swab	**Tissue**	**Fetal gastric fluid**
Preparation of the sample	Mix the **swab** with **1 ml** of **1X PBS buffer.** Transfer **200 µL** in a microtube.	Put **20-30mg** of **tissue** in a microtube.	Transfer **200 µL** in a microtube*.
Lysis	Add **180 µL** of **T1 buffer**, **25 µL** of **proteinase K (+ 5 µL EPC-Ext)****. Vortex. Incubate **30 minutes** at **+70 °C** (or a night at +56 °C).		
	Add **200 µL** of **B3 buffer**. Vortex. Incubate **10 minutes** at **+70 °C**.		
Binding preparation	Add **200 µL** of **ethanol 100%**. Homogenise the mixture by pipeting (~10 times) or by vortexing (~15 secondes).		
Transfer to columns and binding to the membrane	Identify columns, apply the **whole** obtained solution to the corresponding column. Centrifuge at 10 000 g/1 minute. *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 **BW buffer** to the column. Centrifuge 1 minute at 10 000 g.		
2nd wash	Change the collection tube and add **600 µL** of **B5 buffer** 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 **BE buffer**. 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.		

* if too viscuous, dip a swab in the matrix and process with the “swab” protocol
** optional

3. Using ADIAMAG kits - DNA/RNA magnetic beads kit

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

V. Amplification

Determine the number of analysed samples including the controls (e.g., positive and negative extraction controls, positive control of amplification (CTL+) and No Template Control (NTC)).

b- Defrost the A5 solution at room temperature. Vortex.

If EPC-Ext was added during extraction step:
Dispense **10 µL** of the A5 solution in each PCR tubes or PCR plate wells with a micropipette with a Nuclease-Free tip.

If EPC-Ext was NOT added during extraction step:
Dispense (n+1) x **10 µL** of « A5 » reagent in microtube.
Add (n+1) x **0.5 µL** of **« EPC-Amp »**.
Dispense **10 µL** of the mix in each PCR tubes or PCR plate wells with a micropipette with a Nuclease-Free tip.

c- **Immediately replace the A5 tube at <-15 °C and in darkness.**

d- For each sample, the extraction negative control (required) and the extraction positive control (recommended), add **5 µL** of purified extract to the **10 µL** of **A5** reagent.
For the "SALMO CTL+", add **5 µL** of the solution (§ II.3.) to the **10 µL** of **A5** reagent.
For the No Template Control (NTC), nothing is added to **A5** reagent.
Immediately replace the purified DNA at +2/8 °C or at <-15 °C. Ensure the wells bottoms are bubbles-free.

e- Once all the tubes have been prepared, run the real-time PCR amplification.
The *Salmonella* target is read in FAM. The internal control is read in VIC or HEX. The solution contains a passive reference ROX for the ABI machines. The fluorescence is read at the end of the elongation step at 60 °C.

The following programs are defined for **ABI Prism** thermalcyclers (like 7500, QuantiStudio 5,StepOne…) from **Applied Biosystems** (check the "emulation 9600" option if available), for the **MX3005P** and **AriaMx** from **Agilent** and for the **CFX96** from **BioRad**.

<table>
<tr><th colspan="2">Standard Program</th><th colspan="2">Fast Program</th></tr>
<tr><td colspan="2">2 min 50 °C
10 min 95 °C</td><td colspan="2">2 min 95 °C</td></tr>
<tr><td>15 sec 95 °C</td><td rowspan="2">45 cycles</td><td>5 sec 95 °C</td><td rowspan="2">45 cycles</td></tr>
<tr><td>1 min 60 °C</td><td>30 sec 60 °C *</td></tr>
</table>

*32 secondes 60 °C for the 7500 Thermofisher

Contact us for other thermalcyclers.

VI. 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.

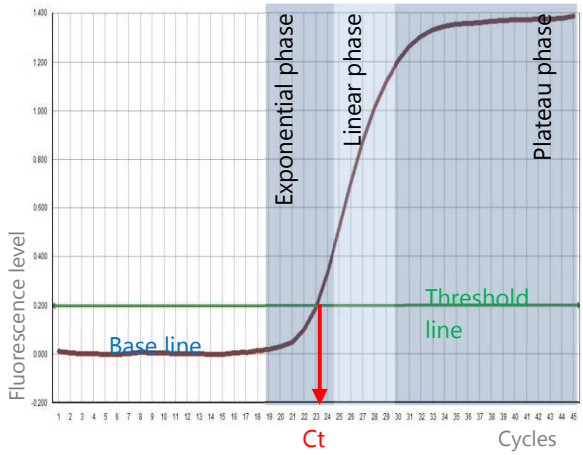
The **« Characteristic amplification curve »** qualifies a fluorescence curve with an exponential phase, a linear phase and a plateau phase.

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

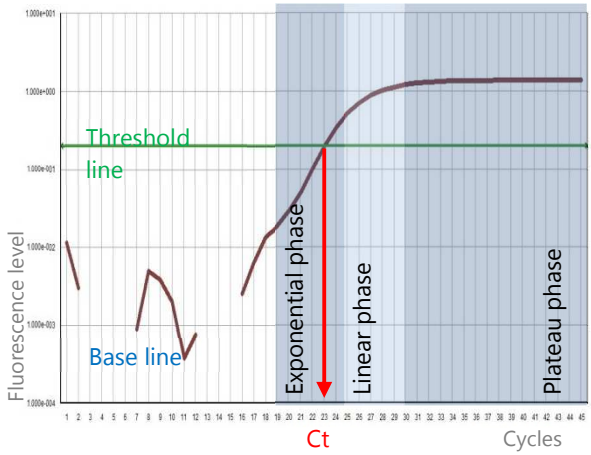
The **« threshold cycle » (Ct)** of a well corresponds, for each detected fluorophore, to the crossing point of the threshold line and fluorescent curve. The Ct value expressed by the machine for each well depends on the threshold position and on the quantity of the target sequence initially present.

Example of characteristic amplification curve

Arithmetic scale of Y axis



Logarithmic scale of Y axis



2. Validation and interpretation of results

Display the FAM curves from the plate and set the threshold value as indicated above. Repeat for the VIC or HEX curves.

A. Results validation

Amplification is considered valid if the following results are obtained according to the controls included:

Controls		No Template Control (NTC)	Amplification positive control (SALMO CTL+)	Extraction negative control	Extraction positive control
FAM Amplification		No	Yes	No	Yes
HEX Amplification	If EPC-Ext used	No	No	Yes	Yes
	If EPC-Amp used	Yes	Yes	Yes	Yes
Validation of		Amplification free of contamination	Amplification of *Salmonella enterica spp.* target	Extraction free of contamination	Extraction and amplification step

The values of Ct expected in FAM and VIC/HEX for the positive control ("CTL +") are indicated on the certificate of analysis of the kit.

B. Results interpretation

DNA extraction and amplification are considered valid for each sample if at least one characteristic amplification curve is observed for *Salmonella spp* (FAM) or internal control (VIC/HEX).

Exemple	A	B	C
FAM amplification	No	Yes	No
HEX amplification	Yes	Yes / No	No
Results	Undetected	Detected	Undetermined

The sample is considered **undetected** if a characteristic amplification curve is observed in VIC or HEX but not in FAM (example A).

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

The absence of a characteristic amplification curve for a sample in both FAM and VIC or HEX (example C) indicates an issue with DNA extraction (loss or destruction of the DNA) or a defective real-time RT-PCR (presence of inhibitors in the sample, program error or lack of sample). In this case, we recommend repeating the test using pure DNA extract and a ten-fold dilution in Nuclease-Free water. If the test is still not validated, a new DNA extraction is recommended.

VII. Symbols index

Symbole	Signification
	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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