

# Thermo Scientific SureTect System: Stability of lysates and their use in SureTect System protocol

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## Key Words

Real-time PCR, Listeria, Salmonella, Food analysis, Lysate, Thermo Scientific SureTect Real-time PCR System

## Goal

The goal of this study was to evaluate the stability of lysed samples (lysates) stored at +4°C with and without the presence of lysis particles in the lysates.

## Abstract

A study was conducted to evaluate the stability of lysed samples stored at +4°C with and without the presence of lysis particles in the lysates. Enriched food samples spiked with target organisms of Thermo Scientific™ SureTect™ Salmonella species, Thermo Scientific™ SureTect™ Listeria monocytogenes and Thermo Scientific™ SureTect™ Listeria species PCR assays and lysed according to the SureTect lysis protocol were subjected to storage at 4°C and then tested on the SureTect Real-Time PCR assays at predefined time points.

Lysates were shown to be stable for at least for 24 hours following storage at +4°C. When comparing the average Cq values obtained from the different time points to freshly prepared lysates, no difference was noticed (>95% stability left). The ability to use the same lysates for reanalysis or for subsequent analysis with different PCR assays reduces the amount of work by removing the need to repeat the lysis step or to aliquot the lysate into separate tube away from the lysis particles.

## Introduction

Until now the storage time for the lysates has been restricted to 30 minutes due to concerns of the lysis particles' degenerative effects on the performance of the lysates. From a user perspective, ability to store lysates at +4°C for extended periods could reduce extra work needed to reanalyze the samples or to analyze a single sample with several assays in a series (e.g. first with the SureTect Listeria species assay followed by the SureTect Listeria monocytogenes assay in the case of a positive result with the first test). Currently, the SureTect assay Instructions For Use (IFU) state that lysates can be stored for up to 30 minutes and if reanalysis is found necessary, a



sample should be prepared by going back to the enriched sample and repeating the lysis steps to prepare a fresh lysate. The possibility of conducting further tests from stored lysates would remove the need for additional lab work.

## The Study

A beef sample was enriched in Buffered Peptone Water (ISO) overnight and then spiked with *Salmonella enterica* subsp. *enterica* serotype Typhimurium cells to a final concentration of approximately 10<sup>4</sup> CFU/ml (results in 10 copies of target DNA to Real-Time PCR reaction). Samples of salami and brie cheese were similarly prepared by enrichment overnight in 24 LEB and then spiked with *Listeria monocytogenes* cells to the same level. These samples were then lysed according to the SureTect lysis protocol by pipetting 10µl of the enriched sample to strip tube wells prefilled with 170µl of Lysis Reagent 1 and 10µl of added Proteinase K. 10µl of Lysis reagent 2 was also added to the strip wells to be used for the Listeria reactions prior to sample addition. 10 replicate lysis reactions were

made by adding 10µl from each enriched sample to the appropriate strip wells. Samples were then lysed by incubating them for 10 min at +37°C and 5 min at +95°C.

After the lysis, 140µl of lysate sample was removed from five replicate strip wells of each matrix without disrupting the lysis particles at the bottom of the wells. These lysates were transferred into clean tubes and closed with appropriate caps. The transferred lysates and the remaining five lysates with lysis particles still at the bottom of the strip wells were then stored at +4°C for further analysis.

Immediately after the lysis step (time point zero), Real-Time PCR runs were performed from five replicate lysates with and without lysis particles by transferring 20µl of the lysates into Real-Time PCR tubes prefilled with lyophilized SureTect Real-Time PCR reagents for detection of *Salmonella* spp., *Listeria* spp. or *Listeria monocytogenes*. The beef sample lysates were analyzed with the SureTect *Salmonella* species Assay; the salami lysates with the SureTect *Listeria monocytogenes* Assay and the cheese lysates with SureTect *Listeria* species Assay. After pipetting the sample into the appropriate PCR strips, the lysate strips were sealed and put back for storage at +4°C.

The five lysates of each sample were analyzed at time point zero and after 3h, 5h, 8h and 24h storage at +4°C in separate Real-Time PCR runs.

A positive control containing 2,000 copies of target DNA was analyzed in each run to observe any variation between the separate Real-Time PCR runs. The positive control was stored at -20°C between the Real-Time PCR runs. Each Real-Time PCR run was conducted using the same Real-Time PCR instrument.

Real-Time PCR strips were sealed with optical clear caps and analyzed on Thermo Scientific™ PikoReal™ Real-Time PCR instruments using the PikoReal software. All runs were analyzed using the same threshold level.

Stability of the lysates was evaluated by comparing the Cq values obtained at each time point compared to those obtained from the time point zero.

$$\% \text{ stability} = \text{Cq average (Fresh)} / \text{Cq average (Stored)} \times 100$$

In theory, all percentage values below 100% indicate loss in the stability. Taking account for the variation within and between the analytical method as well as other possible non-stability related factors, percentage values <95% are regarded significant, indicating actual decrease in the stability.

## Results

### SureTect *Salmonella* species Assay

The average Cq values of five replicate reactions from lysates stored with or without lysis particles are presented in Table 1 and Chart 1. These results are presented in percentages in Table 2 and Chart 2.

### SureTect *Listeria monocytogenes* Assay

Average Cq values of five replicate reactions stored with or without lysis particles are presented in Table 3 and Chart 3. These results are presented in percentages in Table 4 and Chart 4.

### SureTect *Listeria* species Assay

Average Cq values of five replicate reactions stored with or without lysis particles are presented in Table 5 and Chart 5. These results are presented in percentages in Table 6 and Chart 6.

Table 1. Average Cq values of *Salmonella* species PCR runs obtained from beef lysates stored with or w/o lysis particles at +4 °C.

<i>Salmonella</i> spp. Cq values					
	Fresh	3 h	5 h	8 h	24 h
with lysis particles	40.1	39.7	39.7	38.9	40.3
w/o lysis particles	39.5	40.0	40.3	40.2	40.3
+control	32.0	32.8	32.9	32.7	32.9

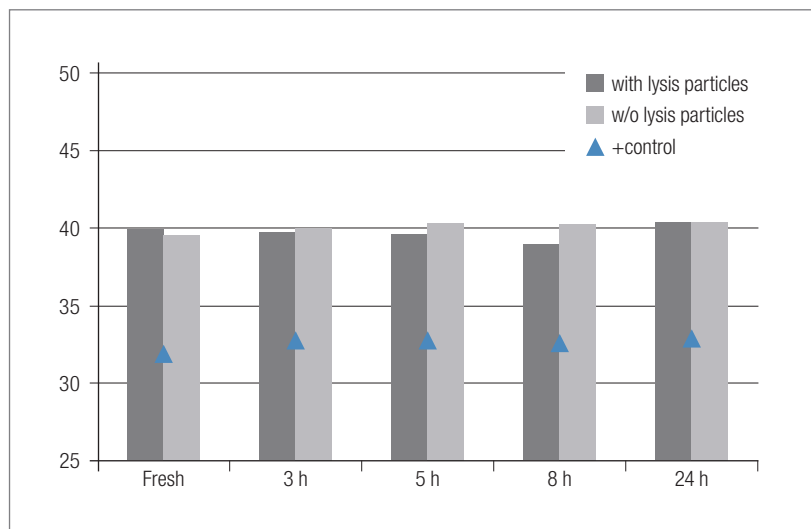


Chart 1. Average Cq values of *Salmonella* species PCR runs obtained from beef lysates stored with or w/o lysis particles at +4 °C.

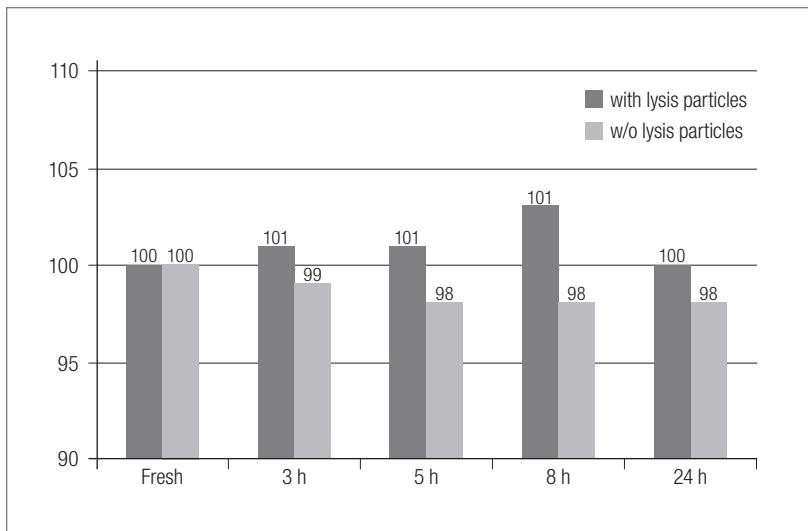


Chart 2. Percentage performance of *Salmonella* species lysates at tested time points.

Table 2. Percentage performance of *Salmonella* species lysates 3 at tested time points.

<i>Salmonella</i> spp. stability compared to fresh (%)					
	Fresh	3 h	5 h	8 h	24 h
<b>with lysis particles</b>	100	101	101	103	100
<b>w/o lysis particles</b>	100	99	98	98	98

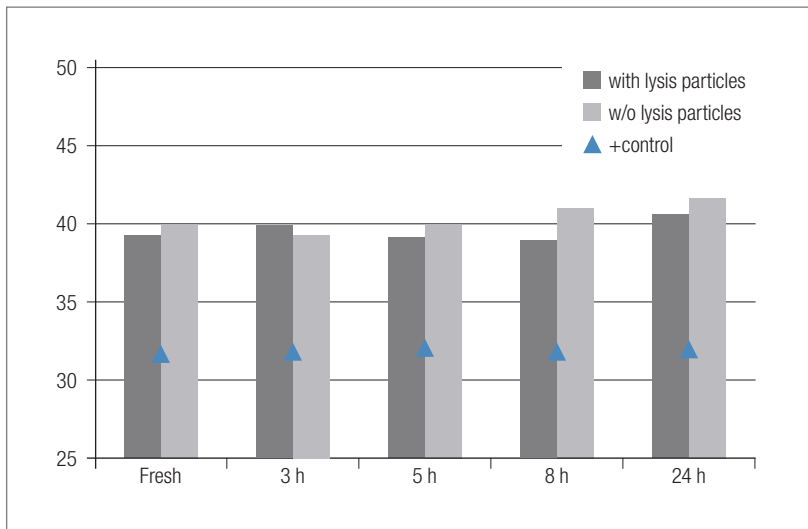


Chart 3. Average Cq values of *Listeria monocytogenes* PCR runs obtained from salami lysates stored with or w/o lysis particles at +4 °C.

Table 3. Average Cq values of *Listeria monocytogenes* PCR runs obtained from salami lysates stored with or w/o lysis particles at +4 °C.

<i>L. monocytogenes</i> Cq values					
	Fresh	3 h	5 h	8 h	24 h
<b>with lysis particles</b>	39.3	40.0	39.2	38.9	40.6
<b>w/o lysis particles</b>	39.9	39.2	40.0	41.0	41.6
<b>+control</b>	31.7	31.7	32.0	31.8	32.0

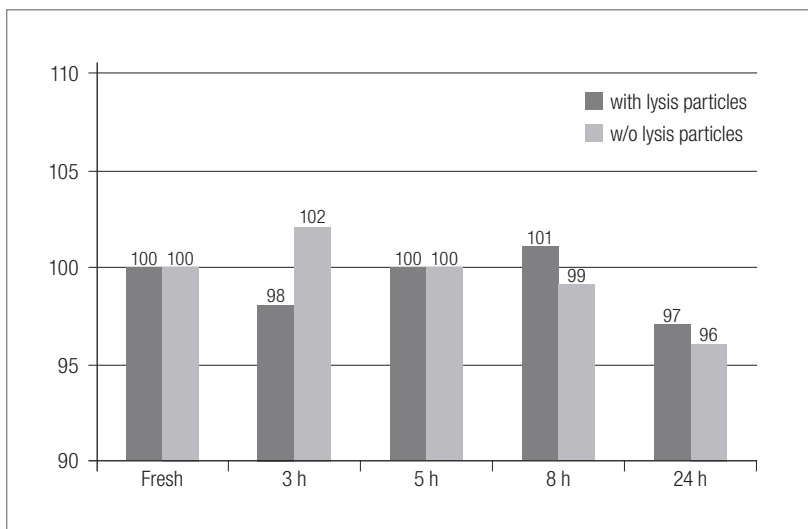


Chart 4. Percentage performance of *Listeria monocytogenes* lysates at tested time points.

Table 4. Percentage performance of *Listeria monocytogenes* lysates at tested time points.

<i>L. monocytogenes</i> stability compared to fresh (%)					
	Fresh	3 h	5 h	8 h	24 h
<b>with lysis particles</b>	100	98	100	101	97
<b>w/o lysis particles</b>	100	102	100	99	96

Table 5. Average Cq values of *Listeria* species PCR runs obtained from cheese lysates stored with or w/o lysis particles at +4 °C.

<i>Listeria</i> spp. Cq values					
	Fresh	3 h	5 h	8 h	24 h
<b>with lysis particles</b>	41.3	40.9	40.9	40.7	40.5
<b>w/o lysis particles</b>	41.0	41.0	40.7	40.2	40.5
<b>+control</b>	31.8	31.9	32.1	31.8	32.2

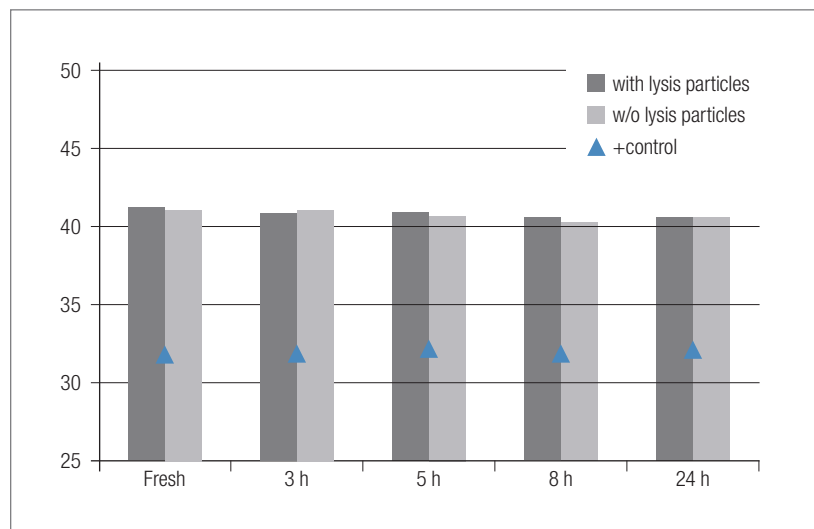


Chart 5. Average Cq values of *Listeria* species PCR runs obtained from cheese lysates stored with or w/o lysis particles at +4 °C.

## Conclusions

According to average Cq values obtained from the lysates stored with or without the lysis particles with the SureTect assays, lysates can be stored at +4°C for up to 24 hours without compromising the performance of the assays. Cq values received during the study indicate that the stability of the lysates remains constant regardless of the presence of the lysis particles. This enables a single lysate to be utilized for multiple PCR runs over a 24 hour time period without the need to aliquot it into separate tubes.

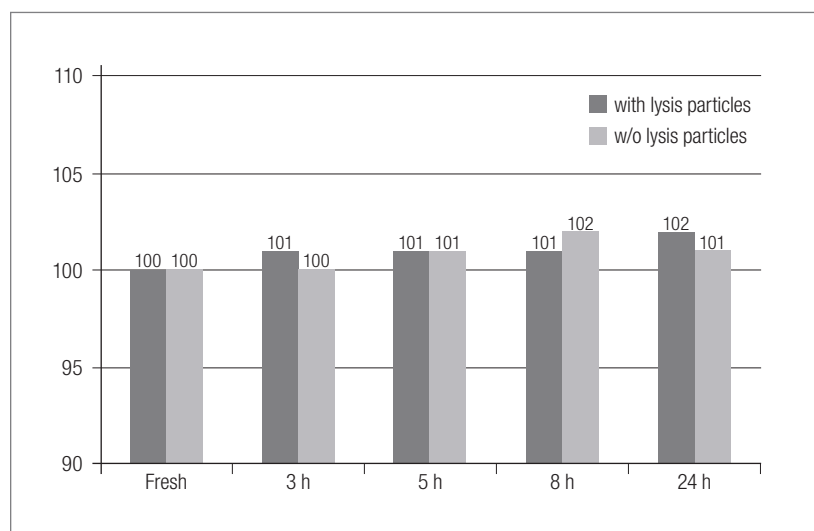


Chart 6. Percentage performance of *Listeria* species lysates at tested time points.

Table 6. Percentage performance of *Listeria* species lysates at tested time points.

<i>Listeria</i> spp. stability compared to fresh (%)					
	Fresh	3 h	5 h	8 h	24 h
<b>with lysis particles</b>	100	101	101	101	102
<b>w/o lysis particles</b>	100	100	101	102	101

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