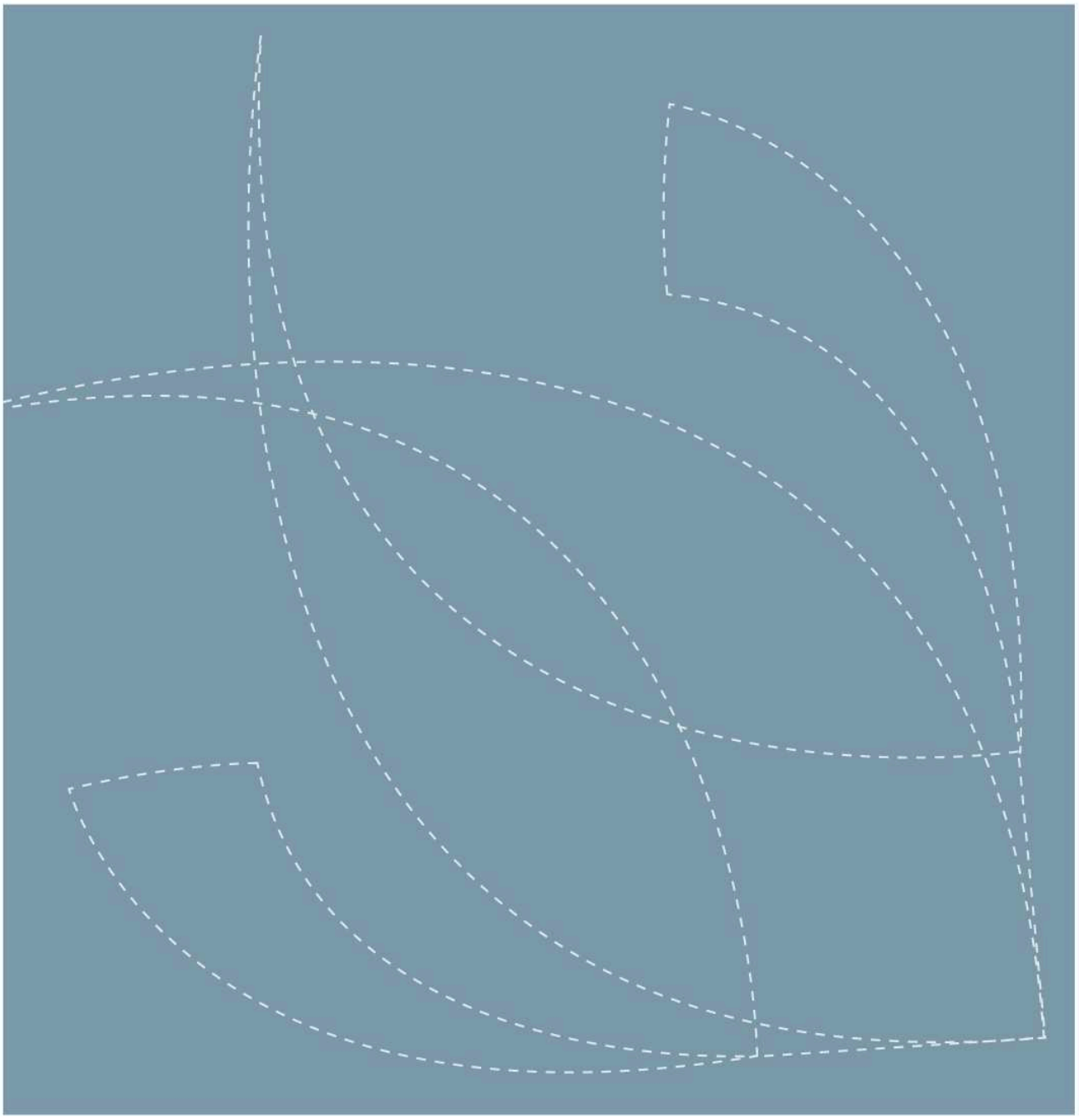
Report 47/2014 • Issued December 2014

# Measures for increased control of listeria in the salmon industry

Final report

Even Heir and Solveig Langsrud



Nofima is an industry-oriented research institute conducting research and development for the aquaculture, fisheries and food industries.

Nofima has around 350 employees.

The head office is located in Tromsø and research activities take place at six different locations: Ås, Stavanger, Bergen, Sunndalsøra and Tromsø

**Central contact information:**
Tel.: 02140
E-mail: post@nofima.no
Internet: www.nofima.no

**Company registration no.:**
**NO 989 278 835**

**Head office, Tromsø:**
Muninbakken 9–13
Postboks 6122 Langnes
NO-9291 Tromsø

**Ås:**
Osloveien 1
Postboks 210
NO-1431 ÅS

**Stavanger:**
Måltidets hus, Richard Johnsensgate 4
Postboks 8034
NO-4068 Stavanger

**Bergen:**
Kjerreidviken 16
Postboks 1425 Oasen
NO-5828 Bergen

**Sunndalsøra:**
Sjølseng
NO-6600 Sunndalsøra

# Report

ISBN: 978-82-8296-245-2 (printed)
ISBN: 978-82-8296-246-9 (pdf)
ISSN 1890-579X

| | |
|---|---|
| *Title:*<br>**Measures for increased control of listeria in the salmon industry**<br>**Final report** | *Report no.:*<br>47/2014<br>*Availability:*<br>**Open** |
| *Author(s)/Project Manager:*<br>Even Heir (Project Manager) and Solveig Langsrud | *Date:*<br>31/12/2014 |
| *Department:*<br>Safe and sustainable food | *Number of pages and appendices:*<br>43 pages + one appendix (Guide) |
| *Client:*<br>The Norwegian Seafood Research Fund (FHF) | *Client's ref.:*<br>FHF#900521 |
| *Keywords:*<br>Listeria monocytogenes, salmon, measures, hygiene, incidence, infection, guide | *Project no.:*<br>4144 |

*Summary/recommendations:*

Listeria is one of the largest microbiological challenges faced by the salmon industry. The aim of this project was increased knowledge and an improved basis to achieve increased control of listeria in the salmon industry. The project is based on the pilot project "Surveying corporate practices that inhibit and promote the incidence of listeria in Norwegian salmon products" (Norwegian title: "Kartlegging av bedriftspraksis som hemmer og fremmer forekomst av listeria i norske lakseprodukter", FHF no. 900315) and has been deeply rooted in FHF's strategic investment in quality as a prioritised R&D activity. The salmon industry has been an active partner in the project and has contributed valuable results and input. The project has focused on areas in which previous surveys highlighted a need for knowledge to achieve increased control of listeria in the industry. This has included methods for detection of listeria, knowledge about the source of infection and routes of transmission for listeria and measures to achieve increased control of listeria in production environments. The results demonstrated that the cleanliness practised does not eliminate listeria in the plants. This results in salmon being infected during processing at the farms and often early on in the slaughtering process. Analyses from gutted salmon identified regular occurrence of listeria in products from many slaughterhouses. Both general issues and specific issues in individual plants were identified. The results found that improved cleanliness could result in increased control of listeria. Other strategies and often a combination of multiple measures are however necessary to eliminate listeria in production environments and products. Based on the results from the project an industry guide was prepared. The guide can be used as a tool for risk-based monitoring and combating listeria in the salmon industry and is available for download on the FHF website.

*English summary/recommendations:*

Listeria is among the most serious microbial challenges for the salmon industry. The aim of the current project was to provide the salmon processing industry with increased knowledge and an improved basis to obtain enhanced control of listeria in the salmon industry. The project was anchored in the R&D strategy of the Norwegian Research Seafood Fund on seafood quality. The project has included methods for listeria sampling, identification of listeria sources and source tracking, and measures for increased control of listeria in the salmon processing industry. A guideline was prepared as a tool for the salmon industry to obtain increased control of listeria in processing plants. The salmon processing industry has been active partners in the project and provided valuable results and inputs to the project. The project has pointed out specific and general challenges for processing plants and the industry and provided a basis for targeted and risk-based strategies to obtain enhanced control of listeria in the salmon processing industry.

# Contents

# 1 Summary

## 1.1 Norwegian

The aim of this project was increased knowledge and an improved basis to achieve increased control of listeria in the salmon industry. The project is based on the pilot project "Surveying corporate practices that inhibit and promote the incidence of listeria in Norwegian salmon products" (Norwegian title: "Kartlegging av bedriftspraksis som hemmer og fremmer forekomst av listeria i norske lakseprodukter", FHF no. 900315) and is deeply rooted in FHF's strategic investment in quality as a prioritised R&D activity. In this project we have taken a closer look at areas where, based on the pilot project, an increased need for knowledge was identified in order for the salmon industry to achieve increased control of listeria. Four processing plants for salmon/trout (hereinafter collectively referred to as salmon) actively participated in the project; two slaughterhouses, one plant spanning the entire process from slaughtering to smoking and one plant that produced smoked salmon from purchased gutted salmon. There has been excellent collaboration between all parties involved in the project, including representatives from the salmon industry, suppliers of hygiene expertise and research institutions (Nofima and the Norwegian Institute for Public Health). The project comprised methods for sampling, identification of source of infection and routes of transmission for listeria, typing of listeria (performed by the Norwegian Institute for Public Health) and measures to combat listeria. In the latter part of the project a guide was prepared for the purpose of providing an important tool for the industry in the daily work to achieve control of listeria in individual plants.

Listeria sampling and analysis is demanding with respect to both time and costs. An assessment of the commercially available alternative technologies for detection of listeria was therefore conducted. A test of selected rapid cultivation technologies found that the technologies can give a high proportion of false positives while also being less sensitive than standard technologies for sampling and detection. The technologies therefore have limited suitability for use in the salmon industry.

Control of listeria in connection with the processing of salmon is crucial in order to avoid listeria in the final products. The main focus has therefore been to identify the listeria situation in the production process from whole fish to gutted, filleted and smoked products. Visits to four plants provided the basis for systematic sampling in the plants, conducted over a period of 1.5-2 years and comprising sampling from the environment, equipment and fish. The results demonstrated that the cleanliness practised does not eliminate listeria in the plants. This results in listeria in final products as the fish is infected during processing at the farms and often early on in the slaughtering process. Analyses from gutted salmon identified regular occurrence of listeria in products supplied from further processing from many slaughterhouses. Both general issues and specific issues in individual plants were identified. Potential strains with permanent residence in equipment and machinery ("in-house" strains) were identified in all plants where sampling was conducted.

Typing (DNA fingerprint analysis) of all *L. monocytogenes* isolates using MLVA (Multiple-locus variable-number tandem-repeat analysis) was conducted at the Norwegian Institute for Public Health. Typing made it possible to identify routes of transmission and to identify "in-house" strains. Comparison of isolates from the salmon industry with isolates linked to human listeriosis cases found that many isolates from salmon had the same MLVA type that has also been identified from listeriosis patients. Further characterisation is necessary to identify whether *L. monocytogenes* from

the salmon industry are types other than those that cause listeriosis in humans and whether the "in-house" strains have any special properties.

Extended sampling provided increased knowledge of infection sources and the effect of measures. The results found that improved cleanliness can provide increased control of listeria in plants but that other strategies and often a combination of multiple measures are necessary to eliminate listeria in production environments and products. Based on knowledge gained in the project and previous knowledge in the area, an industry guide was created for use as a tool for risk management and increased control of listeria in the salmon industry.

The project has resulted in increased attention and contributed to greater transparency concerning one of the biggest challenges in the Norwegian salmon industry: control of *L. monocytogenes*. The project has identified challenges for individual plants and for the industry and has provided the industry with new evidence and the basis for targeted combating of listeria. This includes preventative measures, risk-based monitoring and problem-solving to eliminate listeria.

## 1.2 English

The aim of the current project was to provide the salmon processing industry with new knowledge and an improved basis to obtain enhanced control of listeria in the salmon industry. The project is a continuation of the preproject «Kartlegging av bedriftspraksis som hemmer og fremmer forekomst av listeria i norske lakseprodukter» (FHF nr. 900315) and is anchored in the R&D strategy of the Norwegian Research Seafood Fund on seafood quality. In the current project, priority was given to previously identified research needs to obtain enhanced control of listeria in the salmon industry. Four salmon processors (slaughter houses and salmon smoke houses) were active participants. There has been good cooperation between all involved partners in the project including salmon industry (including project steering group), suppliers of cleaning agents and hygiene expertise and research institutes. The project has included methods for listeria sampling, identification of listeria sources and source tracking, listeria strain characterization and measures for increased control of listeria in the salmon processing industry. Based on knowledge and experiences obtained in the project and on information from other sources, a guideline was prepared. The guideline can be used as a tool for the salmon industry to obtain enhanced control of listeria in salmon processing plants using a risk based approach.

Evaluation and testing of selected rapid methods for listeria detection were performed. Test results showed two commercial rapid methods to have limitations in practical use, compared to standard analyses, due to false positives and reduced sensitivity.

A main focus in the project were to map the listeria situation in the salmon production process from live salmon to finished product (gutted salmon, filet, smoked salmon). Systematic sampling was performed in four processing facilities during a 1,5-2 years period. The findings showed that the cleaning process did not eliminate listeria, and that listeria present in processing machines, equipment and environment contaminate the salmon during processing. Analyses showed that gutted salmon from different slaughter houses and further processed to smoked salmon, regularly contain listeria. Both general and plant specific listeria problem sites were identified.

DNA-based typing of all *Listeria monocytogenes* isolates were done by MLVA (Multiple-locus variable-number tandem-repeats analysis) at Norwegian Institute of Public Health. Potential house strains of listeria were identified in all plants sampled. Typing provided information on listeria sources and transfer and on potential house strains. Comparison of strains from the salmon industry to isolates from human cases of listerioses showed identity between salmon and human *L. monocytogenes* isolates based on MLVA-typing. Further characterization is needed to conclude if the salmon listeria strains are different from those responsible for listeriosis in humans and to identify specific characteristics of house strains.

A number of measures were evaluated and tested, and additional sampling was done to obtain further knowledge on listeria sources and effect of measures. The results showed that improved cleaning could provide improved control of listeria, but that additional strategies, often applied in combinations, are needed for elimination of listeria in the processing environment and in products.

The project has given increased attention to one of the biggest challenges for the Norwegian salmon industry; control of *L. monocytogenes*. The project has pointed out specific and general challenges for processing plants and the industry and provided a basis for targeted and risk-based strategies to obtain enhanced control of listeria in the salmon processing industry.

# 2 Introduction

Control of listeria is a major challenge for the salmon industry. Listeria is a pathogenic food-associated bacteria and there are therefore strict requirements concerning the level of listeria in products and raw materials. Several countries that import Norwegian salmon have a zero tolerance policy when it comes to listeria in products. Listeria risk products are in particular long-lasting, refrigerated products that are not heat-treated prior to consumption. Typical products include cold smoked and cured salmon. For this type of product, listeria would usually not be eliminated in the production process or during processing by the customer. In processes that do not include steps to eliminate listeria (e.g. heat treating) the listeria quality of the raw materials is of the utmost importance.

Some companies periodically struggle with listeria in the production environment and in final products whereas others have good control. Cost-effective control of listeria in the salmon industry can best be achieved through appropriate and systematic work throughout the entire production chain. It is known that the transmission of listeria from production equipment and production environments is an important cause of listeria in final products. Combating listeria in production plants is therefore crucial for increased control of this bacteria in the industry. Ahead of this project Nofima implemented a pilot project ("Surveying corporate practices that inhibit and promote the incidence of listeria in Norwegian salmon products") in collaboration with FHL and NSL. This project identified areas for improvement and a need for knowledge in order to achieve increased control of listeria in Norwegian salmon production. This project was initiated based on the identified need for knowledge and the primary purpose was to provide the industry with an improved basis for achieving increased control of listeria in the production chain for gutted and smoked salmon.

The Nofima Food Division has been the responsible R&D institution with researcher Even Heir as the Project Manager. The project has been implemented in collaboration with the Norwegian salmon industry. The Norwegian Centre for Public Health has carried out DNA-based typing of all *L. monocytogenes* identified in the project period.

The main focus of the project has been processing plants for salmon in Norway (slaughterhouses and smokehouses) in order to identify the listeria situation in machinery, production equipment and the environment. Four plants with a geographical distribution from Hordaland to Finnmark participated in the project:

Plant 1: Produces smoked salmon/trout products as well as fillets. The raw material is live salmon/trout.

Plant 2: Produces smoked salmon as well as fillets. The raw material is gutted salmon supplied from multiple suppliers.

Plant 3: Salmon slaughterhouse producing gutted salmon.

Plant 4: Salmon slaughterhouse producing gutted salmon.

The plants contributed information about production procedures, sampling, cleaning and listeria detection. They were also excellent hosts when part of the Nofima project group visited the plants. The plants conducted sampling for listeria detection in the project, participated in testing of

measures and provided input to the project. The steering committee for the project consisted of Asbjørn Stensvold, Norway Royal Salmon; Randi Haldorsen, Marine Harvest (participated only in the first part of the project), Ståle Høyem, Suempol Norge AS, Svein Reppe, The Norwegian Seafood Association (NSL) and Kristian Prytz, FHF, as the steering committee coordinator. The Norwegian Food Safety Authority represented by Ivar Hellesnes participated in the project as an observer. A reference group comprising participants from the Norwegian National Veterinary Institute and cleaning suppliers (Aquatic AS, Lilleborg Profesjonell) has been affiliated with the project and provided professional input as needed. Collaboration has been excellent with suppliers of hygiene expertise and cleaning, especially Lilleborg Profesjonell, but also local hygiene suppliers. These companies supplied equipment and participated in the planning and implementation of measures at production plants. The project has also been given access to isolates detected using the plants' routine sampling. We would like to thank the local analysis laboratories for the communication of information and provision of isolates for the project. Nofima has coordinated, planned and been responsible for the implementation of activities in the project. All analysis of submitted samples has been conducted by Nofima. We would like to thank all participants for the excellent collaboration in the implementation of the project.

# 3 Issue and purpose

The main goal of the project has been to achieve increased control of listeria in the production process for gutted, filleted and smoked salmon. The project has attempted to fulfil the main goal using the following objectives (the work packages associated with each objective have been included in brackets):

- Recommended standardised methods for sampling for the detection of listeria in the salmon industry (WP 1)
- Identification of routes of transmission and infection sources for listeria across the entire production chain (WP 2)
- Comparison of listeria detected in salmon products with listeria from outbreaks and other sources (WP 2)
- Identification of measures for increased control of listeria in the salmon industry (WP 3)
- Preparation of a guide for the management and prevention of listeria problems in the salmon industry (WP 4)

Project deliveries have been linked to main goals and objectives:

- Evaluation of methods for sampling and analysis including testing and recommendation of suitable methods (WP 1)
- Report is created on important sources of infection and routes of transmission for listeria in the product chain for gutted and smoked salmon (AP 2)
- Guide for the management and prevention of listeria in the salmon industry including recommended measures to prevent listeria in processing plants
- Dissemination of knowledge about the listeria situation in the Norwegian salmon industry. Dissemination includes popular science publications and scientific publications and presentations at meetings and events aimed at the industry.

The Norwegian salmon industry has been given a knowledge-based foundation for achieving increased control of listeria in the industry. The project can therefore contribute to risk-based and more cost-effective combating of listeria in Norwegian salmon, safer products with improved quality, improved reputation and improved economy in the salmon industry.

# 4 Project implementation

The preceding pilot project "Surveying corporate practices that inhibit and promote the incidence of listeria in Norwegian salmon products" (Norwegian title: "Kartlegging av bedriftspraksis som hemmer og fremmer forekomst av listeria i norske lakseprodukter", FHF project no. 900315) identified knowledge and research needs that formed the basis for focusing the work of this project on certain prioritised areas:

- identify routes of transmission for listeria throughout the entire chain
- optimised methods for sampling and analysis of listeria
- measures to reduce transmission of listeria to products during the production process
- recommendations for the prevention and management of listeria problems in the industry
- dissemination of knowledge about listeria in the industry

Collaboration was established with four salmon processing plants (two slaughterhouses and two smokehouses, of which one also slaughtered salmon/trout) at an early stage of the project. These plants, led by Quality Managers, participated actively as partners in the project and the collaboration was crucial to the implementation. Experience gained from measures and input for testing of measures was discussed and implemented in collaboration with the plants. We also contacted other plants concerning potential testing of CIP (Cleaning-In-Place) and visited one plant in connection with this. However, it was not possible to perform the testing as part of this project. This was due to the implementation of other measures in parallel with the implementation of CIP, making it difficult to implement testing and evaluation of the effects of the measure.

Results and progress have been discussed in steering committee meetings. Project meetings with representatives from the industry, the Norwegian Food Safety Authority and other specialist groups have also provided valuable input to the project. Three master's students have completed their master's theses on issues that are partially relevant to the project. Dissemination from the project has been a prioritised task and has been ensured through popular science publication in industry journals, participation in and talks at industry meetings and through scientific publications. Project results were also disseminated in meetings with the French smokehouse association (See Chapter 6 Deliveries).

The work has been implemented using four work packages (WP1-WP4).

## 4.1 WP 1: Evaluation and creation of standardised methods for sampling of raw materials and production environments

The criteria for evaluation of alternative methods for sampling and detection of listeria were determined in consultation with the industry. Commercially available methods were evaluated based on selected criteria. Two rapid technologies were tested in practice in the industry and compared with conventional methods for sampling and analysis. Testing was implemented in close collaboration with the industry. The rapid technologies tested were selected because they were extensively used in the industry.

## 4.2 WP 2: Identification of infection sources and routes of transmission for listeria in production plants. Characterisation of listeria from salmon and salmon production

Production conditions and procedures were reviewed through visits, reviews and sampling at the four plants and the challenges associated with listeria in the plants were discussed with the Quality Managers. A plan had been established with regard to which information to obtain and which samples to take, and the same procedure was used for all visits. Infection sources and routes of transmission for listeria were surveyed by accessing historic sampling plans and results and by conducting systematic sampling during the project period. Some listeria strains detected after routine sampling at the plants were also received from external analysis laboratories. Results and evaluations from sampling were forwarded to the respective plants.

Characterisation of listeria from salmon and salmon projects has focused on the typing of *L. monocytogenes* using genetic fingerprint analysis. The typing conducted at the Norwegian Centre for Public Health had a dual-purpose: 1) Identify routes of transmission in the plants 2) Comparison of the types detected in the salmon industry with the types detected from listeriosis cases in patients.

Some additional characterisation of the sampling material has also been conducted in loose connection with other projects. Certain isolates from the project have been further characterised with regard to whether these have properties associated with the ability to survive in production environments. This includes the ability for biofilm formation and the ability to survive in environments where cleaning and disinfection agents are used. Characterisation has been performed partly in connection with master's theses and this work will also be scientifically published. (See Chapter 6 Deliveries).

## 4.3 WP 3: Identification of measures for increased control of listeria in the salmon industry

Systematic review of the plants and sampling over a period of 1.5-2 years in WP 2 provided knowledge of important infection sources. This provided the basis for the selection of measures, identification of new knowledge needs and the basis for the preparation of the guide for practical combating of listeria in the salmon industry (WP 3 and WP 4). Key areas for measures were assessed based on results from sampling in the project, feedback from plants and experiences gained from visits to the plants. Measures included physical/chemical measures targeted at problem areas at the plant but also measures that involve changes to procedures or production conditions in order to provide increased control of listeria in the production of salmon.

The focus was on measures associated with increased control of listeria in production plants. This largely comprised hygiene measures where the WP 2 results identified needs in this area. Further sampling was conducted to document the listeria status in gutted salmon from various slaughterhouses used as raw material in listeria risk products.

Measures were predominantly tested at plants with the exception of testing of measures during the concept stage, which was conducted at Nofima (see Chapter 5.3). Testing at plants necessitated the possibilities for implementation of testing and sampling at plants. Measures linked to the effect of CIP cleaning necessitated plants where this could be installed and where sampling could be

conducted to test the effect of the measure. Nofima visited a plant where a CIP system was due to be installed, but the parallel replacement of a substantial amount of equipment at the plant during the same period as well as other circumstances meant that testing could not be performed.

There was limited interest for testing of certain measures. This was due to previous experience, inadequate documentation associated with cost-benefit effect and/or restrictions in the use of the measure in connection with applicable regulations. Another issue was the difficulty in measuring the effect of specific measures at the plant as the plants more or less continuously implement changes or optimisation of production that could affect the microbiological status, including listeria status. It can therefore be difficult to measure the effect of specific measures.

## 4.4 WP 4: Preparation of a guide with recommendations for the management and prevention of listeria problems

A guide containing recommendations for the management and prevention of listeria problems in the salmon industry has been prepared based on the results from the project and information provided in previous guides and reports in the area (scientific articles, guides and regulations). The purpose of the guide is to gather the key elements for targeted combating of listeria in salmon processing plants. This will be an important tool for the industry and for individual plants when working to achieve increased control of listeria. There are a number of detailed guides available but it can be challenging for individual companies to identify the areas in which it is most important to invest resources. It was a conscious choice to only provide detailed descriptions for the most important measures to prevent and combat listeria even if this does not provide all the answers for all companies and situations. The content has been discussed in various forums and has been reworked following input from members of the steering committee and the industry. The aim has been to create a guide that the industry considers useful.

It is important to note that such a guide will not cover all desires and needs as production conditions, procedures, experience and knowledge levels vary. Each plant is also often faced with specific listeria problems. Advice and measures recommended in the guide must therefore be adapted for each plant. The guide is intended to be an important tool in this work.

## 4.5 Dissemination of knowledge and communication

Dissemination of knowledge and communication has been a key part of the project. See Chapter 6 Deliveries for details.

# 5 Results

The project was split into four work packages (WP 1-4). The results and conclusions from work associated with the work packages can be found below.

## 5.1 WP 1: Evaluation and creation of standardised methods for sampling of raw materials and production environments

Suitable methods for sampling and detection of listeria are crucial for the salmon industry to be able to conduct cost-effective self- monitoring for listeria in the production chain for Norwegian salmon. With the use of standard sampling methods and analyses (based on ISO 11290 or NMKL 136) it takes a minimum of 4-5 days from sampling until test results are available. Due to the handling of risk material the analyses usually require the use of external analysis laboratories. This necessitates shipping of samples and contributes to lengthy analysis times and high costs. There are a number of commercially available methods that may be suitable as a supplement/alternative to standard methods for sampling and analysis. This could be because they are cheaper, provide quicker test results, allow for concurrent analysis of a large number of samples and/or because they are easier to use and analyse. In the salmon industry there is a need for evaluating which methods could be suitable and to provide recommendations associated with sampling methods and analyses.

The work package comprised:

- An overview of the available methods for sampling of raw materials and production environments including principles for detection, speed and price
- Testing of selected methods through sampling in companies, analysis and evaluation of the suitability of the methods
- Dissemination of knowledge and recommendations linked to the use of methods for sampling at plants

### 5.1.1 Overview of available methods

There are a great number of methods available for the detection of listeria in food and food production environments and new rapid technologies frequently enter the market. It is therefore difficult to provide a complete overview of the rapid technologies available. Table 1 provides an overview of different methods distributed by analysis principle (cultivation methods, immunological methods, molecular methods). Based on key criteria for analysis methods, these were compared with standard methods for listeria analysis (ISO 11290, NMKL 136).

*Table 1 Examples of methods for the detection of L. monocytogenes and other species of listeria*

| | Analysis principle | *Detects Listeria* spp. | *Detects Listeria monocytogenes* | Approximate time in hours (h) for results* | Comments<br>Advantages and disadvantages |
|---|---|---|---|---|---|
| Reference methods | Cultivation for the enrichment of *Listeria* followed by plate counting using selective agar media | X | X | PE: 24 h<br>E: 48 h<br>V: 24 h<br>Total time: ± 4 days | Recognised and robust standard methods. The methods take a long time. They require a lot of handling, laboratory facilities and knowledge. Contagious material must be handled. Verification of presumptive *Listeria*-positive samples is necessary. Only suitable for laboratories with specialist expertise. Reasonable material costs but the analyses are still expensive for the companies.<br>*Examples:* NMKL 136: ISO 11290 |
| Rapid cultivation methods | Sampling, enrichment and cultivation in a single system. Enzymatic reaction results in colour changes for *Listeria*-positive samples | X | | E: 48 h<br>V: 24-48 h<br>Total time: ± 3 days | Often easier to perform, requires little handling and laboratory equipment. Interpretation of positive (based on colour reaction) samples in tubes may be difficult. May be suitable for surveying. Verification of presumptive positive samples is necessary. Suppliers frequently do not recommend these methods for product and raw material control. Contagious material must be handled but is considered possible due to the closed system. Analyses are reasonable.<br>*Examples:* InSite; Path-Chek |
| Rapid immunology methods | Based on binding between antigens and antibodies is | X | X | 10 min-1 h<br>Enrichment required first:<br>E: 48 h<br>V: 24-48 h<br>Total time: ± 3 days | The detection tests are easy to perform and read and take only a short time but this requires a preceding step for the enrichment of *Listeria*. This is time-consuming and requires handling, suitable laboratory premises and equipment with a view to the risk of infection. The price for analysis varies.<br>*Examples:* DuPont™ Lateral Flow System; RapidChek® *Listeria*; Singlepath® L'mono; Reveal® for *Listeria* One-Step |
| Rapid molecular methods | Based on the detection of *Listeria*-specific DNA or RNA | X | X | 1 – 5 h<br>Enrichment required first:<br>E = 24-48 h<br>V = 24-48 h<br>Total time: ± 3 days | The methods are relatively sensitive (extremely small quantities of *Listeria* can be detected) and quick but often require a preceding step for enrichment of *Listeria*. Positive test results should be verified through cultivation/other methods. Investment in specialist equipment and training/expertise in such techniques is necessary. The method is considered most suited to large routine analysis series and less suited for use in individual companies. The analyses necessitates investment in equipment. The analysis price per sample varies depending on method.<br>*Examples:* BAX® system; iQ-Check™; TaqMan® Detection kit; GeneQuence |

*Time consumption for pre-enrichment (PE), enrichment (E) and verification (V) respectively has been specified.

### 5.1.2 Testing of selected methods

The criteria for selection of methods for testing was determined in consultation with industry representatives. Key criteria were that the methods must:

- be available for use across the entire industry
- be cheaper than sending samples to an external analysis laboratory for test results (NOK 300-700 per sample: based on information from the four processing plants that were involved as active partners in the project)
- require only minor investments in specialist equipment for sampling and analysis
- be easy to use in practice
- provide rapid test results (≤ 2 days)
- have a high sensitivity (capable of detecting low levels of listeria)
- be specific (only detect *L. monocytogenes* or other listeria species, not other types of bacteria)
- be safe to use in companies (e.g. not necessitate handling of risk material)

Testing was performed for two alternative methods in the rapid cultivation methods category. The methods were selected based on the abovementioned criteria. In addition, two of the four processing plants that actively participated in the project also informed us that they used these methods for self-monitoring. The methods were compared with standard methods for analysis (ISO11290: NMKL136) and the suitability for use in the salmon industry was evaluated.

*Figure 1* *Rapid cultivation methods Includes swabs for sampling and tubes with growth medium into which the swab is transferred after sampling. The presence of listeria in the sample will cause an enzymatic colour reaction that can be observed visually following an incubation period of 24-48 hours at 30 or 37°C. Potentially listeria-positive samples (no. 2, 4 and 5 from the left) develop a colour change from straw-coloured to brown/black, while listeria-negative samples will should not change colour.*

A total of 163 samples were taken from three salmon processing plants using the InSite and Path-Chek methods. Sampling locations included processing equipment, environment samples (primarily floor and drains) and fish (whole salmon, fillet and smoked salmon). The results from sampling using the rapid methods have been summarised in Table 2

The results found:

- Colour change in 67 of 163 samples (41% of samples were presumptive listeria-positive samples)
- Verification of the 67 presumptive listeria-positive samples found that only 16 contained listeria
- The highest proportion of real positives (10 of 14 samples) came from environment samples
- There was a lower number of false positives (25%) in samples taken after cleaning than from samples taken during production (50%)
- There were no substantial differences in the number of real and false positives between the two rapid methods tested

*Table 2* *Results from sampling and analysis performed using two rapid cultivation methods (InSite and Path-Chek) at three processing plants.*

| Sampling location | Number of locations sampled/total number of samples | Presumptive listeria-positive samples/total number of samples | | Confirmed listeria-positive samples | |
|---|---|---|---|---|---|
| | | InSite | Path-Chek | InSite | Path-Chek |
| Machinery and equipment | | | | | |
| - after cleaning | 26/51 | 7/26 | 7/25 | 0 | 1 |
| - during production | 19/38 | 10/19 | 11/19 | 2 | 2 |
| Environment | | | | | |
| - drains | 11/22 | 5/11 | 7/11 | 4 | 4 |
| - floor | 4/8 | 1/4 | 1/4 | 1 | 1 |
| Fish | | | | | |
| - raw unprocessed salmon | 13/26 | 5/13 | 0/13 | 0 | 0 |
| - raw processed salmon | 7/14 | 4/7 | 6/7 | 0 | 0 |
| - smoked salmon | 2/4 | 1/2 | 2/2 | 0 | 1 |
| Total | *82/163* | *33/82* | *34/81* | *7* | *9* |

**The cause of false positives**

Presumptive positive InSite and Path-Chek samples (dark brown/black tubes following an incubation period of 48 hours) in which listeria was not detected through follow-up tests were considered false positives. Up to eight bacterial isolates from tubes containing false positives were isolated and added to new InSite and Path-Chek tubes. Following incubation the bacteria from the tubes with positive colour reaction were identified to determine which bacteria gave rise to false positives. Table 3 provides the distribution of different types of bacteria that gave rise to false positives in the 37 tubes examined.

*Table 3* *Incidence of bacteria that gave rise to false positive InSite and Path-Chek tubes from sampling from three salmon processing plants.*

| Bacteria | Cause of false positive (% of samples)a |
|---|---|
| Carnobacterium maltaromaticum | 43.6 |
| Carnobacterium divergens | 10.3 |

| | |
|---|---|
| Cellulolosimicrobium sp. | 5.1 |
| Enterococcus sp. | 12.8 |
| Pseudomonas sp. | 12.8 |
| Staphylococcus sp. | 2.6 |
| Stenotrophomonas maltophilia | 2.6 |

[a] In six of 37 samples no bacteria that could give rise to false positive reactions were detected.

*Table 4 Detection of listeria following swabbing using rapid methods or rags.*

| Sampling location | Confirmed listeria-positive samples/total number of samples using the different methods | | |
|---|---|---|---|
| | InSite | Path-Chek | Rags |
| Machinery and equipment | | | |
| - after cleaning | 0/26 | 1/25 | 4/24 |
| - during production | 2/19 | 2/19 | 4/15 |
| Environment | | | |
| - drains | 4/11 | 4/11 | 6/11 |
| - floor | 1/4 | 1/4 | 0/4 |
| Positive (%) | 12 | 14 | 26 |

**Comparison of sampling using rapid methods and standard swabbing using rags**

Comparison of methods for swabbing and analysis was performed. From the same sampling locations samples were initially taken using the rapid methods (InSite and Path-Chek) and then using rags (Sodibox, 3M Food Diagnostics). A total of 54 of 60 sampling locations were sampled using all three methods. The results showed that 26% of samples taken using rags were listeria-positive while the corresponding figures for sampling using InSite and Path-Chek were 12% and 14% respectively (Table 4). Sampling using rags improved the possibility of detecting listeria in the sampling locations. Rags allow for a larger area to be sampled and more force can be applied during sampling. This may have affected the test results.

## Conclusions - methods for sampling

- The rapid methods tested have limited benefits for use in the salmon industry. The reasons for this are
  - The methods give rise to false positives
  - Presumptive positive samples require verification (additional costs and analysis time)
- The use of rapid methods is less likely to detect listeria than swabbing using rags

**Literature**

The results have been published in industry publications and as scientific articles:

Heir E, Hagtvedt T, Langsrud S (2011). På jakt etter Listeria – med egnede metoder. Norsk Sjømat 6

Heir E, Langsrud S (2012). Påvisning av Listeria i laksenæringen: Er alternative metoder egnet for bedriftens egenkontroll? Norsk sjømat 1

Schirmer BCT, Langsrud S, Møretrø T, Hagtvedt T, Heir E (2012). Performance of two commercial rapid methods for sampling and detection of Listeria in small-scale cheese producing and salmon processing environments. Journal of Microbiology Methods 91, 295-300.

## 5.2 WP 2: Routes of transmission and infection sources for listeria across the entire production chain. Characterisation of listeria from salmon and salmon production

Knowledge of important infection sources and routes of transmission in the production chain for salmon and salmon products is key in order to target measures and achieve control of listeria. Systematic reviews were conducted of production plants and production procedures as well as sampling at four salmon processing plants (Plant 1-4). The plants were sampled 4-5 times over a period of 1.5-2 years.

The results for routes of transmission and infection sources are presented below (Chapter 5.2.1 – 5.2.4). Further details can be found in Heir & Langsrud, Nofima report series (20/2013): Smitteveier og smittekilder for listeria i produksjonskjeden for sløyd og røkt laks ("Routes of transmission and infection sources for listeria in the production chain for gutted and smoked salmon") (http://www.fhf.no/prosjektdetaljer/?projectNumber=900521).

All *L. monocytogenes* detected in the samples were typed using MLVA methods to identify infection sources and routes of transmission. *L. monocytogenes* detected from the salmon industry were compared with isolates from human listeriosis cases. Typing and comparison with human isolates were performed at the Norwegian Centre for Public Health. Results from the characterisation can be found in Chapter 5.2.5.

### 5.2.1 Visits and sampling at plants

Each plant was visited and sampling was planned and implemented in accordance with the following process:

- Prior to visits:
  - Submitted by the company: process description, sampling regime, cleaning schedules, overview of listeria findings at the plant

- During visits:
  - Meetings with Quality Manager, Cleaning Manager, Maintenance Supervisors, Production Supervisors, review of documentation
  - Review of processes and premises
  - Determination of sampling locations and methods
  - Sampling was predominantly performed using rags (Sodibox). Swabs were also taken in connection with some sampling
  - Sampling after cleaning and during production
  - Logging of temperature and humidity
  - Visual assessment of cleaning

- After visits:

- Microbiological analyses for the detection of listeria. Methodology in accordance with ISO 11290 for the detection of listeria was used. *L. monocytogenes* was verified using PCR-based methods.
- Anonymised isolates of detected *L. monocytogenes* were typed by the Norwegian Centre for Public Health. Typing was performed using the DNA methodology MLVA. This method assigns a genetic fingerprint to each individual *L. monocytogenes* strain and makes it possible to differentiate between different varieties of *L. monocytogenes*. The method can therefore be used to examine infection sources and routes of transmission for *L. monocytogenes*.
- Compilation and reporting of findings to the plants.

- Later sampling from the same plants:
  - Selected sampling locations were chosen for follow-up sampling at each plant over time (4-5 samplings per plant over a period of 1.5-2 years). The criteria for selection of sampling locations have been included below.
  - Initial sampling was performed by project employees from Nofima. Subsequent sampling in the plants was performed by quality employees at the plants in accordance with instructions provided by Nofima.

- Every effort was made to implement sampling in the same way at the different plants and on the different sampling dates.
  - Sampling was performed using sterile rags (Sodibox)
  - Defined areas were swabbed when possible: approximately 900 $cm^2$ (30 x 30 cm) on level surfaces if possible
  - For certain sampling locations swabs were used in addition to rags. The data from these sampling locations have been included in the analyses. Samples from one sampling location are defined as one sample even if the sampling location has been sampled with both rags and swabs.
  - For whole fish the gills, sides and tail were swabbed. For gutted fish the gills, abdomen and tail were swabbed. For fillets the fillet surface was swabbed.
  - Samples were stored in cool conditions and sent to Nofima for analyses and typing.
  - Compilation and reporting of findings to the plants were performed after each sampling round.

- Other listeria isolates included in the typing trial:
  - *L. monocytogenes* found through routine sampling at certain plants. *L. monocytogenes* isolated in 2001 from Plant 2 from a previous project (Nofima's strain collection).

### 5.2.2 Background for the selection of sampling locations

At each plant sampling was performed in the process from raw material to final product. Sampling included samples from the production environment, production equipment and fish (raw material, fully processed).

Environment samples: Samples from the production environment in addition to processing equipment (drains, floors, floor mats, trolley wheels, footwear, gloves, cleaning equipment, condensation and samples from wellboat).

Equipment samples: Samples from production equipment (conveyor belts, bleed table, gutting machinery, filleting machinery, vacuum equipment, grates on smoke carriages, slicing machinery, etc.).

Fish: Samples from whole salmon prior to processing, salmon during processing and fully processed salmon. Raw materials can include live fish (Plant 1, 3 and 4) and gutted fish (Plant 2). Samples from fish largely comprised pooled samples. The number of fish sampled is therefore higher than the number of samples.

Sampling locations were selected based on:

- Knowledge of important niches for listeria according to scientific literature
- Review of the plants to ensure sampling from presumed important niches for listeria in the production process for each plant, including any problem areas experienced by the company
- Potential risk areas for listeria in production observed during reviews/visits
- In addition to problem areas where listeria was frequently detected, samples were also taken from similar sampling locations where listeria had not previously been detected
- Sampling from fish included fish in the entire process from raw material to final product but with the main emphasis on raw material sampling
- Some common sampling locations were selected for the different plants (e.g. conveyor belts, drains, vacuum systems in gutting machinery)

The main emphasis was on the sampling of equipment and production environments after cleaning and disinfection (i.e. prior to production commencing) but samples were also taken from some of the same locations during production (>3 hours after production commencing).

### 5.2.3 Sampling and findings of *L. monocytogenes*

In the results provided in the tables below, an overview of the incidence of listeria in different environment, equipment and fish samples in Plant 1-4 can be found at the top. An overview has also been provided of findings of *L. monocytogenes* from each of the four plants as well as evaluations linked to infection sources and routes of transmission for listeria at each plant. The results formed the basis for further sampling in selected areas to obtain additional documentation and knowledge of the listeria situation in the Norwegian salmon industry. Sampling was also conducted to document the effect of selected measures. The results from such sampling have been provided in Chapter 5.3.

Distributed across 4-5 sampling rounds at four plants over a period of 1.5-2 years a total of 824 samples were taken. The samples were grouped into the sample types Environment, Equipment and Fish. Total number of samples and percentage of *L. monocytogenes* positive samples have been provided in Table 5. The figures from the different plants are not directly comparable (e.g. variations in different types of samples taken from different plants). The results from this investigation combined with the actual figures from the companies' monitoring systems indicated that there are variations in the incidence of *L. monocytogenes* between the plants.

*Table 5 Incidence of L. monocytogenes in samples from Environment, Equipment and Fish at Plant 1-4*

| Sample type | Plant 1 | Plant 2 | Plant 3 | Plant 4 |
|---|---|---|---|---|
| | % pos. (number) | % pos. (number) | % pos. (number) | % pos. (number) |

| | | | | |
|---|---|---|---|---|
| Environment | 50.0 (76) | 63.8 (80) | 8.5 (71) | 20.9 (43) |
| Equipment | 14.7 (75) | 23.9 (46) | 4.7 (106) | 23.9 (46) |
| Fish | 11.9 (671) | 18.2 (442) | 4.8 (1243) | 35.6 (454) |
| Total | 26.1 (218) | 41.2 (170) | 6.0 (301) | 29.1 (134) |

[1] Total of 320 fish sampled (pooled samples)
[2] Total of 184 fish sampled (pooled samples)
[3] Total of 530 fish sampled (pooled samples)
[4] Total of 225 fish sampled (pooled samples)

**Environment samples**

A total of 270 environment samples were taken, of which 189 were taken after cleaning (prior to production commencing). The results from samples taken after cleaning have been summarised in Table 6 The results show a high incidence of *L. monocytogenes* at multiple sampling locations after cleaning. Samples taken during production (n=81) show a somewhat higher incidence from floor-related samples (drains, floors and trolley wheels). The sample number for the other sampling locations was low. See Nofima report series 20/2013 for further details.

*Table 6 Environment samples taken after cleaning, number of samples from different sampling locations and % of L. monocytogenes positive samples are shown*

| Sampling location | Number of samples/number of positive | % *L. monocytogenes* positive samples |
|---|---|---|
| Drains | 70/34 | 49 |
| Floor | 38/11 | 29 |
| Footwear | 4/4 | 100 |
| Boot cleaner | 3/1 | 33 |
| Floor mats | 13/5 | 38 |
| Wheels, trolleys | 24/11 | 46 |
| Condensation | 8/1 | 13 |
| Wellboat | 20/0 | 0 |
| Other | 9/3 | 33 |
| Total | 189/70 | 37 |

**Equipment samples**

A total of 270 samples were taken from equipment, of which 181 samples were taken after cleaning (prior to production commencing). The results have been summarised in Table 7. *L. monocytogenes* was detected from one or more conveyor belt at all plants. Two of the plants had conveyor belts where *L. monocytogenes* was detected after cleaning two or more times during the sampling period. For one plant this was a conveyor belt used in the process after slicing smoked salmon. There was a higher incidence of *L. monocytogenes* on conveyor belts made from plastic materials (19% positive after cleaning) than conveyor belts made from metal (only one of 11 samples positive). See Nofima report series 20/2013 for further details.

*Table 7 Equipment samples taken after cleaning*

| Sampling location | Number of samples/number of positive | % L. monocytogenes positive |
|---|---|---|
| Conveyor belts | 81/14 | 17 |
| Filleting machine | 3/0 | 0 |

| | | |
|---|---|---|
| Gutting machine | 21/1 | 5 |
| Vacuum systems, gutting | 28/3 | 11 |
| Smoke carriages | 6/2 | 33 |
| Slicing machines | 9/1 | 11 |
| Other | 33/5 | 15 |
| Total | 181/26 | 14 |

**The effect of cleaning**

*L. monocytogenes* detected in samples from the environment and equipment taken after cleaning and during production is shown in Table 8. The figures indicate that there is a relatively high proportion of *L. monocytogenes* positive sampling locations after cleaning. *L. monocytogenes* was detected in a boot cleaner from Plant 2. This is a device that is intended to contribute to better hygiene but that may contribute to increased dissemination if listeria is able to survive in the equipment. Some higher listeria detection in the environment during production compared with before production can be caused by listeria being disseminated from infection sources via water and product flow.

*Table 8 Proportion of positive samples from environment and equipment after cleaning and during production*

| Sample type | After cleaning (% *L. monocytogenes*) | During production (% *L. monocytogenes*) |
|---|---|---|
| Environment | 36 | 47 |
| Equipment | 14 | 15 |

**Fish**

A total of 280 fish (salmon, trout) samples were analysed. Each sample is predominantly a pooled sample comprising 3-5 units. In total, samples were taken from 1259 salmon/trout. Sampling includes samples from live fish prior to processing (wellboat, waiting weir) and during processing: from the commencement of processing in the plant, during production and from the final product.

Number of samples, fish and samples with *L. monocytogenes* has been provided in Table 9.

*Table 9 Samples from fish: Number of samples taken/number of fish sampled in different parts of the production process*

| | Wellboat | Waiting weir | After electrical anaesthesia | Gutted in process[1] | Gutted in boxes[2] | Fillet | Slicer waste |
|---|---|---|---|---|---|---|---|
| Number of samples/fish[3] | 48/200 | 8/40 | 127/627 | 5/25 | 70/290 | 20/774 | 25/- |
| *L. monocytogenes* (n) | 0 | 0 | 10 | 4 | 17 | 7 | 1 |

[1] Gutted salmon, halfway through production (before sorting, from Plant 3 only)
[2] Includes both gutted fish as raw material (Plant 2) and gutted fish as product (Plant 3 and 4)
[3] Samples from fish predominantly consist of pooled samples from 3-5 fish
[4] A total of 76 fillets were analysed. In addition to one pooled sample of fillet remnants at Grader (Plant 2)
[5] Two samples from slicer waste from cold-smoked salmon

Conclusions from listeria findings in salmon processing plants:

- The incidence of listeria varies between the different plants
- Cleaning does not eliminate listeria in the plants
- Salmon is often infected during processing. Gutted salmon can therefore be an important source of infection for plants using gutted salmon as a raw material

Further details can be found in Nofima report 20/2013 Smitteveier og smittekilder for listeria i produksjonskjeden for sløyd og røkt laks ("Routes of transmission and infection sources for listeria in the production chain for gutted and smoked salmon")

### 5.2.4 Infection sources and routes of transmission in Plants 1-4

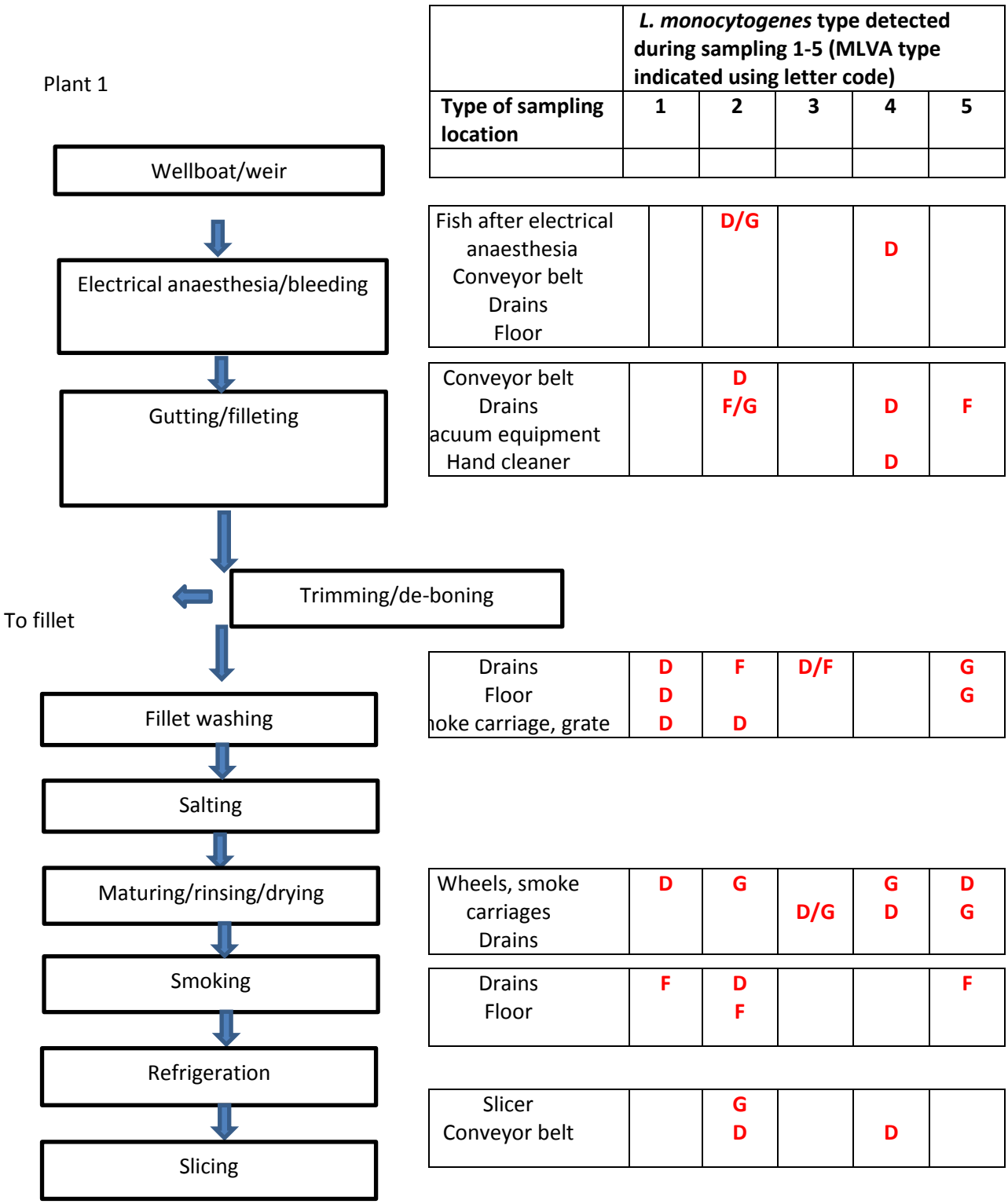
To obtain knowledge of routes of transmission and infection sources, the findings from each plant were systematised with regard to infection site, sampling date and type *L. monocytogenes*. An overview of the production process from raw material to final product was created for each plant. Typing of *L. monocytogenes* using molecular biology methods known as MLVA was used to identify infection sources and routes of infection at each plant. In addition to samples taken during the project period, some isolates from previous sampling at the plants were included. These were typed in order to obtain knowledge of infection sources and routes of transmission. During the sampling at the four plants, which was conducted over a period of 1.5-2 years, a total of 218 *L. monocytogenes* isolates were typed. In addition, 10 isolates were typed from Plant 2 that had been isolated during previous sampling in 2001. Figure 2 specifies the sampling locations used in each of the 5 sampling rounds conducted at Plant 1. The type of *L. monocytogenes* detected in each sampling location/date has been specified (letter code for MLVA type *L. monocytogenes*). Corresponding figures for all plants 1-4 have been provided in Nofima report 20/2013. Below you can find an overview of each plant in respect of findings and potential routes of transmission.

**Plant 1**

In Plant 1 three different types of *L. monocytogenes* were detected (figure 2):

- Type D and G was were detected on fish early in the process (after electrical anaesthesia) during one sampling and in drains. These types were also detected in slicer waste from smoked salmon and conveyor belts after the slicer (type D) and on the slicer machine (type G).
- Type F was detected only in drains/floor

All three types of *L. monocytogenes* detected at the plant are present in the drains. A single sample from the drains contained multiple *L. monocytogenes* types and multiple drains were positive when samples were repeated over time. During four of the five sampling rounds, *L. monocytogenes* was detected on the smoke carriage wheels. The same smoke carriages had metal grates (onto which the salmon is placed after salting) where *L. monocytogenes* was detected during the first two sampling rounds. These grates are situated at multiple levels and the lowest level is situated near floor level. The smoke carriages were positioned next to a drain where listeria was detected in four of the five sampling rounds. This drain was a known problem area for the company. *L. monocytogenes* was also detected late in the production process with type D and type G: detcted in slicer for smoked salmon and conveyor belt after slicer.




| | ***L. monocytogenes* type detected during sampling 1-5 (MLVA type indicated using letter code)** | | | | |
|---|---|---|---|---|---|
| **Type of sampling location** | **1** | **2** | **3** | **4** | **5** |
| | | | | | |
| Fish after electrical anaesthesia<br>Conveyor belt<br>Drains<br>Floor | | **D/G** | | **D** | |
| Conveyor belt<br>Drains<br>acuum equipment<br>Hand cleaner | | **D**<br>**F/G** | | **D**<br><br>**D** | **F** |
| Drains<br>Floor<br>oke carriage, grate | **D**<br>**D**<br>**D** | **F**<br><br>**D** | **D/F** | | **G**<br>**G** |
| Wheels, smoke carriages<br>Drains | **D** | **G** | **D/G** | **G**<br>**D** | **D**<br>**G** |
| Drains<br>Floor | **F** | **D**<br>**F** | | | **F** |
| Slicer<br>Conveyor belt | | **G**<br>**D** | | **D** | |

*Figure 2* *Results from five sampling rounds (1-5) in Plant 1. MLVA type in detected L. monocytogenes in sampling locations is indicated using letter code.*

This indicates that *L. monocytogenes* was transferred in the plant from slaughtering to final, sliced product. *L. monocytogenes* in product contact surfaces late in the process constitutes a substantial risk of transmission to products. Strains of *L. monocytogenes* with type D, F and G were detected

from the same sampling locations over time. The results show that drains are habitats for these strains that are presumed to be "in-house" strains in the plants.

**Plant 2**

There was many different types of *L. monocytogenes* present in the plant after cleaning (type A, B, C, F, H, I, J, unspecified (+)). Dominant types were type H and to some extent type I. The plant has numerous raw material suppliers for gutted salmon and *L. monocytogenes* was detected in the gutted salmon raw materials. This was a different type (G) than other *L. monocytogenes* types detected in the plant. Several drains were positive in all four sampling rounds. *L. monocytogenes* was also detected in samples from wheels on trolleys/smoke carriages and from floor mats. Footwear and jack trolleys (wheels) were positive in all sampling rounds. One conveyor belt (filleting machine) tested positive to *L. monocytogenes* in multiple sampling rounds. The plant had identified problems in this sampling location in connection with the rollers for the conveyor belt. Several trolleys were used for transport from unclean zones via the clean zone. *L. monocytogenes* was detected in smoked and cured salmon and in brine during routine sampling in the plant. These belonged to the same MLVA types that were detected in the plant (MLVA type F and I (smoked salmon), F (cured salmon) and B (brine)).

From Plant 2, Nofima had isolated *L. monocytogenes* 12 years previously. These were dominated by type H, the same type that was dominant in 2012. This could indicate that *L. monocytogenes* type H is established as a persistent type ("in-house" strain) in the plant and has been present in the plant for more than ten years. MLVA typing also indicated that type F and I could be potential "in-house" strains in the plant.

**Plant 3**

In Plant 3 only one type of *L. monocytogenes* was detected, type B, (plus one sporadically detected variant of this, B*) from sampling locations that were systematically sampled over time. In addition an isolate was also detected from an environment sample in the wellboat. This isolate was a different type (H) to the isolates detected in the production process.

In the second sampling round, all fish samples (five samples) taken before packaging tested positive to *L. monocytogenes*. Other positive samples from fish were only detected during the initial sampling round, where two samples from raw material salmon after electrical anaesthesia contained *L. monocytogenes*. At the time of the third sampling there had been a six-week shutdown at the plant. *L. monocytogenes* was still detected in sampling locations at the plant (drains, wheels), demonstrating that the chance of survival of *L. monocytogenes* is excellent on surfaces in production premises for salmon. In the last two sampling rounds (4 and 5) *L. monocytogenes* was only detected in samples from the floor/floor mats in the packaging department.

After the sampling period the plant conducted its own sampling of salmon throughout the process. The results found that salmon was frequently infected very early on in the slaughtering process (during pumping/bleeding). Typing of *L. monocytogenes* from these samples all showed type B with the exception of one sample from packaged, gutted salmon (type Z). Type B can be characterised as an "in-house" strain in the plant. The plant has initiated a number of measures to achieve increased control of listeria (see Table 14). The plant has also shown that the incidence of listeria on floors in the slaughtering department is high, while listeria was not detected on floors in drier parts of the plant (packaging department, storeroom).

**Plant 4**

Several different types of *L. monocytogenes* were detected in Plant 4. It is worth noting that for the fiirst sampling only basic flushing of the plant was performed before sampling. Ordinary cleaning including washing and disinfection was therefore not implemented between two production days. Later sampling with ordinary cleaning showed a lower incidence of *Listeria*. This demonstrates that cleaning is very important for control of listeria in production plants (Table 10).

In the initial sampling round all samples of finished, slaughtered fish in boxes tested positive to *L. monocytogenes*. *L. monocytogenes* was identified in all four sampling rounds for finished, slaughtered fish. The *Listeria* types found on products were not always identical to the types isolated from production equipment and the infection source and route of transmission was therefore not identified. The vacuum suction in the gutting machine tested positive in two of four sampling rounds. The plant reports that vacuum systems are a problem area. These were, for a period, cleaned by running ice through them. Cleaning and disinfectant agents are now used.

*Table 10* *Results from sampling performed in the same locations in two different sampling rounds (1 and 2) in Plant 4. Sampling locations where L. monocytogenes has been detected are highlighted in red. Ordinary cleaning was not performed in connection with sampling round 1 (only flushing). Cleaning and disinfection was performed before samples were taken in connection with sampling round 2. All samples were taken prior to production commencing.*

| Sampling location | Sample type | Sampling round 1 | | Sampling round 2 | |
|---|---|---|---|---|---|
| | | *L. monocytogenes* | *L.* spp. | *L. monocytogenes* | *L.* spp. |
| 2 | Equipment | | | | |
| 6 | Environment | | | | |
| 9 | Environment | | | | |
| 12 | Equipment | | | | |
| 15 | Equipment | | | | |
| 19 | Equipment | | | | |
| 20 | Environment | | | | |
| 24 | Equipment | | | | |
| 25 | Environment | | | | |
| 26 | Equipment | | | | |
| 27 | Equipment | | | | |
| 28 | Environment | | | | |
| 31 | Equipment | | | | |
| 32 | Environment | | | | |

**Conclusions regarding infection sources and routes of transmission**

- Each plant has its own specific problem areas
- Cleaning does not eliminate listeria in the plants
- "In-house" strains are present in the plants and linked to specific problem areas in each plant (examples: drains, gutting machines/vacuum systems, conveyor belts, intake pipes)
- High incidence in floor-related samples, especially from gutting departments (floors, floor mats, drains, trolley wheels)
- Production procedures contribute to the transmission of listeria from problem areas to salmon products, including risk products such as smoked or cured salmon
- Key areas for measures were identified

Further details can be found in Nofima report 20/2013 Smitteveier og smittekilder for listeria i produksjonskjeden for sløyd og røkt laks ("Routes of transmission and infection sources for listeria

### 5.2.5 Characterisation of listeria from salmon and salmon production

The Norwegian Centre for Public Health has constructed a database including type data for *L. monocytogenes* isolated from patients with listeriosis. The database is an important tool in the event of e.g. investigation of foodborne infection outbreaks linked to *L. monocytogenes*.

In this project the types detected in isolates from the salmon industry were compared with types of *L. monocytogenes* isolated from patients with listeriosis. The purpose of this was to gain knowledge of whether there are differences in the types of *L. monocytogenes* detected in the salmon industry and types linked to cases of illness in humans. Please note that the methods can show whether the isolates are different but not if they are 100% equal/the same strain.

Typing was also performed on all *L. monocytogenes* detected in connection with the testing of measures and surveying of the incidence of *L. monocytogenes* in gutted salmon from various suppliers. Overall results from typing of isolates from the four plants and MLVA types associated with registered listeriosis cases in patients in Norway have been provided in Table 11.

*Table 11 Types of L. monocytogenes detected based on genetic fingerprint analyses (MLVA) and comparison with other types detected in connection with listeriosis cases in patients. A bold cross (X) indicates that L. monocytogenes with this MLVA type is a potential "in-house" strain in the plant.*

| MLVA type | Detected in plant | | | | Isolated from[1] | Profile detected in human listeriosis cases (since 2006)[2] |
|---|---|---|---|---|---|---|
| | 1 | 2 | 3 | 4 | | |
| A (05-08-13-12-06) | | x | | | M | 3x |
| B (05-08-15-10-06) | | x | X | X | M, LF, LS, U | 9x |
| B* (05-08-14-10-06) | | x | x | x | M, LF, LS, U | 9x |
| C (06-00-14-10-06) | | x | | | LF | 5x |
| D (06-10-05-16-06) | X | x | | | M, LF, LS, Other[3] | 8x |
| E (06-07-13-10-06) | | x | | | M | 1x |
| F (06-07-14-10-06) | X | X | | x | M, LF, LR, U, Other[3] | 11x |
| G (06-08-14-18-06) | X | x | | x | M, LF, LR, U | 1x |
| G*(07-08-17-18-06) | | x | | | LS | Not previously detected |
| H (06-09-18-16-06) | | X | x | | M, LF, LS, U | 14x |
| I (07-07-10-10-06) | | X | | X | M, LF, LS, U, Other[3] | 37x |
| J (08-08-17-19-06) | | x | | | M | 4x |
| K (06-10-02-22-06) | | x | | | LS | Not previously detected |
| L (06-09-04-10-06) | | | | | | Not previously detected |
| M (07-08-01-12-16) | | | | | | Not previously detected |
| O (05-08-16-10-06) | | x | | x | M, LF, LS, U | Not previously detected |
| P (06-09-26-16-06) | | | | x | LF, U | Not previously detected |
| Q (07-07-11-10-06) | | x | | x | M | 3x |
| R (08-08-17-18-06) | | x | | | M, U | Not previously detected |
| S (06-07-15-10-06) | | x | | | LF, LS | 1x |
| T (06-08-14-10-06) | | x | | | LS | Not previously detected |
| U (06-09-26-18-06) | | x | | | LS | Not previously detected |
| V (06-07-14-06-09) | | x | | | LS | 2x |
| V* (06-07-15-06-09) | | x | | | LS | 1x |
| W (08-08-17-16-06) | | x | | | LF, LS | Not previously detected |
| X (06-11-05-18-06) | | | | x | LS | Not previously detected |
| Z (06-10-01-21-06) | | | x | | LS | Not previously detected |
| Z* (06-10-17-21-06) | | x | | | LS | 6x |
| + (08-08-03-09-00) | | x | | | M | 3x |

[1] M: Environment, LR: Gutted salmon (including gutted salmon as product from slaughterhouses and gutted salmon as raw material in smokehouses (Plant 2), LF: Filleted salmon or salmon during processing, U: Equipment

[2] Indicates the number of listeriosis cases in which the different MLVA types have been detected. Data based on cases recorded by the Norwegian Centre for Public Health.

[3] Other: MLVA type D: slicer waste from smoked salmon during slicing (Plant 1). MLVA type F: cured salmon and cold-smoked salmon. MLVA type I: Smoked salmon

The typing results show that *L. monocytogenes* with the same MLVA types that have been detected in patients with listeriosis also are widespread among isolates from the salmon industry. This means that it cannot be ruled out that *L. monocytogenes* from the salmon industry may be identical to

isolates that have caused listeriosis in humans. It is important to note that the results cannot be interpreted as *L. monocytogenes* from salmon being the cause of these listeriosis cases. The results also do not provide the basis for ruling out listeria-infected salmon as a potential source of listeriosis. Different L. monocytogenes strains have different potential for causing disease. Of the total of 30 MLVA types detected, 12 types have never been detected from patients with listeriosis. This may indicate that these are not associated with a great potential for causing disease. *L. monocytogenes* with MLVA type I has been isolated from around half the recorded listeriosis cases in Norway (including the outbreak in 2007 that was linked to organically produced soft cheese) and *L. monocytogenes* with this MLVA type is also detected in the salmon industry. Typing has been performed using a single typing method only. This is an area where it could be relevant to perform further studies to clarify the extent to which *L. monocytogenes* from salmon and the salmon industry differs from *L. monocytogenes* from other sources and whether these have potential to cause disease.

It would be desirable to find out more about the characteristics of listeria that establishes itself in production environments and is a constant source of infection of raw materials. Some characterisation has been conducted as part of three master's theses relating to issues addressed in the project. Further characterisation of some isolates has been planned to identify whether these have any characteristics that means that they will become established as "in-house" strains. The characterisation will include full genome sequencing and investigation of biofilm formation and tolerance to cleaning agents. This work will be performed in part through other projects and the deliveries will be finally recorded after completion of this project (see Chapter 6 Deliveries).

**Literature:**

Lindstedt *et al.* (2008). Journal of Microbiological Methods 72 (2), 141-148.

Heir & Langsrud (2013). Nofima Report series 20/2013. Smitteveier og smittekilder for Listeria i produksjonskjeden for sløyd og røkt laks.

- Many different types of *L. monocytogenes* are present in the salmon industry
- Many of the types detected from the salmon industry have also been detected in *L. monocytogenes* that have caused listeriosis
- Further studies must be conducted to establish whether *L. monocytogenes* from salmon

## 5.3 WP 3: Measures

Based on the results from WP 2 (Chapter 5.2-5.4), a need for further documentation and measures was identified to achieve increased control of listeria in the salmon industry.

Input linked to the type of measures and the possibility of testing and implementation of different measures was investigated by the steering committee and among the four salmon producers participating in the project. It was decided that the focus would be on measures in the production environment. In addition, the questionnaire from the previously mentioned pilot project and results from sampling as part of this project found that processed salmon could be an important source of infection for listeria in plants that used processed salmon as a raw material. The following measures and investigations were therefore prioritised:

- Establish whether gutted salmon is an important potential source of infection for listeria in the salmon industry by documenting the incidence of listeria in gutted salmon from slaughterhouses supplying raw materials to Plant 2
- Test the hygienic effect of automatic cleaning of conveyor belts
- Test if increased user concentration of detergent would give an increased cleaning effect and thus increased effect of subsequent disinfection
- Evaluate the effect of citric acid used in drains and on floors to reduce the level of listeria
- Test and evaluate new cleaning concepts using antimicrobial agents in rinse water
- Document the effect of CIP cleaning for reduction of listeria in problem areas (not implemented)
- Collect information about the companies' own experiences of the effect of measures

### 5.3.1 Gutted salmon as a source of infection for listeria

Results from WP 2 found that listeria is often introduced to salmon early in the slaughtering process. For risk product producers the incidence of listeria in gutted salmon or fillet purchased from different suppliers and used as a raw material in their own plant could be of great importance. A high incidence of listeria on raw materials results in an increased supply of listeria to the plant, increased risk of listeria establishing itself in the plant and risk of transmission of listeria to final products. Knowledge of the incidence of listeria in raw materials from salmon used at the plant is therefore crucial.

The incidence of listeria in gutted and filleted salmon from different suppliers to Plant 2 was investigated. The plant receives gutted salmon from a number of different slaughterhouses that is processed for filleted, smoked and cured salmon. Sampling was performed directly on salmon in boxes delivered by the supplier. Sampling was performed through rag swabbing and each sample was a pooled sample from five salmon. The gills, skin side and abdomen of each gutted salmon was swabbed. The fillet side of fillets was swabbed. During each sampling five pooled samples (25 salmon) were predominantly taken from each slaughterhouse. The suppliers investigated were randomly selected based on which suppliers delivered salmon to the plant during the sampling periods. In the final sampling round the focus was on sampling from specific plants with a low and high incidence of listeria based on results from the initial sampling rounds. The different sampling rounds for each plant were conducted on different days and therefore represent different production batches. All samples were sent to Nofima for analysis. One isolate from each listeria-positive sample was MLVA typed.

The results show that there is great variation in the incidence of listeria in gutted salmon from different slaughterhouses (Table 12). From certain plants, *L. monocytogenes* is detected in connection with each sampling, while *L. monocytogenes*was not detected from certain other plants. The results also found that the MLVA types detected over time in each plant were often identical. From supplier 22 for example, the same MLVA type (F) was detected in 37 of 38 isolates during the sampling period, which took place over a period of one year in this plant. This indicates that the plant has an "in-house" strain of *L. monocytogenes* infecting the salmon during the slaughtering process.

*Table 12* *Incidence of L. monocytogenes in gutted salmon from 12 different slaughterhouses. Only slaughterhouses from which 10 or more samples were analysed have been included. Each sample was a pooled sample comprising five salmon from the same production batch. The colour codes are used to visualise the incidence of listeria in the samples: Listeria not detected (green); listeria detected in <50% of the samples (pink); listeria detected in >50% of the samples (red). MLVA types have been indicated for listeria-positive samples. See details provided in the text for the implementation of sampling.*

| | Slaughterhouse (*L. monocytogenes*-positive/number of samples) (MLVA types) | | | | | | | | | | | | |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| Sampling round | 17 | 18 | 22 | 25 | 30 | 46 | 50 | 51 | 66 | 76 | 81 | 85 | 95 |
| 1 | 0/5 | 1/5 (H) | 3/5 (F) | 0/5 | 0/5 | 0/5 | 2/5 (G*) | 0/5 | 0/5 | 0/5 | 0/5 | 5/5 (F) | 5/5 (H,S) |
| 2 | 0/1 | 0/0 | 3/5 (F,T) | 0/5 | 2/5 (I) | 1/5 (W) | 5/5 (G*) | 2/5 (L) | 2/5 (G) | 0/5 | 0/5 | 5/5 (F) | 4/5 (H) |
| 3 | 0/5[1] | | 1/5 (F) | 0/5 | 3/5 (B,I) | 0/5 | 3/5 (G*) | | 0/5 | 0/5 | | | |
| 4 | 0/5[1] | | 3/5 (F) | 1/5 (K) | 4/5 (I,G) | 0/5 | | | | 0/5 | | | |
| 5 | 3/5 (D,V*) | | 4/5 (F) | | | | | | | | | | |
| 6 | 2/5 (D,V) | | 1/5 (F) | | | | | | | | | | |
| 7 | 0/5 | | 6/10 (F) | | | | | | | | | | |
| 8 | | | 5/5 (F) | | | | | | | | | | |
| 9 | | | 3/5 (F) | | | | | | | | | | |
| 10 | | | 4/5 (F) | | | | | | | | | | |
| 11 | | | 5/5 (F) | | | | | | | | | | |
| % positive | 16 | | 63 | 5 | 45 | 5 | 67 | 20 | 13 | 0 | 0 | 100 | 90 |

[1] Taken from fillet

In total, samples were taken from gutted salmon from 24 slaughterhouses supplying raw materials to Plant 2. From only seven of these *L. monocytogenes* was not detected in gutted salmon from the slaughterhouse.

**Gutted salmon as a source of infection**

- Gutted salmon could be an importance source of *L. monocytogenes*
- The incidence of *L. monocytogenes* in gutted salmon from different slaughterhouses varies
- Several salmon slaughterhouses have "in-house" strains of *L. monocytogenes* that infect the salmon during the slaughtering process
- Plants that further process gutted salmon must implement quality requirements for

### 5.3.2 Measures for improved cleaning

**Automatic cleaning of conveyor belts**

Effective cleaning of production equipment is an important measure to achieve good production hygiene. In collaboration with Plant 1 the effect of automatic cleaning of conveyor belts was examined and compared to manual cleaning. Nozzles for automatic cleaning of conveyor belts were installed at the plant. Automatic cleaning was performed for different types of conveyor belts (intralox and woven belts). Belt sampling was conducted in the period before automatic cleaning was initiated (zero samples) and in the period after automatic cleaning was implemented. In addition, samples were taken from equivalent conveyor belts that were manually cleaned throughout the entire period. All sampling was conducted prior to production commencing (after cleaning). The results have been provided in Table 13.

*Table 13* *Total germination index on Intralox belts (A) and woven belts (B) after manual cleaning and automatic cleaning. Sampling was conducted from both the top and bottom of woven belts (U). Germination indices have been categorised using the following levels (bacteria/cm$^2$): Category 0: <0.3; Category 1: 0.3-50; Category 2: 51-500; Category 3:>500. All samples taken after manual cleaning have been shaded in blue. Red text indicates that listeria was detected in the sampling location during sampling.*

A: Intralox belts

| | Manual cleaning | | | | Automatic cleaning | |
|---|---|---|---|---|---|---|
| Week | 1 | 2 | 3 | 4 | 5 | 6 |
| Belt no. | | | | | | |
| B1 | 0 | **3** | 1 | 0 | 0 | 1 |
| B2 | 1 | 3 | 0 | 0 | 1 | 1 |
| B3 | 0 | **3** | 1 | 1 | 1 | 1 |
| K1 | 1 | **3** | 1 | 0 | 1 | 1 |
| K2 | 0 | **3** | 1 | 0 | 1 | 1 |

B: Woven belts

| | Manual cleaning | | | | Automatic cleaning | |
|---|---|---|---|---|---|---|
| Week | 1 | 2 | 3 | 4 | 5 | 6 |
| Belt no. | | | | | | |
| B4 | 2 | **3** | **2** | 0 | **1** | **3** |
| B4U | | **3** | **2** | 0 | 1 | **2** |
| B5 | | 3 | 1 | 0 | 2 | 1 |
| B5U | | 2 | 2 | **1** | **1** | 3 |
| K3 | | | 0 | 0 | 1 | 1 |

| | | | | | |
|---|---|---|---|---|---|
| K3U | | 2 | 3 | 1 | 2 |

The results found that manual cleaning can be extremely effective and can result in a low bacteria count but that there can be great variation in the quality of manual cleaning. In some of the belts there were more than 10,000 bacteria/cm$^2$. Week 2 of the sampling stood out with a high bacteria count and many listeria-positive samples. The plant reported that new cleaners with inadequate training had been involved in manual cleaning this week. In general, the bacteria count was higher on woven belts than Intralox belts. Automatic cleaning generally provided more even cleaning results but did not eliminate listeria from the belts. The automatic cleaning conducted did not result in substantially improved belt cleanliness in the plant. There are a number of factors that could affect cleaning efficacy and the extent to which the automatic cleaning in the plant was optimised is not known. The plant suspected that spraying caused by automatic cleaning resulted in increased listeria infection rates in fish. Automatic cleaning is therefore not in itself a solution to the listeria problems, even if optimised automatic cleaning could, in principle, provide better and more even results than manual cleaning.

- Automatic cleaning of conveyor belts did not significantly improve hygiene
- The quality of manual cleaning can be very good but substantial variations were detected
- It is important to avoid transmission via spraying both during manual and automatic

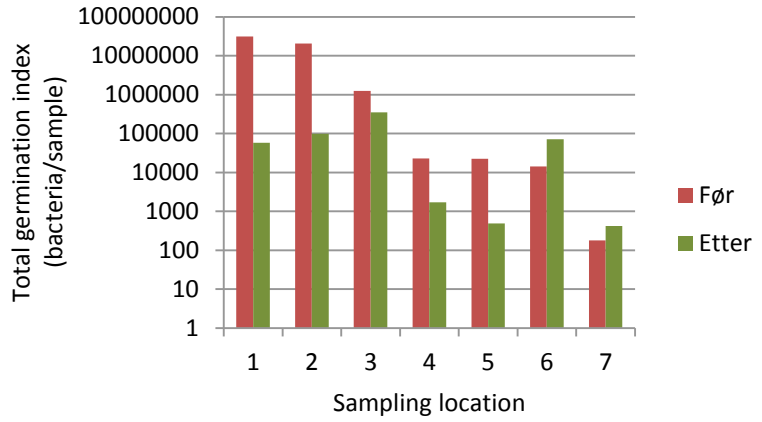
**Use of detergents with increased active ingredient concentrations**

Results from WP 2 found that listeria was relatively frequently detected from equipment and production environments and that listeria is also often detected following ordinary cleaning. In collaboration with Plant 2, Lilleborg and the plant's local hygiene supplier, experiments were therefore conducted to investigate whether increased user concentrations of active ingredients in detergents could provide increased cleaning effect (dissolution of biofilm, removal of dirt) and thus also increase the effect of subsequent disinfection.

Prior to the experiment Lilleborg made changes to the cleaning satellites through the replacement of nozzles and titration to determine the concentration of detergent applied. Around double the concentration of the foaming detergent Addi SU 932 (alkaline, hypochlorite-based) was applied during the four-week test period. Cleaning would otherwise be conducted as normal before, during and after the action period.

Sampling of selected locations on equipment and in the environment was conducted before (weekly sampling over a period of four weeks) and after the measure was initiated. All sampling was conducted after cleaning and the swab samples were analysed for total germination index and listeria. The results showed a consistently lower germination index on both conveyor belts and floor-related samples (floors, drains, floor mats, wheels) during the period after cleaning with a double dose of detergent was initiated (Figure 3 and 4). Listeria analyses found that sampling locations in which *L. monocytogenes* was present were almost halved during the period in which the increased detergent dose was used. The results also found that there was a lower number of *L. monocytogenes* in the positive sampling locations in the period after the measure (results not shown).

A




B

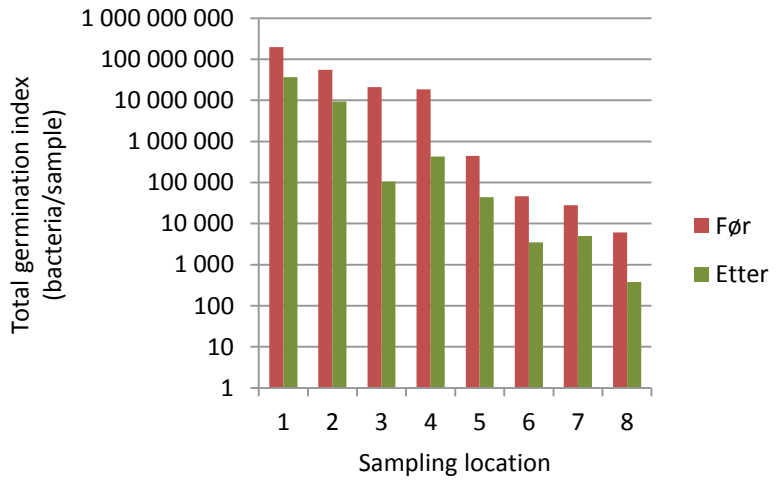



*Figure 3* *A: Total germination index in samples from seven different conveyor belts before the measure (red) and after the measure (green) with increased detergent dose. B: Total germination index in samples from eight floor-related samples before the measure (red) and after the measure (green) with increased detergent dose.*

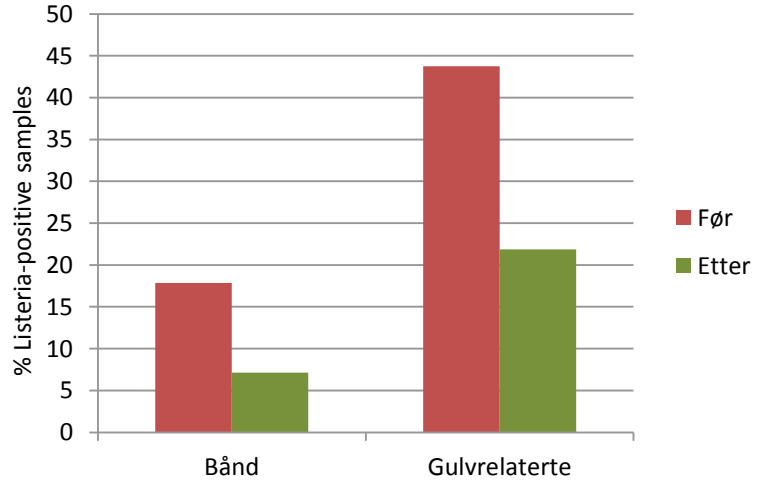



*Figure 4* *Listeria-positive samples from conveyor belts and floor-related sampling locations in the period before the measure (red) and after the measure (green) with increased detergent dose.*

**Resetting**

The results from sampling conducted in connection with the measure where double detergent dose was used showed a high total germination index in multiple sampling locations (Figure 3). It was therefore decided that the plant would be examined in respect of deposits on the equipment as this could contribute to the attachment of bacteria and formation of biofilm. Site inspections at Plant 2 indicated good cleaning all over but some "blue tinting" was detected on stainless steel. The deposits could be removed using a strong hypochlorite solution and indicated that there were protein deposits on the equipment and most probably also on the conveyor belts. It was therefore decided that the plant would be "reset" using a strong solution comprising an alkaline, hypochlorite detergent. During resetting the conveyor belts were dismantled and placed in vessels containing approximately 20% detergent. The belts were rinsed clean after a few hours in the vessels. A strong hypochlorite solution was also sprayed on equipment and rinsed off after being left to work for around 3-5 minutes. Belts and equipment were sampled for microbiological analysis in the period before and after resetting. All sampling was conducted after cleaning. The results found that three of the seven belts had a total germination index of <100/sample after resetting. However, the results also found that resetting was not effective for all belts (Fig. 5 and 6). For other equipment and floor-related samples, including drains, there was no obvious reduction of bacteria in the sampling locations after resetting (data not shown).

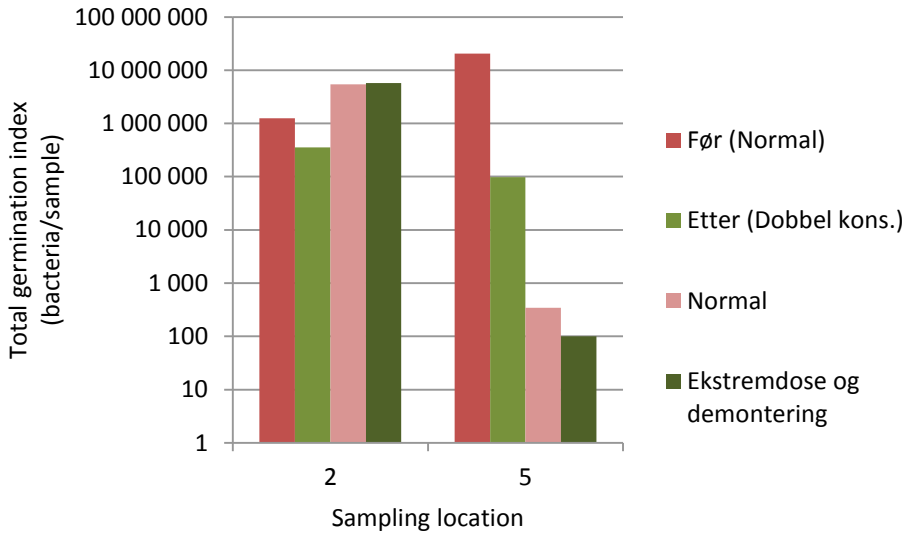



*Figure 5* *Total germination index in samples from two belts (2 and 5). Data from sampling conducted before and after measures using double detergent dose, in the period after the measure and after resetting using a high dose and dismantling of belts was performed.*

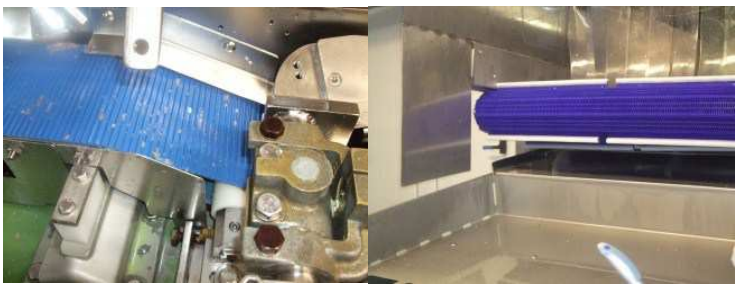


*Figure 6 Picture of conveyor belts 2 and 5.*

- Cleaning using detergents with an increased concentration of active ingredients can result in reduced bacteria levels and incidence of listeria on surfaces
- Resetting of the plant using extreme concentrations of hypochlorite-based, alkaline detergents can have good effect but is not always effective for all types of equipment and areas

**Other cleaning measures**

Floor mats were a sampling location with consistently high bacteria count throughout the entire sampling period in Plant 2. These also frequently tested positive for listeria. Floor mats are often made from porous material and are difficult to clean and dry. In an attempt to achieve improved hygiene standards for floor mats these were hung up to dry after cleaning. This resulted in a substantially lower bacteria count (Figure 7). The conclusion is that high bacteria levels are difficult to avoid but that the combination of cleaning and drying of mats would have a positive effect.

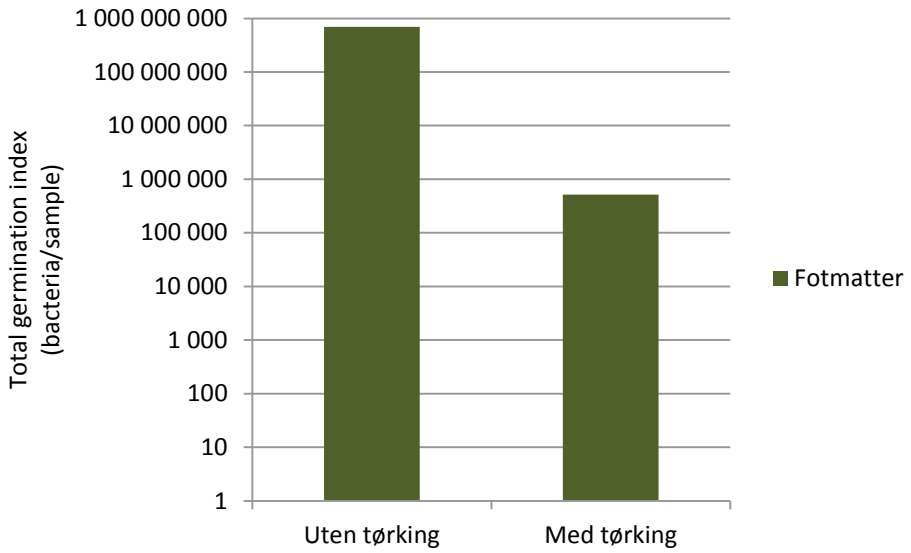



*Figure 7 Total germination index on floor mats after cleaning but with and without drying of the mats before sampling.*

**Improved cleaning using low concentrations of hypochlorite or chlorine dioxide in rinse water?**

Regular detection of listeria from sampling locations in plants that had recently been cleaned found that the cleaning performed is often not sufficient to eliminate listeria in the plants. It was therefore investigated whether better effect of cleaning could be achieved through the use of antimicrobial agents in rinse water. During ordinary cleaning disinfectants are rinsed from the surface using clean water. We wanted to investigate whether the use of low concentrations of hypochlorite or chlorine dioxide within the framework of current regulations could contribute to an increased reduction of listeria on surfaces and thus also increased effect of cleaning. Concentrations of hypochlorite or chlorine dioxide of 0.7 and 0.5 ppm respectively were used and, which is within the permitted levels for use in drinking water.

The experiments were conducted as laboratory experiments in which *L. monocytogenes* biofilms (6 strains in a mix) on steel stubs were exposed to disinfectants (hypochlorite, peracetic acid or benzalkonium chloride). After disinfection the stubs were rinsed using water (control), chlorine dioxide or hypochlorite. Rinsing using hypochlorite or chlorine dioxide contributed to a 90-99% increased reduction of attached listeria on stubs compared to rinsing using water (Figure 8). Synergistic effect was detected using peracetic acid as the disinfectant and chlorine dioxide in rinse water. The use of low concentrations of these agents in rinse water used for cleaning can provide an increased reduction of listeria on surfaces. Further testing of this concept in plants should be considered.

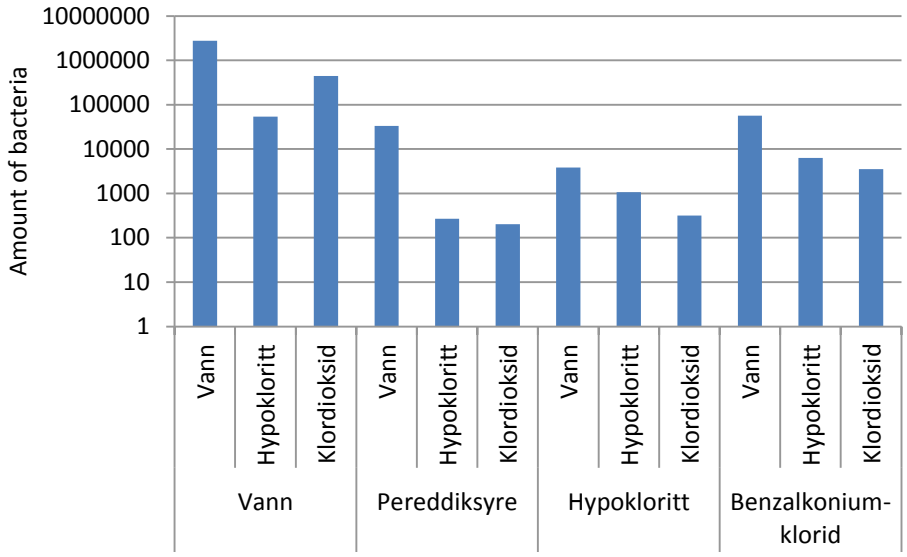



*Figure 8* *The amount of bacteria present on steel stubs after exposure to disinfectants or water (control) followed by rinsing using water (control) or low concentrations of hypochlorite or chlorine dioxide.*

**Use of citric acid in drains and on floors for increased control of listeria**

Multiple guides recommend the use of citric acid as a measure against listeria on floors in production plants. Nevertheless, documentation of the effect of citric acid against listeria is inadequate. Testing of citric acid on floors and in drains was therefore conducted in collaboration with Plant 2.

Testing was conducted in two drains where listeria had previously been regularly detected. Citric acid was also tested on a defined floor area adjacent to the drains. For control, samples were taken from two drains and one floor not treated using citric acid. Treatment and sampling were conducted during a period of three weeks.

Citric acid was applied daily after cleaning in the plant on production days. Citric acid in powder form was added directly to drains (approximately one tablespoon). Citric acid was also drizzled into drain troughs and the transition between the drain and the floor (25 ml/2000 $cm^2$). On floors, 10 ml/2500 $cm^2$ floor area was used. The amount of citric acid used was based on testing of drains at Nofima.

Sampling through swabbing was performed twice per week and took place prior to production commencing. Rags with neutralising buffers were used. Identical areas of drains/floors with citric acid and drains/floors without citric acid were sampled. Gauze pads were also placed in the drains twice per week. These were placed in the drains after cleaning and removed before subsequent cleaning

the next day. All samples were analysed at Nofima for total germination index and listeria. Additionally, pH readings were taken from the drains.

The results found that listeria was frequently detected in the drains. There was little difference in the incidence of listeria in drains treated with citric acid and untreated drains (Table 14). Listeria was detected in both swab samples and samples from gauze pads placed in the drains. Only two of the floor samples tested positive to listeria during the sampling period. Quantitative measurements were not performed to establish whether treated drains had a lower listeria count than untreated drains. There were large variations in total germination indexs from drains and floors, but the germination index was consistently not lower in treated drains or floors compared to untreated drains and floors.

Results from pH readings have been provided in Table 15. It is desirable to have a low pH (<4) in areas that are treated to prevent possible listeria growth. Readings taken after daily production but before cleaning and application of the daily dose of citric acid showed small pH differences (pH 6-7) in drains treated with citric acid and control drains. This was the case for drains (no. 11 and 37) in areas with heavy water consumption (filleting/salting department). In drains in drier areas of the plant where there was less run-off, the pH value was 2 and 6 respectively in citric acid-treated (drain A) and untreated drains (drain B) when pH was measured after daily production (before cleaning). Application of acid to drains and floors resulted in an immediate pH decrease to between 1 and 2 for drains and a pH of around 2 for floors. The results from our experiments indicate that citric acid did not reduce the incidence of listeria in drains where the incidence is already high. Treatment of drains and floors using citric acid does therefore not appear to be an effective immediate measure against listeria. With a longer sampling/treatment period and optimisation of treatment we cannot exclude that citric acid could have a certain positive effect and that it could prevent the establishment of listeria in treated areas. Further investigations are necessary to determine this.

*Table 14* *Effect of citric acid treatment on listeria in drains and floors during two weekly sampling rounds (swab samples) over a period of three weeks. Red fields indicate listeria-positive samples. The + symbol indicates detection of L. monocytogenes, while * indicates that L spp was detected in the samples but not L. monocytogenes.*

| Drains | Treated using citric acid | Sampling round[1] | | | | | |
|---|---|---|---|---|---|---|---|
| | | 1 | 2 | 3 | 4 | 5 | 6 |
| Drain A | Yes | | + | | + | | |
| Drain B | No | | + | | | | + |
| Drain 11 | Yes | + | + | | + | + | + |
| Drain 37 | No | * | * | * | | + | * |
| Floor 17[2] | Yes | | | * | | | |
| Floor 17[2] | No | | | | | + | |

[1] Samples marked with (+) indicate that *L. monocytogenes* was detected in the sample, (*) indicates that listeria was detected but species other than *L. monocytogenes*

[2] Two defined areas of the same floor were sampled. One was treated using citric acid while the other was untreated

*Table 15 Results from pH readings in drains. Readings were taken after daily production but before cleaning.*

| Drain (Department) | Treated using citric acid | pH |
|---|---|---|
| A (RTE[1]) | Yes | 2 |
| B (RTE[1]) | No | 6 |
| 11 (Salt) | Yes | 6 |
| 37 (Fillet) | No | 6-7 |

[1]RTE = Ready-To-Eat

- The combination of good cleaning and adequate drying has a good effect on listeria
- New cleaning concepts such as the use of antimicrobial agents in rinse water may provide an increased reduction of listeria but testing in processing plants will be necessary to assess whether the method has potential
- The use of citric acid to achieve a reduced incidence of listeria in drains and on floors had little effect in our experiments

**Effect of other measures**

**Circulation cleaning (CIP)**

Pipe systems connected to vacuum or water could be habitats for listeria. Several plants have reported good effect on listeria using circulation cleaning, so-called CIP (Cleaning-In-Place), of such systems. The project therefore wanted to evaluate the effect of this type of cleaning as there is little documentation that quantifies the effect of circulation cleaning on the hygiene status of plants. In addition, the installation of such systems may be expensive and documentation was therefore desired. The project visited a plant where circulation cleaning was due to be installed. Unfortunately it was not possible to conduct sampling and analysis for the effect of circulation cleaning. This was in part due to several other measures being implemented in the plant at the same time. When implementing optimised circulation cleaning customised for the system that will be cleaned, good effect is expected to be achieved compared to conventional procedures for the cleaning of such equipment. Circulation cleaning has been addressed in more detail in the report "Veiledning for forebygging, overvåking og fjerning av listeria i laksenæringen" (guide for the prevention, monitoring and elimination of listeria in the salmon industry).

**Effect of measures: Experiences from processing plants**

Through visits to five plants the project surveyed the experiences each plant had gained of listeria measures implemented in the plants. Table 16 provides an overview of measures said to have been tested and their experiences with the effect of the measure in respect of solving the listeria problems in the plant. Measures that have solved listeria problems based on information from the plants include:

- Replacing worn equipment/poorly designed equipment (gaskets, rollers, conveyor belts)
- CIP cleaning of gutting machines
- Complete dismantling and cleaning of conveyors - sometimes works
- Heat disinfection (70°C, humid heat, new problems may arise)
- Improved hygienic design of equipment

In addition to the measures in Table 16 the plants also report that measures include employing more cleaners, coordinating cleaning responsibilities (employing supervisors), improved logging of dismantling and listeria detection in equipment, improved hygienic solutions (e.g. removal of air supply to cooling tanks and thorough wash down/dismantling of the plant during shutdown).

*Table 16 Effect of measures to solve listeria problems in plants based on information provided by five plants. For some measures the effect is uncertain, in part because multiple measures have been implemented at the same time (highlighted?).*

| Area | Measures | Result | Comments | Plant |
|---|---|---|---|---|
| Drains | Chlorine tablets<br>Additional scrubbing | No effect | Poorly maintained and cleaned drains | 1 |
| Portion cutter | Machine replaced | Solved | | 2 |
| Antibacterial belts | Use of belts with antibacterial materials | Did not work as intended | Belts improved after use | 2 |
| Worn conveyor belts | Belts replaced | Solved | | 2 |
| Conveyor belt rollers | Rollers removed, vulcanised rollers replaced | Solved | Poorly designed | 2 |
| Conveyor | Repeated complete dismantling including cleaning and disinfection | Sometimes solves the problem | | 4 |
| Conveyor | Automatic cleaning | Not solved | Result from this project | 4 |
| Conveyor - degrees | Heating, humid heat | Solved | New problems arise | 4 |
| Conveyor belt to refrigeration | Cleaned twice | Not solved | | 3 |
| Conveyor belt, pipes, gutting machines | More frequent dismantling interval | ? | | 3 |
| Conveyor belts, | Replace with stainless steel belts | ? | | 3 |
| Joystick | Gasket removed | Solved | | 3 |
| Switch panels | Disposable plastic covers | ? | | 3 |
| Parts of Baader | Cleaned in dishwasher | Not solved | | 5 |
| Baader - vacuum system | CIP | Solved | | 4 |
| Vacuum system | Systematic cleaning sequence | ? | | 3 |
| Hand scraper | Dismantling | ? | | 3 |
| Refrigeration tank | Air circulation pipe removed | Solved | | 3 |
| Conveyor belts | Automatic flushing | Not solved | Increased dissemination of listeria suspected | |
| Vacuum systems | Ice | ? | | 4 |
| Vacuum systems (not on Baader) | CIP | Solved | | 5 |
| Premises | Heated to 25°C at the weekend | ? | | 5 |

## 5.4 Work package 4: Guide

A guide was prepared for the purpose of providing a tool in practical work to achieve increased control of listeria in plants that produce gutted, filleted, smoked and/or cured salmon and trout.

The guide was structured around the areas of prevention, monitoring and problem-solving:

1. **How to prevent listeria problems from occurring?** How to prevent establishment and transmission of listeria in plants.
2. **How to monitor listeria in plants?** How to establish risk-based monitoring and implement sampling in practice.
3. **How to get rid of listeria?** How to solve listeria problems using measures.

Recommendations in the guide were based on results and experiences from this project and the preceding pilot project "Kartlegging av bedriftspraksis (produkt, prosess og organisering) som hemmer og fremmer forekomst av listeria i norske lakseprodukter" (Surveying corporate practices (product, process and organisation) that inhibit and promote the incidence of listeria in Norwegian salmon products). The recommendations were also based on other reports and guides in which further information about the prevention and control of listeria can be found.

The guide forms a good basis for targeted work to prevent listeria in the salmon industry. However, the guide is relatively general and measures and procedures at each individual plant can be specified based on the guide and experiences/conditions at the individual plant. It is also important to note that the contents of the guide should be updated regularly based on new knowledge, experiences, measures and production technologies achieved through the continuous work to combat listeria in the industry.

- The report "**Guide for the prevention, monitoring and elimination of listeria in the salmon industry" (Norwegian title: Veiledning for forebygging, overvåking og fjerning av listeria i laksenæringen)** will be published on the FHF's website. It has also been enclosed

## 5.5 Evaluation of the project's usefulness for the salmon industry

The project has put the spotlight on listeria in the salmon industry. This is considered the most important contribution of the project with regard to usefulness for the Norwegian salmon industry. Listeria is probably the largest microbiological challenge faced by the salmon industry. The reason for this is that the bacteria can cause listeriosis, an infection associated with very serious complications that results in death in around 20% of cases. A number of outbreaks have been caused by listeria-infected risk products. Products such as smoked and cured salmon belong to the category of potential risk products. Investigations have also found that potential salmon risk products have a higher incidence of *L. monocytogenes* than a number of other products. The Norwegian Food Safety Authority's report from the monitoring and control programme for *L. monocytogenes* in ready-to-eat food (Norwegian title: Mattilsynets overvåknings- og kontrollprogram for forekomst av *L. monocytogenes* i spiseklar mat(2014)) found that 8.3% of ready-to-eat fish and fish products (including salmon) contained *L. monocytogenes* and that 1.2% (2 out of 169 samples) contained *L. monocytogenes* levels >100/g. Thankfully, products originating from Norwegian salmon and trout

have not been documented as a source of listeriosis outbreaks or serious disease. Any outbreaks linked to salmon could however, in addition to having serious health-related impact, have major consequences for individual salmon suppliers and for the entire Norwegian salmon brand. It is therefore of the utmost importance to highlight listeria challenges in the salmon industry and this will hopefully contribute to Norwegian salmon products with less listeria. This will contribute to the increased competitiveness of Norwegian salmon in international markets.

The salmon industry must comply with regulations and customer requirements to document control of *L. monocytogenes* in their own products. The project has contributed important documentation and knowledge, providing the industry and individual suppliers with an improved basis to achieve increased control of listeria.

Through systematic sampling and analysis at multiple processing plants it has been documented that machines and equipment contribute to the transmission of listeria to products. The cleaning conducted is often not adequate for the elimination of listeria. Important infection sources and problem areas for listeria have been documented. The effect of potential measures has also been documented. This knowledge provides the industry with a good basis for implementing measures in key areas. The results from the project also provide a basis for practical testing of new cleaning concepts (such as the use of rinse water with low concentrations of hypochlorite or chlorine dioxide). The industry's experience of various measures (Table 16) can also provide the basis for further investigations to document and optimise the effect of measures with the potential for solving listeria problems in the industry.

It has been especially important to present results from the project in venues where the salmon industry is represented. In the project this has in particular included events arranged by FHF, at which listeria has been on the agenda. A separate themed listeria workshop was also arranged for the industry. The workshop attracted more than 50 participants from various parts of the Norwegian salmon industry. These events have also been important venues for discussing listeria challenges and experiences among industry representatives.

Based on the knowledge gained through the project a guide has been created: "Guide for the prevention, monitoring and elimination of listeria in the salmon industry" (Norwegian title: Veiledning for forebygging, overvåking og fjerning av listeria i laksenæringen). The guide describes important measures and solutions to achieve increased control of listeria in the salmon industry within the areas of prevention, monitoring and elimination of listeria in salmon processing plants. The guide will be a tool for targeted work and prioritisation of measures to combat listeria in production plants.

**Usefulness for the salmon industry**
The project has:

- Put the spotlight on listeria in the Norwegian salmon industry
- Contributed venues for discussion and exchange of listeria experiences
- Procured knowledge and documentation as the basis for targeted, risk-based and cost-effective monitoring and combating of listeria
- Prepared a guide as a tool for listeria work in individual plants

# 6 Deliveries

Results from the project have been disseminated via written reports, popular science articles in industry magazines, talks and presentations at scientific conferences, scientific magazines and presentations at events and specialist meetings with the industry. Three students have completed their master's theses on issues that have been partially relevant to the project. We aim to publish more scientific articles based on the results of the project. Several of these articles will, in addition to the results from this project, also include results from ongoing listeria projects linked to the Norwegian meat industry. The latter project will continue until the end of 2015 and scientific publications will therefore be added as results and processing are completed for these projects.

## 6.1 Expert reports

Heir, Even; Langsrud, Solveig (2013). Smitteveier og smittekilder for Listeria i produksjonskjeden for sløyd og røkt laks. Nofima 2013 (ISBN 978-82-8296-083-0) 21 pages. Nofima report series 20/2013.

Heir, Even; Langsrud, Solveig; Hagtvedt, Therese (2015) Veiledning for forebygging, overvåking og fjerning av listeria i laksenæringen. Available from the FHF website. Appendices to the final report: Measures for increased control of listeria in the salmon industry

Heir, Even; Langsrud, Solveig (2015). Final report: Measures for increased control of listeria in the salmon industry

## 6.2 Popular science articles

Langsrud Solveig (2011). Elektrolysert vann – et nytt desinfeksjonskonsept for matindustrien. Matindustrien 11.

Heir, Even; Hagtvedt, Therese; Langsrud, Solveig (2011). På jakt etter Listeria med egnede metoder. Norsk sjømat 6.

Heir, Even; Langsrud, Solveig (2012). Påvisning av Listeria i laksenæringen. Er alternative metoder egnet for bedriftens egenkontroll. Norsk sjømat 1.

Langsrud, Solveig; Schirmer, Bjørn; Hagtvedt, Therese (2014). Prøvetaking av Listeria i mat. Matindustrien 10.

Heir, Even; Langsrud, Solveig (2014). Smittekilder for Listeria i lakse- og ørretnæringen. Norsk sjømat 4.

Langsrud, Solveig; Møretrø, Trond; Heir, Even (2015). Renhold for bekjempelse av Listeria. Norsk fiskeoppdrett 1.

Langsrud, Solveig; Møretrø Trond; Heir, Even (2015). Proper cleaning can provide improved control with listeria. Will be submitted to English-language industry publication (preliminary title, in development).

## 6.3 Presentations, talks

Heir, Even (2013). Bedre renhold kan gi økt kontroll med Listeria i laksenæringen. FHF event Verdikjede Havbruk, 21-22 Oct.

Heir, Even (2013). Smitteveier og smittekilder for Listeria i produksjonskjeden for laks. FHF event: 12-13 June.

Heir, Even; Langsrud, Solveig; Møretrø, Trond (2014). Kan vi oppnå kontroll med Listeria i laksenæringen? Conference Havbruk 2014, 31 March - 2 April.

Heir, Even (2014). Økt kontroll med Listeria i laksenæringen. Forslag til innhold i bransjeveileder. FHF Havbrukssamling 2014; Hell, 23 Sept.

Heir, Even (2014). Hvorfor overlever Listeria i laksenæringen? Resultater fra bransjeprosjektet. Listeria workshop, Gardermoen 7 Oct.

Langsrud, Solveig (2014). Hvordan bli kvitt Listeria? Listeria workshop, Gardermoen, 7 Oct.

Langsrud, Solveig (2014). *Listeria monocytogenes*. Nor-Fishing, Trondheim, 21 Aug.

Heir, Even (2011). Listeria i laksenæringen. Expert event Kvalitet i Lerøy Seafood Group ASA, Smøgen, 14 Sept.

Heir, Even (2011). Listeria i norsk sjømat. Oppfølging fra bransjen. Veterinære Fagdager, Oslo, 21 May.

Heir, Even (2012). Tiltak for økt kontroll med Listeria i laksenæringen. FHF event, Hell, 26 Nov.

Heir, Even (2011). Listeria eller hysteria i norsk laks? Sjømatdagene, Hell, 9 Jan.

Langsrud, Solveig (2011). Tiltak for økt kontroll med Listeria. Status og videre planer. FHF event, Hell, 11-12 May.

Heir, Even (2012). Listeria in the salmon processing industry. Presentation for the French smokehouse association, Paris, 29 March.

## 6.4 Master's theses

Løype, Marie (2013). Bakterieflora og forekomst av *Listeria monocytogenes* i lakseindustrien: *L. monocytogenes* i multi- og duokultur biofilmer under ulike betingelser.

Fossmo, Sabine (2013). Effekt av ulike desinfeksjonsstrategier mot *Listeria monocytogenes*. Ås: Universitetet for miljø- og biovitenskap, Institutt for kjemi, bioteknologi og matvitenskap.

Simensen, Andreas Lorentzen (2013). *Listeria monocytogenes* - vekst og overlevelse på rustfritt stål under betingelser relevante for matindustriprosesser. Ås: Universitetet for miljø- og biovitenskap, Institutt for kjemi, bioteknologi og matvitenskap.

## 6.5 Posters

Heir, Even; Langsrud, Solveig Heir, Moen, Birgitte; Møretrø, Trond (2014). *Listeria monocytogenes* biofilm formation and dynamics in multigenera biofilms under relevant conditions for food processing. Biofilms 6; Vienna, 11-13 May.

Langsrud, Solveig; Moen, Birgitte; Møretrø, Trond; Heir, Even (2013). Impact of microbiota in fish production facilities on growth and biofilm formation of *Listeria monocytogenes*. IAFP Annual meeting, Charlotte, North Carolina, July 28-31.

Langsrud, Solveig; Moen, Birgitte; Møretrø, Trond; Heir, Even (2013). Impact of microbiota in fish production facilities on growth and biofilm formation of *Listeria monocytogenes*. ISOPOL Conference. Goa, Sept. 19-22.

Schirmer, Bjørn Christian; Møretrø, Trond; Langsrud, Solveig; Heir, Even (2012). Rapid all-in-one swabs for detection of Listeria in cheese producing and salmon processing environments. Food Micro, Istanbul, Sept. 3-7.

## 6.6 Scientific articles (published and in development)

Schirmer, Bjørn Christian; Langsrud, Solveig; Møretrø, Trond; Hagtvedt, Therese; Heir, Even (2012). Performance of two commercial rapid methods for sampling and detection of Listeria in small-scale cheese producing and salmon processing environments. *Journal of Microbiological Methods* 2012; Volume 91(2), 295-300.

Solveig Langsrud; Birgitte Moen; Trond Møretrø; Marie Løype; Even Heir. Microbial dynamics in biofilm of *Listeria* spp. and bacteria surviving sanitation of conveyor belts in salmon processing plants (manuscript).

Schirmer, Bjørn Christian; Langsrud, Solveig; Møretrø, Trond; Heir, Even. Persistence of *Listeria monocytogenes* in food industry premises is not correlated with presence of genes associated with disinfectant resistance and biofilm formation. (Preliminary title, in development).

Schirmer, Bjørn Christian; Langsrud, Solveig; Møretrø, Trond; Heir, Even. Critical points of *Listeria* control in the meat and fish processing industry. (Preliminary title, in development).

Heir, Even; Møretrø, Trond; Birgitte Moen; Simensen, Andreas L.; Langsrud, Solveig. Dynamics of *L. monocytogenes* in single-species and multiculture biofilms under food industry relevant conditions. (Preliminary title, in development).

# 7 Quality assurance of the project implementation and results

The project group at Nofima comprised several researchers and engineers (Solveig Langsrud, Bjørn Christian Schirmer, Trond Møretrø, Anette Wold Åsli, Even Heir). Sampling, visits and review of plants was conducted using the same template for all four plants. For sampling at the plants, identical, appropriate methods for sampling based on recommendations cf. ISO 18593 were used. Analyses for listeria detection was performed using standardised analyses based on ISO 12290. Detected listeria isolates were verified as *L. monocytogenes* or *L.* spp. using additional methods (PCR-based; Wesley *et al.*, 2002). Typing of *L. monocytogenes* was conducted at the Norwegian Centre for Public Health using standardised methodology identical to the typing method used for clinical isolates of *L. monocytogenes.* Results from sampling have been collected, systematised and quality-assured by the project group on an ongoing basis. Some results have been scientifically published in journals with a referee scheme. The project group aims to publish further articles in scientific magazines. This would also be a measure of the research having been conducted based on scientific methods and standards. The final report has been peer reviewed for quality assurance and proofread by the Project Manager. The specialist contents of the report have been reviewed by project employee Solveig Langsrud. The report has been quality-assured in accordance with current templates by the administrative coordinator and final approval has been granted by the Director of Research.

ISBN 978-82-8296-245-2 (printed)
ISBN 978-82-8296-246-9 (pdf)
ISSN 1890-579X