National Institute for Public Health
and the Environment
*Ministry of Health, Welfare and Sport*

# EURL-*Salmonella* Proficiency Test Live Bivalve Molluscs 2020

Detection of *Salmonella* in mussels

RIVM report 2020-0203

R.E. Diddens | K.A. Mooijman

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# Colophon



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# Synopsis

**EURL-*Salmonella* Proficiency Test Live Bivalve Molluscs 2020**
Detection of *Salmonella* in mussels

In 2020, the European Union Reference Laboratory for *Salmonella* (EURL-*Salmonella*) organised a Proficiency Test for the *Salmonella* bacteria in mussels. This was done to check whether the National Reference Laboratories (NRLs) are able to detect *Salmonella* in live bivalve molluscs.

Twenty-one NRLs scored a good performance. One NRL tested the control samples in the wrong order and therefore also reported the results in the wrong order. The performance of this NRL was scored as moderate. One laboratory scored an unsatisfactory performance, because they indicated that a sample contained *Salmonella* when it did not. In a follow-up study, the performance was scored as good.

A total of 23 NRLs for *Salmonella* participated in this Proficiency Test: 20 NRLs from 20 EU Member States and three NRLs from other European countries. The laboratories used an internationally accepted method to detect the presence of *Salmonella* in mussel samples. Each laboratory had to prepare and spike the samples themselves following a protocol from the EURL-*Salmonella*. For this, they received a package of mussels and frozen milk samples. Some milk samples contained a set concentration of *Salmonella* Typhimurium and others did not.

The EURL-*Salmonella* is part of the Dutch National Institute for Public Health and the Environment (RIVM).

Keywords: *Salmonella*, EURL, NRL, Proficiency Test, *Salmonella* detection method, live bivalve molluscs, mussels

# Publiekssamenvatting

## **EURL-*Salmonella* ringonderzoek Levende Tweekleppige Weekdieren 2020**

Detectie van *Salmonella* in mosselen

In 2020 organiseerde het Europese Unie Referentie Laboratorium voor *Salmonella* (EURL-*Salmonella*) een ringonderzoek voor de *Salmonella*-bacterie in mosselen. Hiermee wordt gecontroleerd of de Nationale Referentie Laboratoria (NRL's) in staat zijn om *Salmonella* in levende tweekleppige weekdieren aan te tonen.

Eenentwintig NRL's hebben een goede score behaald. Eén NRL voerde de controlemonsters niet in de juiste volgorde uit, waardoor zij hun resultaten ook in de verkeerde volgorde aangaven. Dit NRL scoorde daarom matig. Eén laboratorium haalde een slechte score, omdat het aangaf dat er *Salmonella* in een monster zat terwijl dat niet zo was. In de opvolgstudie was de score van dit laboratorium goed.

In totaal deden 23 NRL's-S*almonella* mee aan dit ringonderzoek: 20 NRL's van 20 lidstaten van de Europese Unie en drie NRL's van andere Europese landen.
De laboratoria hebben een internationaal erkende analysemethode gebruikt om *Salmonella* in de mosselmonsters aan te tonen. Elk laboratorium moest de monsters zelf voorbereiden en besmetten, volgens een protocol van het EURL-*Salmonella*. Ze kregen hiervoor een pakket mosselen en bevroren melkmonsters. Sommige melkmonsters waren besmet met een vastgestelde concentratie *Salmonella* Typhimurium en andere niet.

Het EURL-*Salmonella* is gevestigd bij het Nederlandse Rijksinstituut voor Volksgezondheid en Milieu (RIVM).

Kernwoorden: *Salmonella,* EURL, NRL, ringonderzoek, *Salmonella*-detectiemethode, levende tweekleppige weekdieren, mosselen

# Contents

# Summary

In March 2020, an EURL-*Salmonella* Proficiency Test (PT) for the detection of *Salmonella* in Live Bivalve Molluscs (LBM) was organised for the NRLs-*Salmonella.* The matrix under analysis was mussels. Due to measures taken in regard to SARS-CoV-2, not all National Reference Laboratories (NRLs) were able to participate in March. To give these laboratories an opportunity to participate in this PT, a second round was organised in August 2020.
In total 23 NRLs-*Salmonella* participated in this PT: 20 NRLs from 20 EU Member States (MS) and three NRLs from third countries (EU candidate MS and members of the European Free Trade Association (EFTA)).
The most important objective was to test the performance of the participating laboratories in their detection of *Salmonella* Typhimurium (STm) in the mussel samples. The prescribed method for the detection of *Salmonella* spp. was EN ISO 6579-1:2017. The participants were asked to report *Salmonella* 'detected' or 'not detected' for each sample (after confirmation).
The laboratories had to prepare the samples for this PT themselves by spiking the mussels with (*Salmonella*) reference materials. The mussels and the *Salmonella* reference materials (RMs) were both provided by the EURL-*Salmonella*.
Prior to the start of the Proficiency Test, pre-tests were conducted to ensure the samples were fit for use. Additionally, the concentration of the natural background flora (aerobic count and *Enterobacteriaceae*) in the mussels was measured. The aim was to spike the mussel samples with customised *Salmonella* reference materials at a concentration of approximately 10 cfu/25 g mussel sample. In the pre-test, three different batches of mussel samples were tested which were inoculated with 9-12 cfu STm/sample. The mussel samples were analysed following EN ISO 6579-1:2017 and *Salmonella* was detected in all spiked samples, but not in the non-spiked mussel samples. In the mussels of all three batches, the concentration of *Enterobacteriaceae* was less than 10 cfu/g. The aerobic count was $1{,}1 \times 10^3$, $1{,}3 \times 10^3$ and $3{,}2 \times 10^3$ cfu/g for the three different batches. In July 2020, another pre-test was performed for the second round of this PT. The results were comparable to the results of the first pre-test.

For this EURL-*Salmonella* PT LBM, each NRL received a 2 kg package of mussels and four vials of customised (*Salmonella*) reference materials. Following a protocol, each NRL had to prepare four 25 g samples of mussel flesh and intravalvular fluid. The laboratories then had to spike each sample with 100 µl of the reference material with the corresponding sample. Three vials of reference materials contained *Salmonella* Typhimurium in a milk matrix and one vial of reference material did not contain *Salmonella* (sterile milk only). The inoculation levels of *Salmonella* Typhimurium in the mussel samples at the EURL-*Salmonella* at the start of the PTs were 13 cfu/mussel sample in March and 12 cfu/mussel sample in August 2020.

The NRLs also had to test two control samples in the PT: a procedure control (only Buffered Peptone Water) and a positive control with *Salmonella*.
Twenty-one laboratories fulfilled the criteria for good performance in the EURL-*Salmonella* Proficiency Test for detection of *Salmonella* in mussel samples. One laboratory (lab code 12), scored a moderate performance, as this NRL performed the control samples in mixed order and therefore reported the results in the wrong order. The raw data showed that the results of the control samples were correct. Laboratory 8 scored an unsatisfactory performance in this PT because this NRL detected *Salmonella* in a *Salmonella*-negative mussel sample. In a follow-up study, the laboratory scored a good performance.

The specificity rate for the negative mussel samples was 96%. The sensitivity rates for the contaminated mussel samples with *Salmonella* was 100% and the accuracy rate of all mussel samples for all participating laboratories was 98,9%.

In addition to the prescribed method (EN ISO 6579-1:2017), the NRLs-*Salmonella* were given the opportunity to analyse the mussel samples with a second detection method, if this method was (routinely) used in the laboratory.
Seven laboratories used a second method for the detection of *Salmonella* in the mussel samples. The methods used were PCR, qPCR and Rapid *Salmonella*. The results of the second detection method were all equal to the results obtained with EN ISO 6579-1:2017.

# 1 Introduction

An important task of the European Union Reference Laboratory for *Salmonella* (EURL-*Salmonella*), as laid down in Commission Regulation EC No 2017/625 (EC, 2017), is the organisation of Proficiency Tests to evaluate the performance of the National Reference Laboratories for *Salmonella* (NRLs-*Salmonella*). The history of the Proficiency Tests on the detection of *Salmonella*, as organised by EURL-*Salmonella* from 1995, is summarised on the EURL-*Salmonella* website (EURL-*Salmonella*, 2019).

The objective of the current study was to test whether the participating laboratories could detect *Salmonella* in mussels. This is important in order to verify that the examination of samples is carried out uniformly in all EU Member States (MS) and that comparable results are obtained by all NRLs-*Salmonella*.

The method prescribed for the detection of *Salmonella* spp. is set out in EN ISO 6579-1:2017.

The Proficiency Test (PT) was organised in March 2020 and NRLs-*Salmonella* which analyse Live Bivalve Molluscs (LBM) were invited to participate. Due to the SARS-COV-2 pandemic, not all laboratories were able to participate in March. Therefore a second round for this PT was organised in August 2020.

The laboratories had to prepare the samples themselves by spiking the mussel samples with (*Salmonella*) reference materials. The mussels and the *Salmonella* reference materials were both provided by the EURL-*Salmonella*.
Four mussel samples had to be tested by each NRL-*Salmonella.* Three samples were spiked with a reference material containing *Salmonella* Typhimurium in a milk matrix and one sample was spiked with reference material without *Salmonella* (only sterile milk). Additionally, two control samples (procedure control and own positive control with *Salmonella*) had to be tested by the laboratories.

# 2 Participants

| Country | City | Institute / NRL-*Salmonella* |
|---|---|---|
| **Albania** | Tirana | Food Safety and Veterinary Institute (FSVI) |
| **Belgium** | Brussels | Sciensano |
| **Bulgaria** | Sofia | National Diagnostic Research Veterinary Institute (NDRVMI) |
| **Croatia** | Split | Croatian Veterinary Institute Zagreb |
| **Cyprus** | Nicosia | Cyprus Veterinary Services |
| **Denmark** | Ringsted | Danish Veterinary and Food Administration |
| **France** | Nantes | Institut français de recherche pour l'exploitation de la mer (IFREMER) |
| **Germany** | Berlin | German Federal Institute for Risk Assessement |
| **Greece** | Chalkida | Veterinary Laboratory of Chalkis |
| **Hungary** | Budapest | National Food Chain Safety Office |
| **Ireland** | Co Kildare | Department of Agriculture, Food and the Marine Laboratories |
| **Italy** | Ancona | Istituto Zooprofilattico Sperimentale dell'Umbria e delle Marche "Togo Rosati" |
| **Latvia** | Riga | Institute of Food Safety, Animal Health and Environment BIOR |
| **Netherlands** | Bilthoven | National Institute for Public Health and the Environment (RIVM), Centre for Zoonoses and Environmental Microbiology |
| **Norway** | Bergen | Institute of Marine Research |
| **Poland** | Puławy | National Veterinary Research Institute (NVRI) |
| **Portugal** | Algés | Instituto Portugues do Mar e Atmosfera, I.P. |
| **Romania** | Bucharest | Institute for Diagnosis and Animal Health |
| **Slovenia** | Ljubljana | National Veterinary Institut, Veterinary faculty, UL |
| **Spain** | Majadahonda | Centro Nacional de Alimentación-AESAN |
| **Sweden** | Uppsala | National Veterinary Institute |
| **Switzerland** | Zürich | Institute for Food Safety and Hygiene, University of Zurich |
| **United Kingdom** | Weymouth | Centre for Environment, Fisheries and Aquaculture Science (Cefas) |

# 3 Materials and methods

## 3.1 Mussel samples and reference materials

### *3.1.1 Mussels*

The matrix used for this PT was mussels. Packages of 2 kg fresh mussels were obtained from a supermarket in the Netherlands. For the two rounds of the PT organised in March and August, two different batches of mussels were obtained. Per batch, the packages of fresh mussels had an identical packing date and expiration date.
One package of mussels of each batch was checked for the absence of *Salmonella*. Per batch, five samples were tested, and for each sample at least 10 mussels were opened and the content pooled. Twenty-five grams of pooled mussel flesh and intravalvular fluid was weighed in a sample bag, to which 225 ml of Buffered Peptone Water (BPW) was added. The samples were then mixed with a homogeniser. For preparation of the samples, the procedures described in EN ISO 6887-1:2017 and EN ISO 6887-3:2017 were followed. After pre-enrichment at 37 °C ± 1 °C for 18 h ± 2 h, selective enrichment was carried out in Muller-Kauffmann tetrathionate-novobiocin broth (MKTTn) and on Modified semi-solid Rappaport-Vassiliadis agar (MSRV) agar. The MKTTn tubes and the suspect growth on MSRV plates were then plated out on Xylose Lysine Deoxycholate (XLD) agar and Brilliance *Salmonella* Agar (BSA). Suspect colonies were confirmed biochemically and serologically.

Important steps in analysing live bivalve molluscs are the opening of the shells and the preparation of the samples. As the EURL-*Salmonella* wanted these steps to be part of the PT, it was not possible to artificially contaminate the mussels at the EURL-*Salmonella* without opening the mussels. Therefore it was decided to spike the mussels with reference materials after opening and preparation at the participating laboratories.

### *3.1.2 Reference materials*

Reference materials (RMs) from Biosisto (the Netherlands), a supplier of (certified) microbiological reference materials were used to spike the mussel samples.

The RMs consisted of vials containing milk with a set concentration of *Salmonella* Typhimurium per ml. Negative RMs were also used, consisting of sterile milk only. The supplier guaranteed a stability of the RMs for at least two years after the production date, if the vials were stored (unopened) at -70 °C to -86 °C.
For the pre-test, RMs from the standard collection of Biosisto were used:

- Batch 20G-1811, consisting of RMs containing 1,5 ml milk with *Salmonella* Typhimurium (NCCB 100483, ATCC 14028) at a concentration of 480 cfu/ml with a standard deviation of 79 cfu/ml.

For the PT, customised RMs were ordered from Biosisto:

- Batch PT LBM 2020 A, B and D, consisting of RMs containing 1,25 ml milk with *Salmonella* Typhimurium (NCCB 100483, ATCC 14028). The concentration of the RMs was 110 cfu/ml with a standard deviation of 18 cfu/ml.

- Batch PT LBM 2020 C, consisting of RMs containing 1,25 ml milk without *Salmonella* spp.

### 3.1.3 *Pre-tests for the preparation of mussel samples spiked with* Salmonella *reference materials*

Three different packages of mussels were used for the pre-tests. RMs with *Salmonella* Typhimurium, batch 20G-1811, were used for spiking.

Tenfold dilutions were prepared from the reference material in peptone saline solution in order to artificially contaminate the mussel samples with approximately 10 cfu/25 g. For the enumeration of the contamination level, 0,2 ml of the diluted reference material was inoculated on XLD agar and incubated at 37 °C ± 1 °C for 24 h ± 3 h.
In addition to the artificially contaminated samples, three mussel samples per batch were prepared without the addition of *Salmonella* to test if the batches of mussels were not naturally contaminated with *Salmonella*.
The artificially contaminated and negative samples were tested for the presence of *Salmonella* following EN ISO 6579-1:2017 (see 3.1.1).

The level of the natural background flora was determined in the three mussel batches by analysing the number of aerobic bacteria and *Enterobacteriaceae* (see 3.1.4).

Additionally, the stability of the RMs was tested during storage at -20 °C (instead of storage at -70 °C to -86 °C , as prescribed by the supplier). The concentration of *Salmonella* in the RMs was tested after one week and after two weeks of storage at -20 °C.

Due to the SARS-COV-2 pandemic, a second PT round was organised in August 2020. For this second round, another pre-test was performed with a new package of 2 kg of mussels and the customised *Salmonella* reference materials, which had been stored at -70 °C since receipt in February 2020.

### 3.1.4 *Determination of the level of background flora in mussels*

The total number of aerobic bacteria and the number of *Enterobacteriaceae* in the mussels were investigated by following EN ISO 4833-1:2013 and EN ISO 21528-2:2017 respectively. For this purpose, an initial suspension was prepared by adding 225 ml of peptone saline solution to 25 g of mussel flesh and intravalvular fluid (EN ISO 6887-1:2017). Finally, tenfold dilutions of the initial suspension were analysed on Plate Count Agar (PCA) and on Violet Red Bile Glucose (VRBG) Agar.

### 3.1.5 *Determination of the concentration of* Salmonella *in the customised reference materials*

The concentration of *Salmonella* in the customised RMs ordered for the PT was determined by enumeration on XLD and on Tryptone Soy Agar (TSA). The concentration was tested in February, in March (one week before the start of the PT) and in July 2020 (before the second round of the PT). Since receipt in February 2020, the RMs had been stored at -70 °C.

## 3.2 Design of the Proficiency Test

### *3.2.1 Number and type of samples*

The materials for the PT, a 2 kg package of mussels and the (*Salmonella*) RMs, were sent in two different packages to the NRLs.
Four vials of (*Salmonella*) RMs were packed with dry ice and sent to each NRL by a door-to-door courier service on 16-03-2020. The vials were labelled A, B, C and D. The laboratories were asked to store these vials at -20 °C until the start of the PT on 18-03-2020. Not all laboratories were able to participate in the March 2020 PT and were therefore asked to store the RMs between -70 °C and -86 °C until the start of the second round of the PT. Those NRLs which did not receive the reference materials in March, were sent the four vials in August 2020.
The fresh mussels were packed with frozen cooling elements and sent with a door-to-door courier service on 16-03-2021. The participants were asked to store the mussels at 5 °C until the start of the PT. The NRLs which participated in the second round of the PT (August 2020) received a new batch of mussels. The packages of 2 kg of fresh mussels were packed under modified atmosphere.

Four samples (numbered A, B, C and D) and two control samples (numbered CTRL1 and CTRL2) had to be tested by each participating laboratory. Table 3.1 gives an overview of the number and type of samples tested by each participant.
For the positive control samples, the laboratories had to use their own positive control that they normally used when analysing routine samples for the detection of *Salmonella*. In addition to this positive control (CTRL2), a procedure control (CTRL1) consisting of only Buffered Peptone Water had to be analysed.

*Table 3.1 Overview of the number and type of samples tested per laboratory in the Proficiency Test Live Bivalve Molluscs 2020*

| **Contamination level** | **Test samples with mussels (n=4)** |
|---|---|
| Negative sample (mussel sample spiked with a reference material without *Salmonella*) | 1 |
| Positive sample (mussel sample spiked with a reference material containing *Salmonella* Typhimurium*)* | 3 |
| | **Control samples (n=2)** |
| Procedure control (only BPW) | 1 |
| Positive control with *Salmonella* | 1 |

### *3.2.2 Shipment of parcels and temperature recording during shipment*

The mussels were packed in a large safety bag and placed in a parcel with four frozen cooling elements. The parcel also included six empty sample bags (labelled A, B, C, D, CTRL1 and CTRL2).
To monitor exposure to excessive temperatures during shipment and storage, temperature buttons were used to record the temperature. These buttons are tiny units sealed in a stainless-steel case, 16 mm in diameter

and 6 mm deep. One button was packed together with the package of mussels in the large safety bag. The loggers were programmed by the EURL-*Salmonella* to measure the temperature every hour. Each NRL-*Salmonella* had to return the temperature recorder to the EURL-*Salmonella* on the day the laboratory started the PT. At the EURL-*Salmonella*, the loggers were read using a computer program and all recorded temperatures from transport and storage were transferred to an Excel sheet.
The parcel was sent to the participants as 'biological substances category B (UN3373)' (IATA, 2020) using a door-to-door courier service.

The four vials of RMs were also packed in a safety bag and sent separately to the mussels. The safety bag was placed in a parcel to which approximately 5 kg dry ice was added. The parcel was also sent as 'biological substances category B (UN3373)' with the addition of dry ice (UN1845). The participants were asked to report if the *Salmonella* reference materials were still frozen on receipt.

Further details about the shipping and handling of the samples and the reporting of the test results can be found in the protocol (EURL-*Salmonella*, 2020a) and in (a printout from) the result form (EURL-*Salmonella,* 2020b).

## 3.3 Methods

The prescribed method was EN ISO 6579-1:2017 and the underlying EN ISO documents, e.g., the EN ISO 6887 series, for the preparation of the test samples.

The laboratories had to prepare the mussel samples themselves and had to spike them with the *Salmonella* reference materials provided by the EURL-*Salmonella*.

The laboratories were provided with the following instructions for the preparation and spiking of the mussel samples. For the correct use of the *Salmonella* reference materials:

- Defrost the vials at room temperature for 30 minutes on the day of the start of the Proficiency Test.
- Store in the refrigerator at 0 – 4 °C, until use.
- Mix well before use.

Preparation of mussel samples (A, B, C and D):

- Per sample, open and pool the content of at least 10 mussels.
- Weigh 25 g of pooled sample in the supplied sample bag. Open more mussels if needed.
- Repeat the preparation for the other three samples.

Spike the mussel samples with the corresponding vial:

- Mix the *Salmonella* reference material well before use.
- Each sample should be spiked with 100 µl of the corresponding vial.
- Sample A should be spiked with 100 µl of vial A.
- Sample B should be spiked with 100 µl of vial B.
- Sample C should be spiked with 100 µl of vial C.

- Sample D should be spiked with 100 µl of vial D.

After sample preparation, the prescribed method had to be followed. In summary:

- pre-enrichment in:
  Buffered Peptone Water (BPW);
- selective enrichment in/on:
  Muller-Kauffmann tetrathionate-novobiocin (MKTTn) broth;
  Modified semi-solid Rappaport-Vassiliadis (MSRV) agar and/or;
  Rappaport-Vassiliadis with Soya (RVS);
- plating-out on two isolation media:
  first isolation medium: Xylose Lysine Deoxycholate agar (XLD);
  second isolation medium (obligatory): medium of choice;
- confirmation by means of:
  appropriate biochemical and serological tests (EN ISO 6579-1:2017) or reliable, commercially available identification kits.

Additionally, the NRLs-*Salmonella* were given the opportunity to analyse the samples using a second detection method if this method was (routinely) used in their laboratories. These results could also be reported, but only the results obtained with EN ISO 6579-1:2017 were used to assess the performance of the NRL.

## 3.4 Statistical analysis of the data

The specificity, sensitivity and accuracy rates were calculated for the mussel samples spiked with reference materials. For the control samples, only the accuracy rates were calculated. The rates were calculated using the following formulae:

**Specificity rate**

$$\frac{\text{Number of negative results}}{\text{Total number of (negative) samples}} \times 100\%$$

**Sensitivity rate**

$$\frac{\text{Number of positive results}}{\text{Total number of (expected positive) samples}} \times 100\%$$

**Accuracy rate**

$$\frac{\text{Number of correct results (positive and negative)}}{\text{Total number of samples}} \times 100\%$$

## 3.5 Criteria for good performance

For the determination of 'good performance', the criteria indicated in Table 3.2 were used. For the determination of good performance per laboratory, the results obtained with all combinations of selective enrichment media and isolation media used by the laboratory were taken into account.

*Table 3.2 Criteria for good performance in the Proficiency Test LBM 2020*

| **Contaminated samples** | **Percentage positive** | **# pos. samples/ total # samples** |
|---|---|---|
| **Negative samples** | 0% | 0 / 1 |
| **Positive samples** | > 50% | ≥ 2 / 3 |
| **Control samples** | **Percentage positive** | **# pos. samples/ total # samples** |
| **Procedure control** | 0% | 0 / 1 |
| **Positive control with *Salmonella*** | 100% | 1 / 1 |

## 3.6 Follow-up study

A follow-up study was organised in August 2020 at the time of the second PT round. The batch of mussels used for the second round was used for the follow-up study.
The design of the follow-up study differed only slightly from the design of the original PT. Also four mussel samples had to be spiked with (*Salmonella*) reference materials. The same (renumbered) reference materials were used, but in the follow-up study two *Salmonella*-negative reference materials were used instead of one, and two RMs containing *Salmonella* Typhimurium were used instead of three. Additionally, the laboratory was asked to spike each mussel sample with 500 µl reference material (instead of 100 µl in the original PT). The samples were coded as follows: follow-up A to follow-up D. In addition, two control samples had to be tested (as in the original PT): follow-up CTRL1 (procedure control) and follow-up CTRL2 (positive control).

# 4 Results and discussion

## 4.1 Mussel samples and *Salmonella* reference materials

### *4.1.1 Pre-tests for the preparation of mussel samples spiked with* Salmonella *reference materials*

Three different batches of mussels were bought at a supermarket in the Netherlands and were spiked with *Salmonella* RMs for the pre-test. For the preparation and spiking of the mussel samples, the instructions in section 3.3 were followed. Two batches of mussels were each inoculated with 9 cfu STm/sample, and one batch was inoculated with 12 cfu STm/sample. The samples were analysed following EN ISO 6579-1:2017. *Salmonella* was detected in all the spiked samples and no *Salmonella* was detected in the samples which were not spiked.

The natural background flora in the mussels was also tested. For all batches, the concentration of *Enterobacteriaceae* was less than 10 cfu/g. The aerobic count was 1,1 x $10^3$, 1,3 x $10^3$ and 3,2 x $10^3$ cfu/g for the three different batches.

The stability of the reference materials (batch: 20G-1811) was also tested during storage at -20 °C. The concentrations were tested after one week and after two weeks of storage. The results are shown in Figure 4.1.

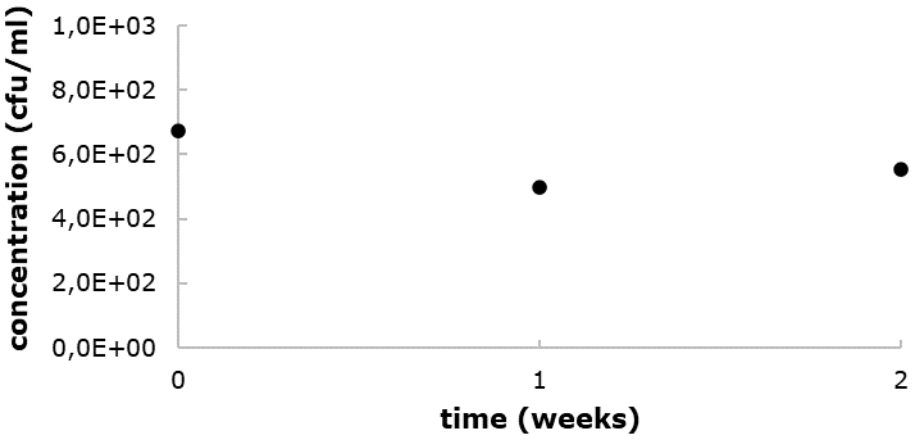



*Figure 4.1 Concentration of* Salmonella *per millilitre of reference material (batch: 20G-1811) during storage at -20 °C*

Based on these results, the aim was to order custom made *Salmonella* RMs to be able to spike the mussel samples with 10 cfu STm/sample. However, the supplier of the RMs was only able to produce *Salmonella* RMs with a minimum concentration of 100 cfu/ml. Therefore the participating laboratories had to spike the mussel samples with 100 µl reference material in order to obtain mussel samples with a contamination level of approximately 10 cfu/sample.

For the second round of this PT, another pre-test was performed in July 2020. The same procedure described in section 3.3 was followed. Six

mussel samples were spiked with the customised *Salmonella* reference material.
Three mussel samples were spiked with 12 cfu/sample and three mussel samples with 18 cfu/sample. The samples were analysed following EN ISO 6579-1 and *Salmonella* was detected in all six samples. In five samples which were not spiked with RMs, no *Salmonella* was detected. The concentration of *Enterobacteriaceae* in the batch of mussels used for the pre-test in July was 30 cfu/g. The aerobic count was 8,6 x $10^3$ cfu/g.

*4.1.2 Preparation of mussel samples for Proficiency Test*

For the PT, packages of 2 kg fresh mussels were obtained from a supermarket in the Netherlands. On 16 March 2020, the packages of mussels were bought and shipped to the participants. Two participants had already indicated that they were unable to participate because of measures taken at their laboratory against SARS-COV-2. All the packages of mussels had a similar packaging date and expiration date, 12-03-2020 and 19-03-2020 respectively.

Later on, seven more laboratories indicated that they were unable to participate in the March 2020 PT, also due to the SARS-COV-2 pandemic. To give these nine laboratories an opportunity to participate in this PT, a second round was organised in August 2020. On 24 August 2020, packages of 2 kg fresh mussels were obtained at the same supermarket in the Netherlands as for the first round, and shipped the same day to the NRLs. The packages of mussels had a packaging date and expiration date of 19-08-2020 and 26-08-2020 respectively.

*4.1.3 Background flora in mussels*

In March and August 2020, an extra package of mussels was used to determine the level of background flora at the start of the PT. Table 4.1 shows the number of aerobic bacteria and *Enterobacteriaceae* in the mussels.

*Table 4.1 Number of aerobic bacteria and* Enterobacteriaceae *per gram mussel flesh and intravalvular fluid*

| **Date** | **Aerobic bacteria (cfu/g)** | ***Enterobacteriaceae* (cfu/g)** |
|---|---|---|
| 18 March 2020 | 7,3 x $10^3$ | <10 |
| 26 August 2020 (round 2) | 9,2 x $10^2$ | 2,5 x $10^2$ |

*4.1.4 Concentration of* Salmonella *in the reference materials*

The *Salmonella* RMs were produced by Biosisto in the Netherlands, a supplier accredited for the production of (certified) reference materials. Two batches of RMs were custom-made for this PT and stored at -70 °C at the EURL-*Salmonella*.

These RMs concerned one batch of vials containing a milk suspension with *Salmonella* Typhimurium, and another batch of vials containing only sterile milk (without *Salmonella*). The batch containing *Salmonella* Typhimurium was labelled A, B and D. The batch without *Salmonella* was labelled C. Every NRL-*Salmonella* received four vials: A, B, C and D.

Table 4.2 shows the concentration of *Salmonella* in the customised reference materials labelled A, B and D. The vials were labelled differently, but were all from the same batch.

In March 2020 the RMs were sent one week before the start of the PT. Due to unexpected circumstances, the parcels with dry ice were kept on hold at the depot centre of the courier service and had to be retrieved. After return to the EURL, the RMs were tested again on 16 and 18 March. The tested RMs experienced similar conditions to those materials used by the laboratories. Section 4.3.2 provides more details of the transport of the materials.
The remaining reference materials were stored at -70 °C at the EURL-*Salmonella* and tested again on 13 July 2020 before the second round of the PT.

*Table 4.2 Concentration of* Salmonella *Typhimurium per millilitre customised reference material (labelled A, B and D) used by the participants to artificially contaminate the mussel samples*

| **Date of testing** | **Concentration of *Salmonella* in the reference materials (cfu/ml)** |
|---|---|
| 12 February 2020 | $1{,}3 \times 10^2$ |
| 11 March 2020 | $1{,}3 \times 10^2$ |
| 16 March 2020[a] | $1{,}3 \times 10^2$ |
| 18 March 2020[b] | $1{,}2 \times 10^2$ |
| 13 July 2020 | $1{,}2 \times 10^2$ |

a. After the materials were sent with dry ice for three days, retrieved by EURL-*Salmonella* and stored at -70 °C.
b. After mimicking shipment of the reference materials by storage of the parcel with reference materials and dry ice successively at room temperature from 09-03-2020 until 12-03-2020, at -70 °C from 12-03-2020 until 16-03-2020 and at room temperature from 16-03-2020 until 18-03-2020.

The NRLs-*Salmonella* were asked to artificially contaminate each mussel sample with 100 µl reference material in order to inoculate the mussels with approximately 10 cfu STm/sample. Table 4.3 shows that the intended inoculation level per 25 gram mussel flesh and intravalvular fluid was reached at the start of the PT in March as well as in August.

*Table 4.3 Contamination levels of* Salmonella *Typhimurium in the mussel samples after inoculation with 100 µl customised reference material, tested at the EURL-*Salmonella *at the start of both rounds of the PT*

| Date of testing | ***S.*** **Typhimurium (cfu per mussel sample)** |
|---|---|
| 18 March 2020[a] Inoculation of mussels with 100 µl reference material at EURL-*Salmonella* | 13 |
| 26 August 2020[b] Inoculation of mussels with 100 µl reference material at EURL-*Salmonella* | 12 |

a. After mimicking shipment of the reference materials by storage of the parcel with reference materials and dry ice successively at room temperature from 09-03-2020 until 12-03-2020, at -70 °C from 12-03-2020 until 16-03-2020 and at room temperature from 16-03-2020 until 18-03-2020.
b. EURL-*Salmonella* PT LBM 2020 – round 2.

The RMs tested on 18 March 2020 were packed in a parcel with dry ice, which was stored under the following conditions at the EURL-*Salmonella*:

- at room temperature from 9 March until 12 March 2020;
- at -70 °C from 12 March until 16 March 2020;
- at room temperature from 16 March until 18 March 2020.

This was done to mimic the shipping of the parcel with RMs to the NRLs.

The reference material labelled C contained sterile milk without *Salmonella*. This material was also tested, and the concentration of *Salmonella* in these vials was always 0 cfu/ml.

## 4.2 Technical data Proficiency Test

### *4.2.1 General*

In total, 23 NRLs-*Salmonella* participated in this PT: 20 NRLs from 20 EU Member States (MS)[1] and three NRLs from third countries (EU candidate MS and members of the European Free Trade Association (EFTA)).
Thirteen laboratories performed the Proficiency Test on 18 March 2020. One participant started the PT, after consulting the EURL-*Salmonella*, on 19 March 2020.
Nine laboratories participated in the second round of this PT in August 2020. Seven laboratories started the PT on 26 August. One laboratory started one day earlier. One laboratory started one day later, on 27 August 2020, after consulting the EURL-*Salmonella*.

### *4.2.2 Accreditation*

Nineteen laboratories are accredited for EN ISO 6579-1:2017. Two laboratories are only accredited for an alternative method: RAPID *Salmonella*. Two laboratories did not specify the method which they have under accreditation.

[1] Including NRL-*Salmonella* from United Kingdom

### 4.2.3 *Transport of samples*

The RMs were sent on Monday 9 March 2020, the week before the start of the first round of the PT in March. Due to unexpected circumstances, the parcels were kept on hold at the depot centre of the courier service. The parcel contained dry ice and the RMs remained frozen until they were retrieved by the EURL-*Salmonella* on Thursday 12 March 2020. The materials were stored at -70 °C at the EURL-*Salmonella* until Monday 16 March 2020 and shipped again, packed with fresh dry ice. The parcels arrived within one to two days at the laboratories. All laboratories indicated that the RMs were still frozen after receipt at the NRL.

On Monday 16 March 2020, the parcels with mussels were sent to 21 laboratories. All parcels were delivered to the NRLs within one to two days.

On Monday 24 August 2020, the parcels with mussels were sent to nine NRLs for the second round of this PT. Eight NRLs received their parcel within one day. Laboratory 1 received the parcel after three days of transport because of a delay in delivery by the courier service. Laboratory 1 started immediately with the PT on receipt of the parcel. Two laboratories which had not received the *Salmonella* RMs in March, also received a parcel with the four vials of (*Salmonella*) RMs, packed with dry ice. Both laboratories indicated that the RMs were still frozen on receipt.

The temperature during transport and storage of the parcels with mussels was registered using a temperature probe. The temperature of eighteen parcels was 5 °C or lower during transport. The measured temperature of two parcels started at 7 °C and after eight hours the measured temperature was 5 °C. The parcel with mussels for laboratory 1 was delivered after three days. In the parcel a temperature was measured of 13 °C. Figure 4.2 shows the temperature record of the parcel for laboratory 1, until receipt and at the start of the PT.
No data were received from two laboratories (laboratories 8 and 23).

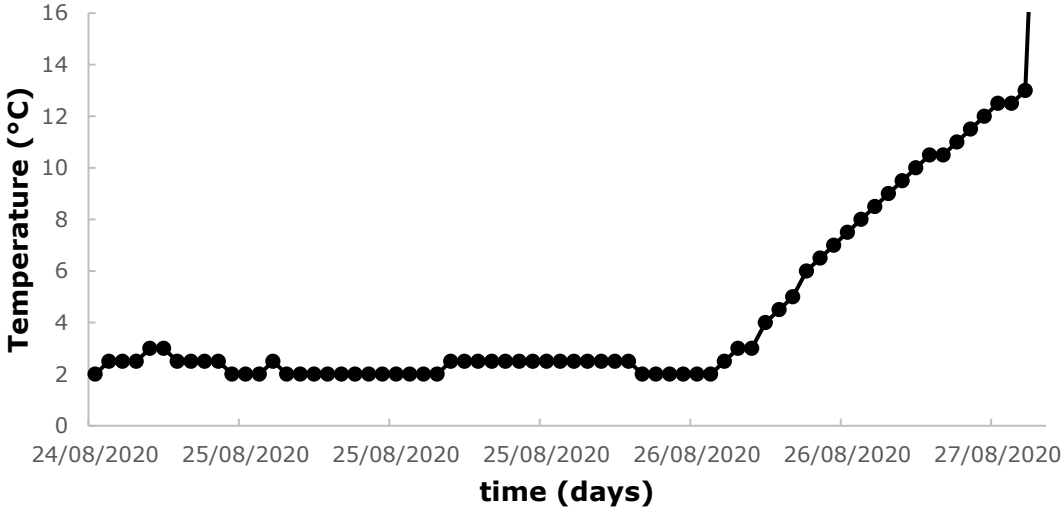



*Figure 4.2 Temperature record of the parcel with mussels sent to laboratory 1*

### *4.2.4 Media*

For this PT, the prescribed method for the detection of *Salmonella* in mussels was EN ISO 6579-1:2017 which stipulates the use of MKTTn and RVS and/or MSRV as selective enrichment media.
Thirteen laboratories used MKTTn and RVS as selective enrichment media (laboratories 2, 4, 5, 8, 9, 12, 13, 15, 18, 19, 20, 21 and 22). Five laboratories used MKTTn and MSRV as selective enrichment media (laboratories 3, 6, 10, 11 and 14).
Three laboratories used all three prescribed selective enrichment media: MKTTn, RVS and MSRV (laboratories 1, 17 and 23).
One laboratory did not use MKTTn as prescribed in EN ISO 6579-1:2017. Laboratory 7 used RVS and MSRV (and not the prescribed MKTTn) as selective enrichment media.
One laboratory did not follow EN ISO 6579-1, but used an alternative method: the Rapid *Salmonella* method (laboratory 16).
Table 4.4 shows the reported values of the incubation times, the concentrations of novobiocin, pH, and the incubation temperatures of the different media. Only those laboratories are shown which reported deviating values from EN ISO 6579-1:2017.

*Table 4.4 Reported technical deviations from prescribed method EN ISO 6579-1:2017*

| | | MKTTn | | | RVS | | MSRV | | |
|---|---|---|---|---|---|---|---|---|---|
| **Laboratory code** | **hours incubation BPW** | **concentration novobiocin (mg /L)** | **pH** | **Temperature (°C)** | **pH** | **Temperature (°C)** | **concentration novobiocin (mg/L)** | **pH** | **Temperature (°C)** |
| EN ISO 6579-1 | 18 ± 2 hours | 40 mg /L | 7 - 8,2 | 37,0 °C ± 1 °C | 5,2 ± 0,2 | 41,5 °C ± 1 °C | 10 mg / L | 5,1 - 5,4 | 41,5 °C ± 1 °C |
| **7** | 18 | | | | 5,2 | 41,5 | 20 | 5,4 | 41,5 |
| **12** | 24 | 40 | unknown | 37 | unknown | 41,5 | | | |
| **14** | 20 | 10 | 8 | 37 | | | 10 | 5,2 | 42 |
| **15** | 18 | 40 | - | 37 | - | 41,5 | | | |
| **16*** | | | | | | | | | |
| **21** | 18 | 40 | unknown | 37 | unknown | 41,5 | | | |
| **23** | 19 | 40 | - | 37 | - | 41,5 | 10 | 5,2 | 41,5 |

Grey cells are deviations from EN ISO 6579-1:2017
- : no information reported
* : did not use EN ISO 6579-1, but an alternative method: the Rapid *Salmonella* method

One laboratory (laboratory 12) used a longer incubation time than prescribed for the pre-enrichment in BPW.
One laboratory (laboratory 14) reported a lower concentration of novobiocin in MKTTn than prescribed.
According to EN ISO 6579-1:2017, the pH of the base medium of MKTTn broth should be 7,8 – 8,2. In addition, it indicates that the complete medium should no longer be used if, after storage, the pH is <7. Four laboratories (laboratory 12, 15, 23 and 23) did not measure the pH of MKTTn. These four laboratories also did not measure the pH of RVS.
One laboratory reported to have used a higher concentration of novobiocin in MSRV than prescribed (laboratory 7).

The selective enrichment culture was plated-out on two isolation media: XLD and an obligatory second isolation medium. The choice of the second isolation medium for the different laboratories can be found in Table 4.5. Several laboratories used Rapid *Salmonella* Agar or BGA or Rambach as a second isolation medium.

*Table 4.5 Second isolation media used by the laboratories*

| **Media** | **No. of users** |
|---|---|
| BGA | 4 |
| BPLS | 3 |
| BSA | 2 |
| Chromogenic *Salmonella* Agar | 3 |
| Compass *Salmonella* | 1 |
| Hectoen Enteric Agar | 1 |
| Rambach | 4 |
| Rapid *Salmonella* Agar | 6 |

Explanations of the abbreviations used are given in the 'List of abbreviations'.

The last step in the procedure for *Salmonella* detection is the confirmation step. All participating laboratories performed one or several confirmation tests for *Salmonella*. An overview can be found in Table 4.6. Twenty-one laboratories performed a biochemical test and the majority performed one or more additional confirmation test(s).
Three laboratories (also) used another confirmation test, such as MALDI-TOF or a combination of multiple tests (*Salmonella* singlepath, gram reaction, oxidase test and RapID ONE).

*Table 4.6 Number of laboratories using different confirmation methods*

| Number of labs | Bio-chemical | Sero-logical | Sero-typing | PCR | Other |
|---|---|---|---|---|---|
| 1 | x | | | | |
| 10 | x | x | | | |
| 2 | x | x | x | | |
| 1 | x | x | | | x |
| 5 | x | | x | | |
| 1 | x | | | x | |
| 1 | x | | | | x |
| 1 | | | | x | |
| 1 | | | | | x |

## 4.3 Control samples

### *4.3.1 General*

Two empty safety bags were sent to each participating NRL-*Salmonella*, which were used for the control samples, being:

- a procedure control consisting only of BPW (CTRL1);
- a positive control with the laboratories' own *Salmonella* control strain (CTRL2).

*Procedure control (BPW only)*

All laboratories but one reported the procedure control sample (no matrix, only BPW) correctly to be negative for *Salmonella*. Only laboratory 12 reported the procedure control as '*Salmonella* detected' and the laboratory was contacted by the EURL-*Salmonella* for an explanation. Raw data showed that the analysis of the control samples were performed in mixed order and therefore they were also reported incorrectly. The raw data showed that the result of the procedure control was, in fact, correctly negative for *Salmonella*.

*Positive control with* Salmonella

The laboratories were asked to use their own, normally used positive control in their routine analysis for the detection of *Salmonella*.
All laboratories but one reported the detection of *Salmonella* in their positive control sample. Only laboratory 12 reported the positive control as '*Salmonella* not detected' and the laboratory was contacted by the EURL-*Salmonella*. Raw data showed that the analysis of the control samples were performed in mixed order and therefore also reported incorrectly. The raw data showed that the result of the positive control with *Salmonella* was correctly tested positive for *Salmonella*.

The *Salmonella* serovars used by the participants for the positive control sample were: *S.* Typhimurium (10 participants), *S.* Nottingham (four participants), *S.* Enteritidis (two participants) and seven participants used other *Salmonella* serovars. More details are given in Table 4.7.

*Table 4.7* Salmonella *serovars used by participants for the positive control samples*

| ***Salmonella* serovar** | **Number of participants** |
|---|---|
| *S*. Typhimurium | 10 |
| *S*. Nottingham | 4 |
| *S*. Enteritidis | 2 |
| *S*. Abaetetuba | 1 |
| *S*. Adabraka | 1 |
| *S*. Alachua | 1 |
| *S*. Derby | 1 |
| *S*. Harleystreet | 1 |
| *S*. Infantis | 1 |
| *S*. Wentworth | 1 |

The concentration of *Salmonella* in the positive control samples used by the different participants varied between 2 and $10^6$ cfu/sample (see Table 4.8). Six laboratories did not determine the concentrations of *Salmonella* added to their positive control sample.

*Table 4.8 Concentration of* Salmonella *in the positive control samples*

| **Concentration *Salmonella* (cfu/sample)** | **Number of laboratories** |
|---|---|
| 2 - 10 | 3 |
| 11 - 120 | 9 |
| 121 - 500 | 3 |
| $10^3$ – $10^6$ | 2 |
| Not Determined | 6 |

A positive control sample for a detection method should demonstrate that media are capable of supporting the growth of the target organisms in low numbers. To obtain information on the sensitivity of a method, the concentration of a positive control sample should preferably be just above the detection limit of the method. Additionally, for a positive control, it may be advisable to use a rarely isolated serovar from the routine samples analysed in the laboratory. In this way, possible cross-contamination can be detected more easily.
Additionally, a more realistic control of the procedure is obtained when the positive control is added to a *Salmonella*–free matrix which is similar to the samples tested.
Four laboratories (lab codes 6, 10, 13 and 23) also used a matrix with their positive control. The matrices used were: minced meat, frozen mussel, dust from a feed factory, and 'raw meat+milk+eggs'.

### *4.3.2 Correct scores of the control samples*

Table 4.9 shows the number of correct scores found with the control samples. The calculations were performed for the results of all participants and for the EU MS only.

*Table 4.9 Correct scores found with the control samples by all participants ('All') and by the laboratories of the EU Member States only ('EU MS')**

| Control samples | | All participants n = 23 | EU MS[a] n = 20 |
|---|---|---|---|
| BPW | No. of samples | 23 | 20 |
| | No. of negative samples | 23 | 20 |
| | Correct score in % | 100% | 100% |
| Own positive control | No. of samples | 23 | 20 |
| | No. of positive samples | 23 | 20 |
| | Correct score in % | 100% | 100% |
| All control samples n=2 | No. of samples | 23 | 20 |
| | No. of correct samples | 23 | 20 |
| | Accuracy in % | 100% | 100% |

* Laboratory 12 switched the reported results of the procedure control and the positive control. The correct scores and accuracy in this table were calculated using the correct raw data.
[a] Including NRL-*Salmonella* from United Kingdom

## 4.4 Mussel samples with reference materials

### *4.4.1 General*

Table 4.10 shows the results of the mussel samples which were spiked with RMs. It shows that the temperature abuse of the parcel with mussels of laboratory 1, as well as the technical deviations (see section 4.2.4), did not influence the final results. *Salmonella* was correctly detected in all positive mussel samples.

*Table 4.10 Number of positive results found with the artificially contaminated mussel samples at each laboratory*

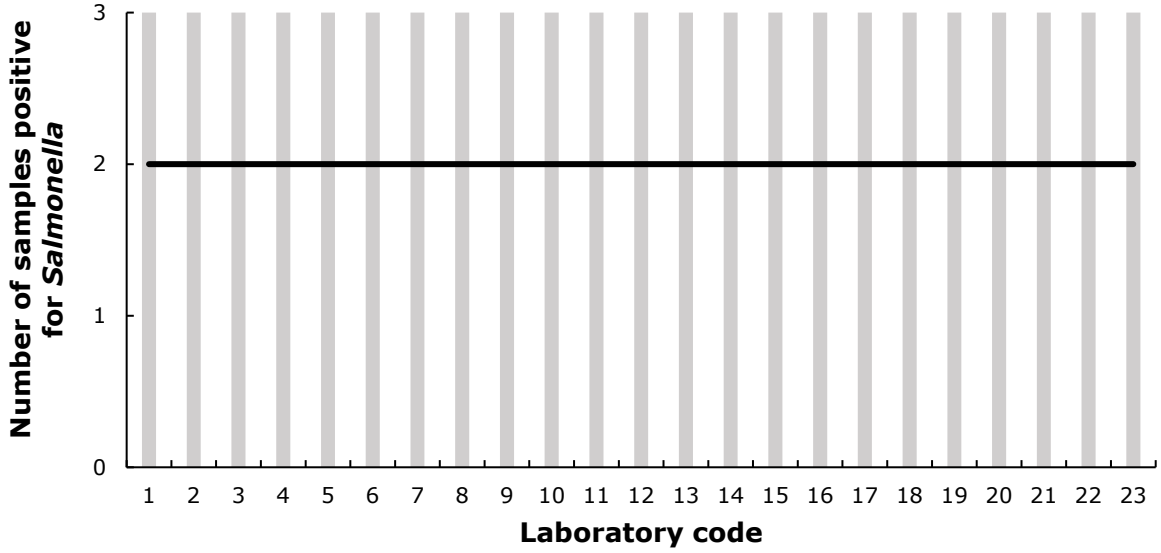
| Laboratory code | Number of samples in which *Salmonella* is detected | |
|---|---|---|
| | negative (n=1) | positive (n=3) |
| **Criteria of good performance** | **= 0** | **≥2** |
| 8 | 1 | 3 |
| All other NRLs-*Salmonella* | 0 | 3 |

*Negative samples*

All laboratories, except laboratory 8, scored the negative mussel sample correctly negative for *Salmonella*. Laboratory 8 detected *Salmonella* in the mussel sample which was spiked with the reference material without *Salmonella*.

*Positive* Salmonella *mussel samples*

All laboratories detected *Salmonella* in all three mussel samples spiked with *Salmonella* RMs. The level of good performance for these PT samples was set at the detection of *Salmonella* in at least two of three samples. Figure 4.3 shows the number of samples in which *Salmonella* was detected per laboratory.




: level of good performance

*Figure 4.3 Number of positive mussel samples (n=3) in which* Salmonella *was detected per laboratory*

### *4.4.2 Specificity, sensitivity and accuracy rates of the artificially contaminated samples*

Table 4.11 shows the specificity, sensitivity, and accuracy rates of the mussel samples tested in this PT. The calculations were performed on the results of all participants and on those of the EU MS participants only.

*Table 4.11 Specificity, sensitivity, and accuracy rates calculated from the results found by all participants ('All') and by the laboratories of the EU Member States ('EU MS') only, with the artificially contaminated mussel samples*

| **Mussel samples** | | **All participants n = 23** | **EU MS[a] n = 20** |
|---|---|---|---|
| Negative samples n = 1 | No. of samples<br>No. of negative samples<br>Specificity in % | 23<br>22<br>96% | 20<br>19<br>95% |
| Positive samples n = 3 | No. of samples<br>No. of positive samples<br>Sensitivity in % | 69<br>69<br>100,0% | 60<br>60<br>100,0% |
| All mussel samples | No. of samples<br>No. of correct samples<br>Accuracy in % | 92<br>91<br>98,9% | 80<br>79<br>98,8% |

[a] Including NRL-*Salmonella* from United Kingdom

## 4.5 Second detection method

Seven laboratories also used a second method for the detection of *Salmonella* in the mussel samples. An overview of the methods used per laboratory is given in Table 4.12. Four laboratories use this second detection method routinely for sample analysis.
The results of the second detection methods were all equal to those obtained with EN ISO 6579-1:2017.

*Table 4.12 Details of the second detection methods used by seven laboratories during the Proficiency Test on detection of* Salmonella *in mussel samples*

| Laboratory code | Second detection method | Vali-dated | Vali-dated by | Routinely used number of tests/year |
|---|---|---|---|---|
| 2 | qPCR | Yes | AOAC Research Institute | NA |
| 4 | qPCR | No | NA | NA |
| 9 | Multiplex qPCR ttrRSBCA-invA (Gonzales-escalona et al, 2012) | No | NA | NA |
| 12 | Real time PCR | Yes | AFNOR | 1000 |
| 14 | Real time PCR | Yes | In-house validation | 329 |
| 15 | RAPID` *Salmonella* method, short protocol | Yes | NMKL-NordVal internatio nal | 200 |
| 20 | BAX System, standard PCR assay for *Salmonella* (a commercial end time PCR-system) | Yes | Nordval | 2521 |

NA: Not Applicable

## 4.6 Performance of the NRLs

### *4.6.1 General*

Twenty-one laboratories fulfilled the criteria for good performance.
Laboratory 8 wrongly detected *Salmonella* in a negative mussel sample and therefore scored an unsatisfactory performance.
Laboratory 12 initially scored an unsatisfactory performance, because they reported to have detected *Salmonella* in the procedure control while *Salmonella* was not detected in their own positive control sample. Raw data showed that the analysis of the control samples were performed in a mixed order and therefore also reported incorrectly. The raw data showed that the results of the control samples were correct, so that the performance of laboratory 12 could be changed to moderate instead of unsatisfactory performance.

### *4.6.2 Follow-up study*

Laboratory 8 detected *Salmonella* in a negative sample. The negative samples was a mussel sample which was spiked with reference material without *Salmonella* (vial C).
Laboratory 8 was asked for a possible technical explanation for their deviating result. The serotyping results were shared and showed that all positive samples were serotyped as *Salmonella* Typhimurium. This serovar was used in the RMs. Additionally, the vials and mussels which were stored when frozen, were tested again by laboratory 8. *Salmonella* was not detected in vial C nor in the mussels. Cross-contamination

during the PT is therefore the most likely explanation for this false positive result.

A follow-up study was organised in August 2020, at the same time of the second round of the PT. The batch of mussels used for the second round of this PT was also used for the follow-up study. The setup of the follow-up study is described in section 3.6 and was dedicated to the problems of laboratory 8 in the full PT (false-positive test result). For that reason, two negative mussel samples (instead of one in the full PT) and two *Salmonella* positive mussel samples (instead of three in the full PT) were included in this follow-up study. The latter samples were artificially contaminated with a slightly higher concentration of *Salmonella* Typhimurium (approximately 60 cfu/sample) than in the full PT (approximately 10 cfu/sample), to test that the laboratory correctly prevented the negative samples from becoming contaminated with the positive samples.

In parallel, similar mussel samples were prepared and tested at the EURL-*Salmonella* on the same date of the performance of the follow-up study. The contamination level of *Salmonella* Typhimurium in the RMs was also checked, and it was determined that an inoculum of 500 µl reference material contained 54 cfu STm/sample.

Laboratory 8 analysed all samples correctly and scored a good performance in the follow-up study. The results are given in Table 4.13.

*Table 4.13 Results of the follow-up study of the EURL-*Salmonella *PT LBM 2020*

| Laboratory code | Number of positive samples / Total number of samples per level | | | |
|---|---|---|---|---|
| | Mussel samples spiked with reference material | | Control samples | |
| | Positive | Negative | Procedure control | Positive control |
| **8** | 2 / 2 | 0 / 2 | 0 / 1 | 1 / 1 |

# 5 Conclusions

Twenty-one laboratories fulfilled the criteria of good performance for the EURL-*Salmonella* Proficiency Test for the detection of *Salmonella* in mussel samples.

One laboratory scored a moderate performance for this EURL-*Salmonella* Proficiency Test.

One laboratory scored an unsatisfactory performance in the Proficiency Test, but scored a good performance in the follow-up study.

The accuracy rate of the control samples was 100%.

The specificity rate of the negative mussel samples was 96%.

The sensitivity rates of the mussel samples artificially contaminated with *Salmonella* was 100%.

The accuracy rate of all mussel samples for all participating laboratories was 98,9%.

Seven laboratories used a second method for detecting *Salmonella* in the mussel samples. The methods used were PCR, qPCR and Rapid *Salmonella*. The results of the second detection methods were all equal to those obtained with EN ISO 6579-1:2017.

# List of abbreviations

| | |
|---|---|
| AFNOR | Association Française de Normalisation (French Standardization Association) |
| AOAC | Association of Analytical Communities |
| ATCC | American Type Culture Collection |
| BGA | Brilliant Green Agar |
| BPLS | Brilliant green Phenol-red Lactose Sucrose |
| BPW | Buffered Peptone Water |
| BSA | Brilliance *Salmonella* Agar |
| CEN | European Committee for Standardization |
| cfu | colony-forming units |
| DG-SANTE | Directorate-General for Health and Consumer Protection |
| EC | European Commission |
| EFTA | European Free Trade Association |
| EU | European Union |
| EURL | European Union Reference Laboratory |
| ISO | International Organization for Standardization |
| LBM | Live Bivalve Molluscs |
| MALDI-TOF | Matrix-Assisted Laser Desorption/Ionization – Time Of Flight |
| MKTTn | Muller-Kauffmann tetrathionate-novobiocin broth |
| MS | Member State |
| MSRV | Modified semi-solid Rappaport-Vassiliadis |
| NCCB | Netherlands Culture Collection of Bacteria |
| NMKL | Nordic Committee on Food Analysis |
| NRL | National Reference Laboratory |
| PCA | Plate Count Agar |
| PCR | Polymerase Chain Reaction |
| PT | Proficiency Test |
| qPCR | quantitative Polymerase Chain Reaction |
| RIVM | Rijksinstituut voor Volksgezondheid en het Milieu (National Institute for Public Health and the Environment) |
| RVS | Rappaport-Vassiliadis Soya broth |
| STm | *Salmonella* Typhimurium |
| TSA | Tryptone Soy Agar |
| VRBG | Violet Red Bile Glucose agar |
| XLD | Xylose Lysine Deoxycholate agar |

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