

SCIENTIFIC REPORT

Proposed technical specifications for a survey on *Listeria monocytogenes* in selected categories of ready-to-eat food at retail in the EU¹

Report of the Task Force on Zoonoses Data Collection

(Question N° EFSA-Q-2008-415)

Adopted on 22 May 2009

SUMMARY

The European Food Safety Authority and its Task Force on Zoonoses Data Collection were requested by the European Commission to produce a proposal for technical specifications on a coordinated monitoring programme (a survey scheme) for *Listeria monocytogenes* in ready-to-eat (RTE) food. This survey should allow the comparison of *L. monocytogenes* contamination in RTE food in the Community and Member States and the verification of the Community food safety criteria for *L. monocytogenes*.

The proposed technical specifications focus on sampling those categories of RTE food in which the highest rates of *L. monocytogenes* contamination have been observed in the European Union (EU): soft and semi-soft cheeses, smoked and gravad fish, and heat-treated meat products that are handled after heat treatment. Two alternatives for an EU wide survey on *L. monocytogenes* in RTE food are proposed. The first option consists of 27 Member State-specific surveys (Member States' specific surveys) and would allow both the *L. monocytogenes* prevalence in each Member State as well as at the Community level to be determined. In this option it is proposed that two RTE food categories would be covered by the survey, i.e. soft and semi-soft cheeses and smoked and gravad fish. The heat-treated meat products could be addressed at a later stage by another optional survey round.

¹ For citation purposes: Report of Task Force on Zoonoses Data Collection on proposed technical specifications for a survey on *Listeria monocytogenes* in selected categories of ready-to-eat food at retail in the EU, *The EFSA Journal*, (2009), 300, 1-66

The second option is a Community-specific survey that would only allow estimation of the *L. monocytogenes* prevalence at the Community level. This proposal requires a Community-specific number of samples to be allocated amongst the Member States in proportion to the size of their human populations resulting in a reduced number of samples to be taken at the Member State-level. This enables the inclusion of a third RTE food category (heat-treated meat products) in the survey as well as the inclusion of the detection method for *L. monocytogenes*.

Sampling of the RTE food categories would be targeted at retail outlets serving the final consumer, with catering and wholesale establishments excluded. A survey undertaken at retail will assess the effectiveness of the implementation of Community *L. monocytogenes* criteria. Such a survey would also provide information to assess the exposure of consumers via these RTE food categories. Food products are suggested to be tested at the end of the shelf-life and additionally in the case of smoked and gravad fish, immediately after sampling. In addition, water activity and pH values are to be measured in the smoked and gravad fish. It is envisaged that the competent authorities in the Member States would construct a sampling plan for the retail sampling in their country.

Standardised analytical methods are proposed to be employed in the analyses of samples. It is recommended that the enumeration method is used to obtain quantitative information on *L. monocytogenes* in the RTE food categories. In addition, in the Community-specific survey the detection method would be used. Isolates of *L. monocytogenes* are suggested to be archived for the purpose of future typing.

It is intended that a modelling and simulation approach be applied in the analyses of the results so that the effectiveness of the implementation of Community *L. monocytogenes* criteria may be assessed. A similar model-based approach will also be used to estimate the growth potential of *L. monocytogenes* in smoked and gravad fish.

Keywords: *Listeria monocytogenes*, ready-to-eat food, survey, microbiological criteria, modelling, soft and semi-soft cheeses, smoked and gravad fish, heat-treated meat products

TABLE OF CONTENTS

SUMMARY	1
TABLE OF CONTENTS	3
A. Background	6
TERMS OF REFERENCE	7
ACKNOWLEDGEMENTS	7
B. Rationale for the choices made in the proposal	8
B.1. The two types of surveys proposed.....	8
B.2. Choice of food categories to be sampled	9
B.3. Choice of the sampling stage (retail)	10
B.4. Choice of testing at the end of the shelf-life and proposal for testing at the time of sampling	11
B.5. Modelling approach	12
B.6. Choice of the analytical methods for the samples	12
B.7. Choice of the sample size	13
B.8. Storage <i>L. monocytogenes</i> isolates for future typing.....	15
C. Use of data from the survey to assess the effectiveness of the implementation of Community <i>L. monocytogenes</i> criteria	18
C.1. Rationale for applying microbiological criteria in food safety management	18
C.2. Use of data from the survey to assess the effectiveness of the implementation of the <i>L. monocytogenes</i> criteria	19
C.3. Proposal for modelling and simulation to assess the effectiveness of the implementation of the <i>L. monocytogenes</i> criteria.....	21
C.3.1. General principles on the model-based approach.....	21
C.3.2. Model building, tuning and validation.....	22
D. I. Proposal for a survey on <i>L. monocytogenes</i> in selected ready-to-eat food at retail in the EU, the Member States' specific surveys	23
D.I.1. Introduction	23
D.I.2. Definitions	23
D.I.3. Objectives.....	25
D.I.4. Sampling frame	26
D.I.4.1. The population to be sampled	26
D.I.4.2. Sampling design	27
D.I.5. Sample size (number of samples) calculation	28
D.I.6. Sample collection	29
D.I.6.1. Type and details of sample.....	29
D.I.6.2. Sample information	30
D.I.6.3. Transport of samples	31
D.I.7. Sampling in the laboratory and analytical methods	31

D.I.7.1. Laboratories.....	31
D.I.7.2. Receipt of samples.....	31
D.I.7.3. Sample preparation and initial suspension preparation.....	32
D.I.7.4. Enumeration method	32
D.I.7.5. Storage of isolates	33
D.I.7.6. Determination of the pH of smoked and gravad fish	33
D.I.7.7. Determination of the water activity (a_w) of smoked and gravad fish.....	33
D.I.8. Reporting.....	34
D.I.8.1. General provisions.....	34
D.I.8.2. Overview of the survey and results	34
D.I.8.3. Overview of laboratory analysis.....	35
D.I.8.4. Detailed sample data	35
D.II. Proposal for a survey on <i>L. monocytogenes</i> in selected ready-to-eat food at retail in the EU, the Community-specific survey	38
D.II.1. Introduction.....	38
D.II.2. Definitions.....	38
D.II.3. Objectives	40
D.II.4. Sampling frame	41
D.II.4.1. The population to be sampled	41
D.II.4.2. Sampling design.....	42
D.II.5. Sample size (number of samples) calculation.....	44
D.II.6. Sample collection.....	45
D.II.6.1. Type and details of sample	45
D.II.6.2. Sample information.....	46
D.II.6.3. Transport of samples.....	46
D.II.7. Sampling in the laboratory and analytical methods.....	47
D.II.7.1. Laboratories	47
D.II.7.2. Receipt of samples	47
D.II.7.3. Sample preparation and initial suspension preparation.....	48
D.II.7.4. Detection and enumeration methods.....	48
D.II.7.4.1. Enumeration	48
D.II.7.4.2. Detection	49
D.II.7.5. Storage of isolates	49
D.II.7.6. Determination of the pH of smoked and gravad fish.....	49
D.II.7.7. Determination of the water activity (a_w) of smoked and gravad fish.....	49
D.II.8. Reporting.....	50
D.II.8.1. General provisions	50
D.II.8.2. Overview of the survey and results.....	50
D.II.8.3. Overview of laboratory analysis	51
D.II.8.4. Detailed sample data	51
E. Task Force on Zoonoses Data Collection members.....	54

F. References.....	54
ANNEX I – SAMPLE SIZE (NUMBER OF SAMPLES) CALCULATION	58
ANNEX II – TYPICAL ASSUMPTIONS UNDERLYING THE MODELS.....	61
ANNEX III – EXAMPLES OF SOFT, SEMI-SOFT AND FRESH CHEESES	63
ANNEX IV – EXAMPLES OF FOOD ADDITIVES (PRESERVATIVES AND ACIDITY REGULATORS).....	65

A. Background

Systemic listeriosis in humans is a severe disease that primarily affects the elderly, people with compromised immune systems, pregnant women and fetuses causing miscarriages, and newborn infants. In the European Union (EU) Member States, a significantly increasing trend in the notification rate of listeriosis cases in humans was observed between 2002 and 2006 (EFSA, 2007a). This notification rate remained at the same level in 2007 according to the Community Summary Report on Trends and Sources of Zoonoses and Zoonotic Agents in the EU (EFSA, 2009). The proportion of food samples exceeding the legal food safety criterion for *Listeria monocytogenes* in EU Member States was reported in this report to be highest in ready-to-eat (RTE) fishery products, followed by RTE meat products and cheeses.

Directive 2003/99/EC on the monitoring of zoonoses and zoonotic agents (EC, 2003) aims to improve and co-ordinate the monitoring of zoonotic agents in the Community. The objective is to make the data collected easier to compile and compare, which will also facilitate an improved contribution to risk assessments of zoonotic agents.

When specific needs are recognised in the Community, Article 5 of the Directive 2003/99/EC allows the establishment of coordinated monitoring programmes, which typically consist of one year surveys (EC, 2003). These coordinated monitoring programmes may be established to assess risks or to establish baseline values related to zoonoses or zoonotic agents at the Member State level or at the Community level. According to Article 13 of the same Directive the Commission has to consult the European Food Safety Authority (EFSA) before establishing any coordinated monitoring programme (EC, 2003).

Regulation (EC) No 2073/2005, as amended by Regulation (EC) No 1441/2007 (EC, 2005 and 2007) lays down the microbiological criteria for foodstuffs to be complied with by food business operators (FBOs) and this includes food safety criteria for *L. monocytogenes* in RTE food. These criteria have been in force since 1 January 2006. The competent authority should verify compliance with the rules and criteria laid down in the Regulation.

The European Commission has requested that the European Food Safety Authority (EFSA) prepares a proposal for technical specifications for a harmonised monitoring scheme for *L. monocytogenes* in RTE food able to support the growth of this microorganism, other than those intended for infants and for special medical purposes, within the framework of Directive 2003/99/EC (EC, 2003) and the implementation of Regulation (EC) No 2073/2005 (EC, 2005). In particular the harmonised monitoring scheme should allow a comparison of the contamination of *L. monocytogenes* in RTE food in the Community, in the different Member States, and the verification of the Community food safety criteria laid down in Regulation (EC) No 2073/2005 (EC, 2005).

The preparation of the technical specifications in EFSA was assigned to the Task Force on Zoonoses Data Collection.

TERMS OF REFERENCE

The Task Force was asked to prepare a coordinated monitoring scheme for *L. monocytogenes* in ready-to-eat food able to support the growth of *L. monocytogenes* other than those intended for infants and for special medical purposes.

This scheme should be designed as a survey protocol and cover, in particular:

- monitoring of fishery products and cheeses, further specified on a risk basis;
- sampling at retail level;
- domestic and imported products;
- collection of qualitative and quantitative analysis results; and
- the possibility for competent authorities to develop a basis that should allow verification of the implementation of Community *Listeria* criteria by the food business operators.

In this report the monitoring scheme takes a form of a survey scheme.

ACKNOWLEDGEMENTS

The Task Force wishes to thank the members of the Working Group for the preparation of this report: Thomas Alter, Billy Amzal, Jens Kirk Andersen, Annie Beaufort, Pierre-Alexandre Beloeil, Frank Boelaert, Corinne Danan, Enne De Boer, Lieven De Zutter, Maria Teresa da Silva Felicio, Ari Horman, Mats Lindblad, Christine Little, Pia Makela, Micheal O'Mahony, Eirini Tsigarida and Martin Wagner.

B. Rationale for the choices made in the proposal

While preparing the survey scheme, some decisions and choices regarding the scope and design of the survey were made. These decisions and their rationales are described below and an overview is set out in Table 4 at the end of this section.

B.1. The two types of surveys proposed

In these technical specifications, two alternatives for an EU wide survey on *L. monocytogenes* in RTE food are proposed, which have distinct, specific features and from which outcomes can be generalized to different populations of food (Table 1).

The sampling plan of one of the proposed EU wide survey consists of 27 Member State-specific surveys (referred to as Member States' specific surveys) and which would allow the estimation of the *L. monocytogenes* prevalence in each Member State and also at the Community level. This proposal requires the same number of samples to be taken in every Member State.

The sampling plan of the other proposed EU wide survey consists of a Community specific survey (referred to as Community-specific survey) and would therefore only allow reliably estimating the *L. monocytogenes* prevalence at the Community level. This proposal requires a Community-specific number of samples to be allocated to every Member State in proportion to the size of the human population in the country (proportionate stratified sampling scheme). This would reduce the number of samples to be taken in each Member State. This sampling strategy is unlikely to reliably estimate the *L. monocytogenes* prevalence in the individual Member States. However, the reduced Member State-specific sample size would enable the inclusion of a third RTE food category (heat-treated meat products) in the survey as well as the inclusion of the detection method in the laboratory analyses. The assumption underpinning the allocation of sample numbers to Member States according to the size of their human populations is that the human population sizes are fairly proportional to the volume sizes of the selected food categories on the market. Several other variables were investigated, including food production and trade figures, of which the human population size proved to be the most reliable stratification variable. Furthermore, it is assumed that there are no major differences between the Member States in the prevalence of *L. monocytogenes* in the selected RTE food categories, allowing for the estimation of a meaningful Community-level *L. monocytogenes* prevalence. This assumption is based on the ubiquitous nature of *L. monocytogenes* and the wide intra-Community trade of the RTE products, especially in case of cheeses and smoked and gravad fish.

Table 1: Features of the proposed surveys

	Member States' specific surveys	Community-specific survey
Sample size (total)	2 700 / Member State Approximately in total 72 900 / Community	12 080/ Community
Results applicable	Each Member State Community level	Community level
RTE food categories	Smoked and gravad fish Soft and semi-soft cheeses	Smoked and gravad fish Soft and semi-soft cheeses Heat-treated meat products
Analyses Methods	<i>L. monocytogenes</i> enumeration method pH and aw measurement	<i>L. monocytogenes</i> detection method and enumeration method pH and aw measurement

B.2. Choice of food categories to be sampled

L. monocytogenes is ubiquitous in the environment and consequently almost all food categories can be contaminated with this organism. Over the past years the Community Summary Reports on Trends and Sources of Zoonoses in the EU (EFSA, 2009) have reported that the highest frequencies of food samples contaminated with *L. monocytogenes* were RTE fishery products, cheeses and RTE meat products. Of these food categories, RTE fishery products, particularly smoked fish, were the most frequently contaminated with *L. monocytogenes* and also had a higher proportion of samples with this organism present at more than 100 cfu/g. Cheeses and RTE meat products then came next in decreasing order of *L. monocytogenes* contamination (EFSA, 2009). The Community Summary Reports in 2006 (EFSA, 2007a) and 2007 (EFSA, 2009) also indicated that soft cheeses were the food vehicle most often implicated in the few food-borne outbreaks of listeriosis reported.

A recent review of sporadic cases and outbreaks of human listeriosis has corroborated that foods associated with transmission are predominantly RTE and capable of supporting the growth of *L. monocytogenes*. Amongst these RTE food, cheeses, meat products and sandwiches were most often identified as the food vehicles in food-borne listeriosis (EFSA, 2007b).

Although smoked fish has only incidentally been linked to food-borne listeriosis, these products have been found to have a high prevalence as well as high contamination levels of *L. monocytogenes*. Additionally, microbiological risk assessment studies have shown a relatively high risk for listeriosis caused by consumption of smoked fish (WHO/FAO, 2004).

Smoked and gravad fish, soft and semi-soft cheeses and heat-treated meat products are targeted in these proposed surveys, as these food types have been shown to exhibit a relatively high prevalence as well as high quantitative levels of *L. monocytogenes*, and have shown this consistently over several years. Fresh cheeses, however, are not included in the survey as they have a relatively short shelf-life and they are usually packed automatically as part of the production process; the risk for *L. monocytogenes* contamination and growth is therefore low.

Sandwiches were also considered for inclusion in the survey. However, pre-packed sandwiches are not commonly consumed in all Member States. Furthermore, sandwiches are a very heterogeneous food category and the fillings were considered to be the source of a potential contamination. The most frequently used types of filling were considered to be already covered by the three RTE food categories included in the survey. For these reasons it was decided not to include sandwiches as one of the RTE food categories in the survey.

It was decided that only smoked and gravad fish packaged by the manufacturer should be included in the survey. The rationale for this is that the results from the survey would reflect the contamination up to and including the production plant, and thereby provide an indication of the hygiene of the product, process, and production environment. Sampling packaged products would also eliminate the risk of contaminating the sample during storage for the end of shelf-life analyses. It is also presumed that packaged products will improve the comparability of the two duplicate samples collected, which are to be analysed immediately after the time of sampling and at the end of the shelf-life.

Cheeses both packaged by the manufacturer and at retail are included in the survey. It is common practice to pack or re-pack cheeses at retail for the purpose of selling these to the final consumer. As shelf-life studies should account for foreseeable handling and storage, it was decided that the survey should also cover these types of products as long as the appropriate labelling information is available.

All the three RTE food categories are heterogeneous comprising of many different types of products that may vary from country to country. In order to collect sufficient information on the exposure of consumers via these food categories in the different Member States, it is essential that the sampling will be done as far as possible according to national market shares of the products.

In the Community-specific survey all three RTE food categories (smoked and gravad fish, soft and semi-soft cheeses and heat-treated meat products) are included, whereas in the Member States' specific survey only smoked and gravad fish and soft and semi-soft cheeses are covered. This proposal was made in order to keep the workload at a manageable level for the Member States' specific survey, and to postpone the RTE heat-treated meat product category for consideration in a possible later survey.

B.3. Choice of the sampling stage (retail)

It is recommended that the sampling site for this baseline survey is at retail. The reason for this is that due to its psychrotrophic nature *L. monocytogenes* can grow in many foods with long shelf-life under refrigeration up to the time of consumption. This fact has complicated the task of setting microbiological criteria, as well as assigning shelf-lives, for RTE refrigerated foods, for the purpose of avoiding high levels at the time of consumption. By choosing retail as the sampling stage it will be possible to assess the exposure of the consumer to *L. monocytogenes* through the food categories targeted, and also to evaluate compliance with the *L. monocytogenes* criteria that apply to RTE food during their shelf-life.

Retail in this report is understood as outlets selling directly to the final consumer for subsequent domestic consumption, i.e. outlets such as supermarkets, specialist shops, markets

and excluding catering activities, restaurants, wholesalers and similar outlets.

B.4. Choice of testing at the end of the shelf-life and proposal for testing at the time of sampling

By analysing samples for *L. monocytogenes* at the end of the shelf-life after storage under specified conditions, compliance with the microbiological limit of 100 cfu/g during shelf-life can be verified in a deterministic way. This means that the data obtained can be used to assess the effectiveness of implementation of the food safety criteria by the FBO in the given storage time and temperature scenario. The analyses at the end of the shelf-life are to be performed on samples from all RTE food categories, i.e. for smoked and gravad fish, soft and semi-soft cheeses and, if the survey is performed at the EU level, heat-treated meat products.

However, testing only at the end of the shelf-life would only provide information on these specific samples, without capturing any variability in storage conditions for the types of products collected, which is vital for performing risk assessments. For this purpose, it is proposed that, in the case of smoked and gravad fish, analyses of *L. monocytogenes* would be performed both directly after sampling and after storage of a duplicate sample. Two packages from the same batch are suggested to be collected at each sampling occasion. One of these would be analysed upon arrival at the laboratory, and the other at the end of the shelf-life (see section D.I.7.2 and D.II.7.2). Water activity and pH would also be measured in the fish sample analysed upon arrival. This duplicate sampling and testing is only suggested for smoked and gravad fish and not for other food categories due to feasibility reasons, i.e. not to make the number of samples to be analysed too high. Smoked and gravad fish is selected because it is the most homogenous food category and also has the highest expected prevalence of *L. monocytogenes*.

By analysing *L. monocytogenes* directly after sampling, compliance with the microbiological criterion could also be verified in a probabilistic way. Data on levels of *L. monocytogenes*, water activity and pH can be used as a starting point for modelling growth of *L. monocytogenes* under different storage scenarios during the remaining shelf-life, accounting for variability in storage times and temperatures. This would enable the estimation of the probability that the level of *L. monocytogenes* in a product will exceed 100 cfu/g at the end of the shelf-life, given a certain concentration found in a product from the same batch analysed directly after sampling at retail. The collection of data on water activity and pH provides possibilities to explore the importance of these parameters as explanatory variables for the occurrence of high levels of *L. monocytogenes* among samples. Also information whether preservatives or acidity regulators (as food additives and as indicated in the label) were used in the smoked or gravad fish products will be collected. This data will be used in the modelling for growth characteristics as the use of the preservatives and acidity regulators are expected to limit the multiplication of *L. monocytogenes*.

The analyses of samples of smoked or gravad fish immediately after sampling at retail is included in the proposed specifications for both the Member States' specific survey and the Community-specific survey. However, due to the substantial number of samples to be collected and analysed, it may be considered if this analysis will be included in the Member States' specific survey. However, the optimum scenario would be that all Member States carry out the analyses in order to obtain comprehensive and reliable datasets. Leaving out the

analyses immediately after sampling would mean that no modelling for the growth characteristics of *L. monocytogenes* in the smoked and gravad fish could be carried out.

B.5. Modelling approach

The sampling scheme for RTE food proposed in this survey is different from that in Regulation (EC) No 2073/2005 (as amended by Regulation (EC) No 1441/2007) (5 samples from a batch versus a single sample, and the sampling site) (EC, 2005 and 2007). Detection of *L. monocytogenes* above a level of 100 cfu/g in a single sample indicates a failure to meet the criterion, whilst a result of equal or below 100 cfu/g in a single sample cannot alone give assurance that the criterion has been met. Therefore, an assessment of the implementation of the criterion will be made by using a model-based approach.

Quantitative models are tools which allow the combination of various data sources and the prediction of contamination under certain scenarios. More specifically, they are necessary here:

- to account for the various sources of variability and uncertainty along the food chain and account for *L. monocytogenes* growth during storage;
- to simulate what levels are likely to be expected under compliance and under various storage scenarios.

The only alternative to a model-based approach for a quantitative assessment of the effectiveness of implementation of the criteria would be very large experimental studies investigating the various scenarios and with many replications to capture all sources of variability.

Once the survey has been carried out and the results are available, the main purpose of the statistical inference will be to assess whether the observed prevalence estimates of high (>100 cfu/g) levels of *L. monocytogenes* in RTE food at the end of the shelf-life are compatible or not with the compliance of the *L. monocytogenes* criteria by FBOs laid down in Regulation (EC) No 2073/2005 (EC, 2005).

If the data on analysing samples immediately after collection is received and modelled, the modelling results obtained can be considered as new reference data for competent authorities, and can be used to estimate if a contaminated food on the market will (or not) exceed the limit during its shelf-life. This is complementary to the shelf-life studies performed at the national level by FBOs on specific products produced under identical conditions.

B.6. Choice of the analytical methods for the samples

In the proposed surveys it is suggested to use standardised ISO methods for the analyses. In the case of the Member States' specific surveys it is suggested that only the quantitative (enumeration) analyses are carried out on the samples and the qualitative (detection) analysis is not performed. It is acknowledged that this will reduce the sensitivity of the analyses, but in the interest of keeping the number of analyses relatively low this is being proposed. However, it is anticipated that this loss of information will not substantially hinder the

analyses of the data and the foreseen outcome.

In the case of the Community-specific survey both the quantitative and qualitative methods are proposed to be carried out. This is because the reduced number of samples per Member States will enable the use of more resources in the laboratory analyses. The adding of the qualitative (detection) method will provide more information especially for the modelling of growth characteristics of *L. monocytogenes* and will also facilitate the evaluation of the compliance with the theoretical criterion of “absence of *L. monocytogenes* in 25g”.

B.7. Choice of the sample size

Estimated sample sizes as opposed to actual number of samples taken

The sample size of a survey is the number of observations (samples) that constitute it. It is typically denoted as n , which is a positive integer (natural number). On the contrary, while planning the survey, it is mandatory to estimate (calculate) a sample size required to yield a survey outcome with a predetermined precision. These calculations require a number of values to be agreed upon and inputted into an equation. The calculated sample size must be regarded as a reference or target number of observations to collect in the field in order to provide an outcome (estimations) with that predetermined precision. The actual survey precision fully depends on the conducted field work with the actual collected number of observations (samples) (and also the scheme according to which the samples are taken) – not on the *a priori* calculated sample size.

Causes of inadequately sized surveys

Typically, all else being equal, a larger sample size leads to increased precision in estimates of various properties of the population. Fewer samples (a lower sample size) translate to a lower precision. Precision being the lack of random error (error due to the play of chance) of an estimated population characteristic (EFSA, 2006). It means that a lower sample size translates to qualitative inferior measurements that are not providing the required study outcome.

Generally, the sample size:

- decreases for finite population sizes (numbers of total units in the populations) and a finite population correction factor is applied in these cases;
- increases when the studied characteristic is not homogeneously spread amongst the population (clustering effect, over-dispersion, heterogeneity);
- varies when previous years' results can be taken into account (for then no worst case scenario of 50% expected prevalence needs to be taken account of).

Furthermore non-response (missing samples) must also be anticipated. Then the calculated sample sizes are strictly minimum numbers, and the survey must be adequately sized so as to prevent a lower-than-expected survey accuracy.

Estimated sample sizes for rare disease/contamination phenomena

General sample size calculations assume that the outcome variable of interest (zoonotic agent)

is prevalent in the population. When the investigated frequency of contaminated foodstuffs is rare (below 0.1%), these general sample size calculations are less valid and more appropriate methodology, like risk-based sampling, should be taken account of.

In this Member States' specific surveys it is expected, based on the Community Zoonoses Summary Reports from 2007 and before, that the prevalence of RTE food contaminated with *L. monocytogenes*, based on quantitative (enumeration) analyses, will be low (between 1% and 10%) to very low (between 0.1% and 1%) in most of the Member States. More specifically an expected (worst case scenario, with high sample size) prevalence of 5% was assumed. In Table 6, Annex I, a sample size calculation table is presented for several options of accuracy and confidence levels. An accuracy of 1.5% and a desired confidence level of 95% are assumed to provide sufficient data for the prevalence estimates at the Member State level. It results that to adequately size the survey, 900 samples must be taken for every specified food category, per analyses stage (analysing at time of sampling or at end of the shelf-life), per Member State. A summary of the calculated sample sizes with different levels of accuracy and of confidence are presented in the Table 2. In case of small Member States, lower sample sizes may be considered. However, smaller sample sizes may jeopardise adequate prevalence estimation because of low accuracy levels.

Table 2. The Member State-specific sample sizes with different levels of accuracy, assuming an expected prevalence of 5% and a confidence level of 95%, Member States' surveys

Accuracy	Confidence interval (CI)	Sample size	Sample size accounting for non-response (+10%)	Rounded up final sample size
1%	2%	1,825	2,008	2,010
1.5%	3%	812	893	900
2.5%	5%	292	321	330
5%	10%	73	80	80

In the proposed Community-specific survey the same input criteria (expected prevalence, accuracy and confidence level) for sample size calculations, at the Community-level, are assumed. However, in absence of knowledge on any Community-specific survey design effect, the calculated sample size is multiplied by three, to 3,000. Next a proportionate stratified sampling scheme is followed to allocate this number of samples to the Members States according to the size of their human population. The exact Member State-specific sample size is detailed in Table 7, Annex I. However, a harmonised Member State-specific sample size is proposed, as summarized in Table 3. The reason for proposing an allocation scheme based on Member States' human population is that reliable and specific food marketing data were not available for all Member States. The reasons for proposing a pragmatic and harmonised sample size for every Member State are to allow minimal

meaningful data collection at the Member State-level, thus a minimum Member State-specific sample size of 30. Moreover these pragmatic and harmonised sample sizes may limit the impact, on prevalence estimation, of discrepancies between the stratification variable ‘food marketing data’ and ‘human population’. It results that 3,020 samples must be taken for every specified food category, per analyses stage (analysing at time of sampling or at end of the shelf-life), at the Community-level.

Table 3. Member State-specific sample sizes, in the Community-level survey

Member State population (% of EU population)	Harmonised Member State-specific sample size	Number of Member States
≤ 1%	30	8
> 1% and ≤ 5%	60	13
> 5% and ≤ 10%	200	2
> 10%	400	4

B.8. Storage *L. monocytogenes* isolates for future typing

The current survey proposes that isolates of *L. monocytogenes* from samples analysed at the end of the shelf-life would be stored by National Reference Laboratories (NRLs) for further typing studies. It is recommended that all or a representative subset of isolates from each food category would be typed with a maximum of 70 strains per Member State.

Whilst a harmonised survey would in itself be of benefit in providing comparable prevalence data, the further step of typing would ensure that maximal value is derived from the resources devoted to sample acquisition and analysis. It is acknowledged that this would create an additional burden of work on the laboratories involved, including NRLs and the Community Reference Laboratory (CRL), but overall the benefits outweigh the costs. In this context, typing activity is proposed to be included within future working plans of the CRL.

Typing of isolates provides relevant information about strain diversity and information on how closely related the strains are. This is particularly relevant when collecting this information at EU level. It may identify certain clusters of types in different parts of the EU, which may be attributed to specific sources. This may lead to a greater understanding of the factors that select for those strains. Ongoing typing programmes could show the spread of certain types from region to region or from food chain to food chain, over time and the possible evolution of new types including the acquisition of relevant virulence factors.

Moreover, linking typing results of both food isolates and clinical isolates from human cases contributes to improving the understanding of the epidemiology of *Listeria* infections. Such knowledge could help to further target risk management decisions to food that pose the highest risks and hence meaningfully impact on public health.

The availability of various phenotypic and genotypic methods for further typing of *L. monocytogenes* is acknowledged. Molecular methods in general provide more resolution potential, but because of this ability to discern exquisitely minute differences between strains,

active attention to harmonisation is required to ensure inter-laboratory comparability of outcomes across laboratories. Among the more common used *L. monocytogenes* typing methods, Pulsed-Field Gel Electrophoresis (PFGE), has attained an acceptable level of standardisation at an international level. PFGE has been shown to be highly discriminatory for typing of *L. monocytogenes* strains and is commonly considered as a gold standard in epidemiological studies. For PFGE, a standardized protocol is available (Graves and Swaminathan, 2001).

Inter-laboratory comparability of results is a paramount consideration in establishing a harmonised approach across the 27 Member States and potentially greater number of laboratories. Comparison of the typing results in the frame of the survey could be obtained from NRLs using the same typing method, assessed by the results of a proficiency trial organized by CRL (annual proficiency trials are planned for PFGE, multiplex PCR serotyping and agglutination serotyping). Methods should be selected on the basis of these results (which method performs well and which most of the Member States are able to perform). Detailed standardised protocols will be proposed by the CRL, for the strain selection, typing methodology, data interpretation, and for the integration of these data in a central database.

Table 4. Meeting the objectives of the survey by the different options of the survey schemes

Scheme options	Objective 1 Assess effectiveness of implementation of microbiological criteria in a food category	Objective 2 Collect comparable data between Member States	Objective 3 Collect data for risk assessment
Testing at time of sampling	X (modelling scenario data to assess <i>L. monocytogenes</i> growth during shelf-life)	X	X (modelling scenarios data to assess consumer exposure)
Testing at the end of the shelf-life	X	X	X (data to assess consumer exposure)
Enumeration method	X	X (product prevalence contaminated by <i>L. monocytogenes</i> , according to specified cut-off)	X (data to assess consumer exposure)
Detection method		X	X (data for hazard characterisation)
Sample size		X	
Modelling	X	X	X (data for hazard characterisation)
Typing isolates			X (data for hazard characterisation)

C. Use of data from the survey to assess the effectiveness of the implementation of Community *L. monocytogenes* criteria

C.1. Rationale for applying microbiological criteria in food safety management

General principles concerning the role of microbiological criteria in food safety management have been produced in 1997 by the Codex Alimentarius (CAC, 1997). In the Community legislation, Regulation (EC) No 2073/2005 (as amended) (EC, 2005 and 2007) stipulates various microbiological criteria, compliance with which is required of FBOs under Article 4 of Regulation (EC) No 852/2004 (EC, 2004a). Verification of the compliance of FBOs with these microbiological criteria is required from competent authorities, under the framework of Regulation (EC) No 882/2004 (EC, 2004b).

More recently the EFSA's Biological Hazards panel has considered the role of such microbiological criteria in the context of current concepts in the assessment, management and communication of food safety risk (EFSA, 2007c). According to its opinion, some of the current relevant applicability of microbiological criteria in food safety assurance includes:

- validation and verification of food safety management systems;
- informing decisions on the acceptability of batches;
- communication of the level of hazard that may be acceptable under legislation; and
- providing some assurance that microbial hazards are not present at unacceptably high concentrations.

The microbiological criteria described in the Community legislation falls into two groups: Process Hygiene criteria, and Food Safety (FS) criteria. FS criteria provide information about the acceptability of batches of food produced, as regards the presence of certain pathogenic microorganisms. Non-conforming batches should be removed from the market, along with commensurate improvements made to the production process.

The current approach to the applicability of microbiological criteria in the Community legislation has been in place since 2006 (EC, 2005), and follows previous legislative approaches whereby the role of microbiological testing may have assumed a more pivotal role in defining acceptability of batches of food.

Currently the use of microbiological criteria is one method, harmonised at the Community level, by which FBOs should verify the effectiveness of their food safety management systems. It is the food safety management system that ensures the safety of the food. Analysing against the microbiological criteria is required to be carried out for only a small proportion of food batches placed on the market. However, all food should be produced to comply with the relevant microbiological criteria. The effectiveness of food safety management systems at controlling hazards at specific steps of the food chain can be assessed by gathering a range of information. For example, this could include information on the temperature of food processes, the salt, water activity, pH and/or sugar levels of the food, the hygiene of the food processing environment, as well as analysing against microbiological criteria. Microbiological criteria are a useful indicator of the ability of the process, including the associated food safety management system, to produce safe food and represent one form of acceptance criteria for determining acceptability of individual batches of consignments of

food.

C.2. Use of data from the survey to assess the effectiveness of the implementation of the *L. monocytogenes* criteria

EU-wide surveys are considerable undertakings and it would add to their value if their results could be used to assess the effectiveness of the implementation of microbiological criteria by FBOs.

In the specific context of the current survey protocol, there are Community microbiological criteria regarding *L. monocytogenes*: FS criteria set down by Regulation (EC) No 2073/2005 on microbiological criteria for foodstuffs (as amended) (EC, 2005 and 2007). This Regulation also laid down three RTE food categories: RTE food supporting or not supporting *L. monocytogenes* growth, and RTE food for infants or special medical purposes. Table 5 summarizes the FS criteria of *L. monocytogenes* for each relevant food category.

The purpose of these FS criteria is to prevent exposure of consumers to RTE food with hazardous levels of *L. monocytogenes*. They provide harmonised standards in the EU and impact upon the entire food chain because the risk of recall and economic loss for food processors are a strong motivation to meet the criteria. However, as noted previously, application of microbiological criteria is only one of several management activities to ensure that RTE food do not pose a risk related to human illness. Microbiological analyses alone to verify compliance with the criteria may convey a false sense of security due to the statistical limitation of sampling plans (EFSA, 2007c). FS criteria should not be considered without implementation of efficient control programs based on hazard analysis and critical control point (HACCP) principles. In addition to criteria, Regulation (EC) No 2073/2005 also introduced the requirement for FBOs “to conduct studies to investigate compliance with criteria throughout shelf-life” for RTE food able to support growth of *L. monocytogenes*. These investigations should take into account the “reasonably foreseeable storage conditions” (in particular temperature and shelf-life) and should consider the significant variability in refrigeration temperatures observed in Europe, particularly in domestic refrigerators.

Table 5. Food safety criteria regarding *L. monocytogenes* according to Regulation (EC) No 2073/2005 on microbiological criteria for foodstuffs (EC, 2005)

Food category	Micro-organisms/their toxins, metabolites	Sampling plan		Limits	Analytical reference method	Stage where the criterion applies
		n	c			
1.1. Ready-to-eat foods intended for infants and ready-to-eat foods for special medical purposes ²	<i>Listeria monocytogenes</i>	10	0	Absence in 25 g	EN/ISO 11290-1	Products placed on the market during their shelf-life
1.2. Ready-to-eat foods able to support the growth of <i>L. monocytogenes</i> , other than those intended for infants and for special medical purposes	<i>Listeria monocytogenes</i>	5	0	100 cfu/g ³	EN/ISO 11290-2	Products placed on the market during their shelf-life
		5	0	Absence in 25 g ⁴	EN/ISO 11290-1	Before the food has left the immediate control of the food business operator, who has produced
1.3 Ready-to-eat foods unable to support the growth of <i>L. monocytogenes</i> , other than those intended for infants and for special medical purposes ^{5,6}	<i>Listeria monocytogenes</i>	5	0	100 cfu/g	EN/ISO 11290-2	Products placed on the market during their shelf-life

² Regular testing against the criterion is not useful in normal circumstances for the following ready-to-eat foods:

- those which have received heat treatment or other processing effective to eliminate *L. monocytogenes*, when recontamination is not possible after this treatment (e.g. products heat treated in their final package),
- fresh, uncut and unprocessed vegetables and fruits, excluding sprouted seeds,
- bread, biscuits and similar products,
- bottled or packed waters, soft drinks, beer, cider, wine, spirits and similar products,
- sugar, honey and confectionery, including cocoa and chocolate products, live bivalve molluscs.

³ This criterion applies if the manufacturer is able to demonstrate, to the satisfaction of the competent authority, that the product will not exceed the limit 100 cfu/g throughout the shelf-life.

⁴ This criterion applies to products before they have left the immediate control of the producing food business operator, when he is not able to demonstrate, to the satisfaction of the competent authority, that the product will not exceed the limit of 100 cfu/g throughout the shelf-life.

⁵ As in (2) above

⁶ Products with pH ≤ 4,4 or a_w ≤ 0,92, products with pH ≤ 5,0 and a_w ≤ 0,94, products with a shelf-life of less than five days are automatically considered to belong to this category. Other categories of products can also belong to this category, subject to scientific justification.

A modelling exercise, as described further in the next section, would allow accounting for various sources of variability and uncertainty along the food chain and account for bacteriological growth during storage.

C.3. Proposal for modelling and simulation to assess the effectiveness of the implementation of the *L. monocytogenes* criteria

C.3.1. General principles on the model-based approach

The purpose of the model-based approach will be to assess whether the observed prevalence estimates of high (>100 cfu/g) levels of *L. monocytogenes* in RTE foodstuffs at the end of the shelf-life are compatible or not with the compliance by FBOs to the *L. monocytogenes* criteria laid down in Regulation (EC) No 2073/2005 (EC, 2005). This requires some understanding of the statistical link between observed contamination levels at the end of the shelf-life and observed contamination levels at production. A stochastic model is a mathematical tool to formalise this link and can be used to meet the objective.

More specifically, the main sources of variability and uncertainty, such as inter-batch variability at production, sampling uncertainty, test sensitivity, and storage time are represented by probability distributions. Then, given the sampling characteristics (sampling design, batch sizes, total number of batches, etc.) and assuming that the *L. monocytogenes* criteria are met for all produced batches, the distribution of the theoretical prevalence of high (>100 cfu/g) levels of *L. monocytogenes* at retail can be derived for each Member State and at EU level. This distribution is then compared to the estimated observed prevalence from the survey at retail with confidence intervals. This way it is possible to quantitatively assess how likely it is that the *L. monocytogenes* criteria are effectively implemented within a given Member State. Figure 1 illustrates examples of cases where the criterion is met or not met.

Once a model is available, some scenarios can be defined for further prospective (predictive) exercises. For this purpose, a range of possible scenarios needs to be defined (e.g. using alternative pH criteria, temperature scenarios, etc.). Then, Monte Carlo simulation can be performed to predict the likelihood of contamination at the end of the shelf-life.

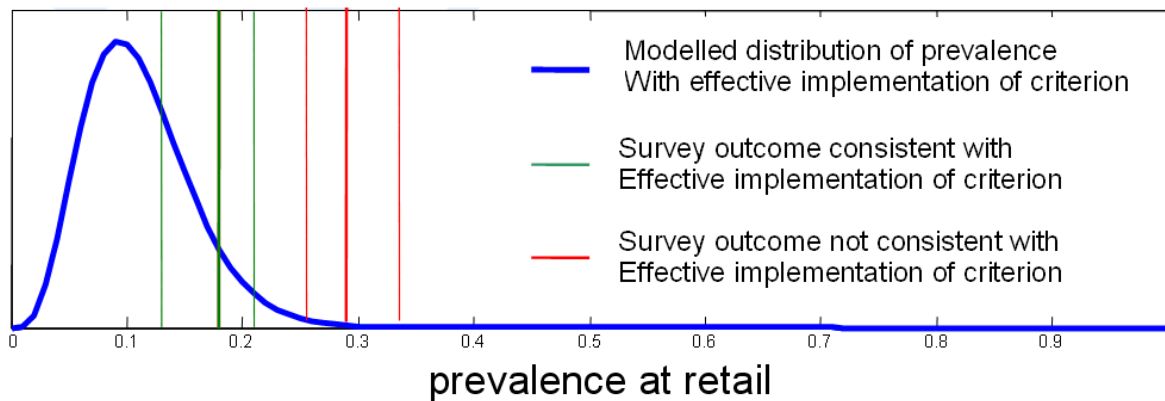


Figure 1. Examples of assessments where baseline survey results at retail at the end of the shelf-life meet and do not meet the predicted (modelled) distribution of prevalence for high (>100 cfu/g) levels of *L. monocytogenes* in RTE food at the end of the shelf-life as regards compliance with the *L. monocytogenes* criteria

C.3.2. Model building, tuning and validation

A specific refined model will be fully developed once all data are available. All assumptions will be assessed in terms of either their realism or the robustness of results with respect to them. For this purpose, a model validation will be performed, e.g. by using simulation and a pilot case where many data are available. All sensitive assumptions will be assessed and adjusted whenever data or prior information allow for it. Such refinements, together with use of literature or industry data, could take place even before the final data are available. An EFSA working group is being established for that purpose.

Predictive growth models and distributions of storage times and storage temperatures will be obtained from literature sources and used in Monte Carlo simulations.

The period of production considered will correspond to the duration of the survey at retail. The statistical evaluation will be made independently for each Member State or at the EU level only, depending on the selected target population of the baseline study. Annex II summarises typical assumptions to be made in the model building.

D. I. Proposal for a survey on *L. monocytogenes* in selected ready-to-eat food at retail in the EU, the Member States' specific surveys

D.I.1. Introduction

Article 5 of Directive 2003/99/EC on the monitoring of zoonoses and zoonotic agents, foresees the establishment of coordinated monitoring programmes to assess risks or to establish baseline values related to zoonoses or zoonotic agents at the Member State or EU level, especially when specific requirements are identified (EC, 2003).

There is a need to determine baseline values for this pathogen in relevant categories of RTE food products in order to obtain information on *L. monocytogenes* contamination levels in RTE food categories recognised to be able to contain the pathogen at high levels, and to enable the consideration of Community measures to combat *L. monocytogenes* in these foods.

In these technical specifications the coordinated monitoring programme takes the form of a one year survey. The specifications, in particular those covering sample collection and analytical methods, are also recommended for undertaking continuous monitoring within the framework of Directive 2003/99/EC (EC, 2003).

D.I.2. Definitions

For the purpose of this document, the following definitions will apply:

Batch – group or set of identifiable products obtained from a given process under practically identical circumstances and produced in a given place within one defined production period (EC, 2005).

Cheeses

- **Soft cheeses** – cheeses that have a percentage moisture, on a fat-free basis, higher than 67% (CAC, 2008a).
- **Semi-soft cheeses** – cheeses that have a texture which is only slighter harder than the soft cheese category. These cheeses have a percentage moisture, on a fat-free basis, ranging from 62 to 67%. Semi-soft cheeses are characterized by their firm but elastic feel.
- **Ripened cheeses** – cheeses which are not ready for consumption shortly after manufacture but which must be held for such time, at such temperature, and under such other conditions as will result in the necessary biochemical and physical changes characterizing the cheese in question (CAC, 2008a).
- **Mould ripened cheeses** – ripened cheeses in which the ripening has been accomplished primarily by the development of characteristic mould growth throughout the interior and/or on the surface of the cheese (CAC, 2008a).
- **Smear-ripened cheeses** – ripened cheeses in which during or after ripening, the cheese rind is treated or naturally colonized with desired cultures of microorganisms, for instance *Penicillium candidum* or *Brevibacterium linens*. The resulting layer or **smear** forms a part of the rind (CAC, 2008a).
- **Brine matured cheeses** – cheeses matured and stored in brine until they are sold or packed.

- **Unripened cheeses** including **fresh cheeses** – cheeses which are ready for consumption shortly after manufacture (CAC, 2008b).
- **Fresh cheeses** – curd-style cheeses which do not undergo any ripening, for example cottage cheese, mozzarella, ricotta, and quark. *Fresh cheeses are not included in this survey.*

Competent authority – means the central authority of a Member State competent for the organisation of official controls or any other authority to which that competence has been conferred; it shall also include, where appropriate, the corresponding authority of a third country (EC, 2004b).

Compliance with microbiological criteria – obtaining satisfactory or acceptable results set in Annex I of the Regulation (EC) No 2073/2005 when testing against the values set for the criteria through the taking of samples, the conduct of analyses and the implementation of corrective action, in accordance with food law and the instructions given by the competent authority (EC, 2005).

Contamination – means the presence or introduction of a hazard (EC, 2004a).

Coordinated monitoring programme – programme referred to in Article 5 of Directive 2003/99/EC, which may be established especially when specific needs are identified to assess risks, or to establish baseline values related to zoonoses or zoonotic agents at the Member State or Community level (EC, 2003).

Country of production – the country indicated in the identification mark referred to in Article 1 of Regulation (EC) No 853/2004 (EC, 2004c).

Food safety criterion – criterion defining the acceptability of a product or a batch of foodstuff applicable to products placed on the market (EC, 2005).

Gravad fish – fish that have been cured in salt and sugar without thermal treatment.

***Listeria monocytogenes* positive food** – a food where *Listeria monocytogenes* is isolated by culture techniques from a sample taken out of it.

Meat products – processed products resulting from the processing of meat or from the further processing of such processed products, so that the cut surface shows that the product no longer has the characteristics of fresh meat (EC, 2004c).

Microbiological criterion – criterion defining the acceptability of a product, a batch of foodstuffs or a process, based on the absence, presence or number of microorganisms, and / or on the quantity of their toxins / metabolites, per unit(s) of mass, volume, area or batch (EC, 2005).

Modified atmosphere packaging (MAP) – removal of air from a food package and replacement with a strictly controlled gaseous mixture of carbon dioxide, oxygen, and/or nitrogen, and then hermetically sealed.

Packaged food – a food that has its entire surface covered in order to prevent direct contact of

the food with the environment. This would include impermeable wrapping such as hermetically sealed plastic, and also include permeable wrapping such as muslin-wrapped cheese.

Preservatives – substances which prolong the shelf-life of foods by protecting them against deterioration caused by microorganisms and/or which protect against growth of pathogenic micro-organisms (EC, 2008).

Processing – any action that substantially alters the initial product, including heating, smoking, curing, maturing, drying, marinating, extraction, extrusion or a combination of those processes (EC, 2004a).

Random sample – sample in which the characteristics of the batch from which it is drawn are maintained (CAC, 2004). It is a sample which is taken under statistical consideration to provide representative data (EC, 1998).

Ready-to-eat food – food intended by the producer or the manufacturer for direct human consumption without the need for cooking or other processing effective to eliminate or reduce to acceptable level microorganisms of concern (EC, 2005).

Retail – the handling and/or processing of food and its storage at the point of sale or delivery to the final consumer (EC, 2002). *In this document retail covers only shops, supermarkets and other similar outlets that sell directly to the final consumer. It does not include distribution terminals or centres, catering operations, institutional catering, factory canteens, restaurants and other similar food service operations and wholesale outlets.*

Sampling frame – complete list of all units of the population, which can be sampled.

Sample size – the number of units randomly chosen from the sampling frame.

Shelf-life – the period preceding the “*Use by*” or the minimum durability date (EC, 2000).

Smoked fish – fish cured by smoking.

Survey – study involving a sample of units selected from a larger, well-delineated population. This (target) population is the entire set of units to which findings of the survey are to be extrapolated. The units to examine are to be selected randomly (Rothman, 1986; Noordhuizen *et al.*, 2001 and EFSA, 2008).

Vacuum packaging (VP) – evacuation of air from a food package that is then hermetically sealed.

D.I.3. Objectives

The objective of the survey is to estimate the prevalence of, and to obtain quantitative data on, the contamination with *L. monocytogenes* in selected RTE food categories at the Member State and EU level. The survey is designed with the aim that results should be comparable amongst the Member States.

This survey includes two selected RTE food categories:

1. smoked and gravad fish; and
2. soft and semi-soft cheeses.

The survey should enable the effectiveness of the implementation of Community criteria established for *L. monocytogenes* to be assessed at the Member State and EU level. The survey should also provide an opportunity for an evaluation of the currently declared shelf-lives of these two RTE food categories in respect to the *L. monocytogenes* criteria.

The results of the survey will provide data on the exposure of consumers to *L. monocytogenes* via the selected RTE food categories and also on growth characteristics of *L. monocytogenes* in the smoked and gravad fish category. These data may be used in microbiological risk assessments that may further assist in the establishment of additional risk management measures aiming at reducing the incidence of human listeriosis.

Packaged RTE heat-treated meat products were also considered for this survey. However, in order to keep the workload manageable for the Member States it is proposed that this food category is investigated in another survey to be carried out later.

D.I.4. Sampling frame

D.I.4.1. *The population to be sampled*

This survey will focus on two categories of RTE food believed to be amongst those most frequently contaminated with *L. monocytogenes*. These two categories are sampled at a retail level.

1) *Packaged, smoked or gravad fish*

Smoked fish includes both hot-smoked and cold-smoked fish. Gravad fish have been cured in salt and sugar without thermal treatment. These products must be packaged by the manufacturer and may be vacuum or modified atmosphere packed (MAP).

The fish may or may not be sliced. The package may contain a whole fish, or half or a part of a fish. The skin of the fish may be present or absent.

2) *Soft or semi-soft cheese*

This category includes cheeses made from raw, thermised or pasteurised milk of any animal species. The cheese may be smear-ripened, mould-ripened, brine-matured or otherwise ripened. Fresh cheeses are not included in the survey.

The cheese may be packaged including wrapped in muslin, or may be unpackaged at retail but packaged at the point of sale for the consumer.

A non-exhaustive list of examples of soft and semi-soft cheeses is included in Table 8 in Annex III.

D.I.4.2. Sampling design

The survey consists of 27 Member State-specific surveys (Member States' specific surveys). The sampling plan within each Member State is based on a multistage cluster design. The first level is composed of the major cities/towns to be sampled (in each Member State). Next at the second level are the retail outlets to be sampled.

Ideally, the central competent authority should draw up a sampling plan following the rules described below and based on the best marketing data available. These marketing data, or assistance with how to obtain the information, may often be available from a national trade association. In the absence of marketing data, the best estimate of market shares should be used to inform the sampling plan at a central level. In the absence of any reliable marketing information it may be necessary for competent authorities to devolve the selection of the type of product to sample within a category to the sampler in the field.

Selection of the retail outlet categories to be targeted

The competent authorities are responsible for choosing the retail outlets to be visited. Typical types of retail outlets that could be included for sampling are: supermarkets, small shops, speciality delicatessens, and street markets (e.g. farmers' or country markets). The following rule shall be used to choose the types of retail outlets to be sampled and needs to be followed for each of the two categories of RTE food:

- if the biggest category of outlets (for example supermarkets) supply at least 80% of the market of a RTE food category then samples only need to be taken from those outlets. Where that is not the case, the second largest outlet category should be added and so on until at least 80% of the market is covered.
- The number of samples that should be taken from each retail outlet category included in the sampling plan should be proportionate to the market share of that outlet category within the targeted outlet categories.

Selection of the cities or towns to be sampled

The sampling shall take place in major cities/towns. The number of major cities/towns to be sampled in each Member State should be at least four and a maximum of eight. These cities/towns should cover at least 30% of the human population in the country. However, if the eight largest cities/towns are included in the plan, the human population coverage could be less than 30%.

Selection of sample timing

As it is possible that the contamination level of *L. monocytogenes* in RTE food may vary over the year, this merits further stratification. For that purpose, the 12-month period should be divided in 12 periods of 1 month. In each of those periods 1/12th of the total sample size should be taken. Taking into account the above described randomisation, it is possible to take more than one sample of soft or semi-soft cheese or smoked/gravad fish during the same visit

to the retail outlet. However, not more than five batches of cheeses and five batches of smoked and gravad fish should be sampled at the same visit.

Selection of the RTE food within the two main categories to be sampled

The RTE food types within the two categories to be sampled should ideally be selected based on the marketing data and detailed in the centrally-devised sampling plan.

However, the diverse nature of the products within a food category may not permit estimation of market share with any degree of reliability. In this case, the competent authorities may choose to adapt an approach whereby the national sampling plan instructs samplers to select within a category for sampling based upon an estimated contribution to market share. Such sampling may be informed by turnover details provided by local retail management, or more crudely by prominence in the marketplace, e.g. what is displayed at the retail outlet. With such an approach competent authorities should provide some direction on approximate market share of major types of food within categories to best approach a sample representative of market e.g. raw/pasteurised milk cheeses.

The retail sampling plan to be prepared by the central competent authority shall define, in line with the reporting requirements set down in section D.I.8, the following:

- the cities/towns to be included in the survey;
- the types of retail outlets covered and their share in sampling;
- the distribution of the samples throughout the year.

and where permitted by available marketing data:

- the types of products to be sampled within each of the two RTE food categories;
- the number of samples taken from each RTE food type;

D.I.5. Sample size (number of samples) calculation

The population size is the number of foodstuff units of the population. In this survey the population is considered to be infinite, as there are more than 100,000 food units constituting the population.

The sample size gives the number of foodstuffs to be tested in every Member State. It is calculated on the basis of the following criteria, assuming simple random sampling:

- expected (a priori) prevalence (**p**): 5%
- the z-score ($Z_{\alpha/2}$)⁷ corresponding to a desired (1- α)% confidence level⁸: for example a

⁷ In statistics, a z-score (also called z-value, standard score, normal score, and standardized variable) is a dimensionless quantity derived by subtracting the population mean from an individual raw score and then dividing the difference by the population standard deviation. This conversion process is called standardizing or normalizing.

⁸ The statistical confidence level is the probability with which one wishes to be certain that an observed change

desired 95% confidence level corresponds to a $Z_{\alpha/2}$ value of 1.96⁹,

- accuracy (L) (or absolute error): 1.5%
- using these values and the formula:
$$n_{\infty} = \frac{(Z_{\alpha/2})^2 p(1-p)}{L^2}$$

An expected prevalence of 5% is based on the Community Zoonoses Summary Reports from 2007 and before, that document that the prevalence of RTE food contaminated with *L. monocytogenes*, based on quantitative (enumeration) analyses, would be low (between 1% and 10%) to very low (between 0.1% and 1%) in most of the Member States (EFSA, 2009).

The resulting sample size is 812 under the specified input values. This number should be augmented by 10% to anticipate non-response which leads to a Member State specific sample size of 900 for every specified RTE food category under investigation, and per analyses stage (analysing at time of sampling or at the end of the shelf-life).

A table presenting sample sizes calculated for combinations of expected prevalences ranging from 0.1% to 50%; accuracy levels ranging from 1% to 5% and confidence levels ranging from 90% to 95%, is presented in Annex I (Table 6). An accuracy of 1.5% and a desired confidence level of 95% are assumed to provide sufficient data for the prevalence estimates at the Member State level.

The total sample size in this survey investigating two selected RTE food categories (smoked and gravad fish and soft and semi-soft cheeses), whereof the former one is analysed at two time periods (analysing at time of sampling and at the end of the shelf-life), is 2,700.

D.I.6. Sample collection

D.I.6.1. Type and details of sample

The objective is to collect samples from the two categories of RTE food (smoked and gravad fish, soft and semi-soft cheeses) taken at random from the customer display e.g. refrigerator shelves in the selected retail outlets. Retail outlets that could be included for sampling are as described in section D.I.4.2, i.e. supermarkets, small shops, specialty delicatessens, and street markets (e.g. farmers' or country markets). At the same visit to a retail outlet, up to five different fish batches and five cheese batches can be sampled.

Only packaged intact (sealed) packs should be collected, i.e. products packaged by the manufacturer. However, in case of cheeses, products repackaged at the retail outlet may also be sampled. The packaged samples must not show evidence of damage to packaging and necessary label information should be present. If the label on the RTE food is not clear or is otherwise damaged then the sample should not be taken. Information on the label should include details of the country of production, batch number, durability date, instructions on

of the magnitude specified did not occur by chance (that is, the level of significance). In other words it is the degree of certainty that a statistical prediction on the change in prevalence of resistance is accurate, or the chance to obtain a prevalence estimate within the desired precision.

⁹ Where $Z_{\alpha/2}$ is the upper critical value of the normal distribution which exceeded with probability $\alpha/2$.

temperature storage conditions, and other information which is normally on the label of packaged RTE food so that this can be recorded. If this is not the case, it will be necessary to ask the proprietor or manager for the information on the required product and labelling details and/or refer to the wholesale pack for this information.

Two samples should be collected from each batch of smoked or gravad fish sampled (labelling information, such as batch numbers, sell by date, etc. should be examined to ensure that the two samples are from the same batch). One of these two samples will be analysed on the day of receipt at the laboratory and the other at the end of the shelf-life. For soft and semi-soft cheeses, only one sample is taken from a batch that will be analysed at the end of the shelf-life. The samples should be placed in a separate sampling bag and sent immediately in refrigerated containers to the laboratory for analysis.

It is essential that cross-contamination is avoided during the collection of RTE food samples. Precautions must therefore be taken at all stages to ensure that the equipment used during sampling, transport and storage are not contaminated with the pathogen *L. monocytogenes* investigated in the programme.

D.I.6.2. Sample information

All relevant information available from the sample should be recorded on a sampling form produced by the competent authority to enable the data requirements in section D.I.8 to be fulfilled. This includes for example information on type of retail outlet, the RTE food category (including the subcategory type, i.e. type of soft or semi-soft cheese, smoked fish), traceability details (i.e. country of production, identification mark number, batch number), production or packing date (if available), durability date and storage temperature and use instructions. In the case of cheese samples repackaged at the retail outlet, it may be necessary to ask the proprietor or manager for the information on the required product and labelling details and/or refer to the wholesale pack for this information.

It is essential to identify the pack-producer codes on the RTE food (and identification marks/supplier codes when possible) for each sample so that the origin of the RTE food can be determined retrospectively as the country of production is not always apparent from examining the label of a retail pack. The co-operation of retailers may be required to permit identification of imported RTE food.

At the point of sampling, the surface temperature of the packaged samples should be taken and recorded on the sampling form. This will provide information on those sampled food products which were not stored at an appropriate refrigeration temperature at the retail premises.

Each sample and its sample form should be labelled with a unique number which should be used from sampling to analyses. The samples of smoked fish from the same batch and outlet have to be identifiable. The competent authority must arrange for the issue and use a unique numbering system.

D.I.6.3. Transport of samples

The samples must be kept at between +2 to +8°C and free from external contamination during transportation. All RTE food samples should reach the laboratory within 24 hours of sampling. In exceptional circumstances (e.g. un-avoidable long journeys) this period may be extended to 48 hours provided the sample would not exceed the use by date before testing.

D.I.7. Sampling in the laboratory and analytical methods

D.I.7.1. Laboratories

National Reference Laboratories (NRLs) for *L. monocytogenes* are the laboratories where normally all types of microbiological analyses described shall take place (detection, enumeration, identification, typing).

However the competent authorities may decide to designate other laboratories involved in official control to perform the analyses in particular the detection and enumeration of *L. monocytogenes* (typing should preferentially be still centrally performed by the NRLs). These laboratories shall have proven experience of using the required methods and shall have a quality assurance system complying with the EN/ISO standard 17025 (ISO, 2005). NRLs may provide advice for the designation of these laboratories, based in particular on their accreditation and their participation in proficiency testing trials. In this last case, the results of these trials shall be communicated to the NRLs.

D.I.7.2. Receipt of samples

On receipt of the samples, laboratories shall check the information recorded by the sampler and complete the relevant sections of the sample form. Digital photographs or photocopies of the label of the packaging may be taken and stored electronically with the appropriate sample number to capture all product information. The photograph or photocopy should be of a high resolution so that the labeling details are clear.

All samples received shall be examined to ensure that the transport packaging is intact before storing.

For smoked and gravad fish, among the two samples:

- One sample unit is analysed (enumeration of *L. monocytogenes*, pH and water activity (a_w)) directly or within 24 h of arrival at the laboratory. This sample needs to be held at 3 °C (\pm 1°C) in the laboratory before analysis.
- The other sample unit is kept under refrigeration until the end of the shelf-life, even if this represents a long period such as three months.

For soft and semi-soft cheeses:

- the sample unit is kept under refrigeration until the end of the shelf-life, even if this represents a long period such as three months

Sample units to be stored until the end of the shelf-life should be stored refrigerated:

- at the storage temperature indicated in the instructions mentioned on the package label. If these instructions mention a temperature interval (e.g. +2-+8°C), this sample unit must be stored at the upper limit of the temperature interval;
- if there is no specific storage temperature mentioned in the package instructions, this sample unit should be kept at the maximum refrigeration temperatures ($\pm 1^\circ\text{C}$) defined by each Member State's national legislation;
- if no national legislation exists; $+8^\circ\text{C} \pm 1^\circ\text{C}$ will be used.

If the end date of the shelf-life is during a weekend, the sample should be analysed on the last working day before the end of the shelf-life.

D.I.7.3. Sample preparation and initial suspension preparation

It is essential that laboratory handlers take care to avoid cross contamination between samples and from the surrounding environment at all stages. Primary samples are discarded once laboratory analyses have been initiated. If the analysis is stopped, e.g. due to unacceptable deviations in the analysis process, new samples must be obtained.

It has been demonstrated that for heterogeneous products, the variability of microbiological quantitative results is essentially due to the sub-sampling of the test portion (see ISO/TS 19036, Annex A) (ISO, 2009a). For these conditions, it is recommended to take either the entire product or a representative 100-150 g subsample. Portions of food reflecting the proportion that would be consumed should be taken. When a product is offered in a sliced condition then the representative sample from more than one slice of product should be taken. The obtained test portions should be cut in small pieces and placed into a stomacher bag, using a sterile instrument and aseptic technique. In accordance to the EN/ISO 6887-2 to 3 standards, the subsample should be homogenized for 1 minute (ISO, 2003a and 2003b).

From this mixture, 10 g sample test should be taken for enumeration. To the volume of the test portion (10 g), 9 volumes (90 ml) of diluent is added and subsequently the mixture is homogenised using a stomacher or a pulsifier for 1-2 minutes. Foaming should be avoided by removing the air as much as possible from the stomacher bag. For the preparation of the initial dilution of cheese samples, a sodium citrate solution, as described in ISO 6887-5 'Microbiology of food and animal feeding stuffs – Preparation of test samples, initial suspension and decimal dilutions for microbiological examination – Part 5: Specific rules for the preparation of milk and milk products', shall be used (ISO, 2009b).

D.I.7.4. Enumeration method

Enumeration shall be performed:

- for smoked and gravad fish samples: immediately after sample collection at retail and at the end of the shelf-life,
- for soft and semi-soft cheese samples: only at the end of the shelf-life.

The enumeration of *L. monocytogenes* shall be performed according to the International Standard ISO 11290-2/Amd I: 2004 (ISO, 2004a).

If contaminated, it is assumed that the majority of products would contain low contamination levels of *L. monocytogenes*. To enable the estimation of low numbers of bacteria in samples (between 10 and 100 cfu/g), 1 ml of the primary dilution shall be tested in duplicate as indicated in the Standard EN ISO 11290-2/Amd I: 2004:

- spread onto the surface of three 90-mm diameter plates;
- or spread onto the surface of one 140-mm diameter plate (ISO, 2004a).
-

Because of the possibility of higher contamination levels of *L. monocytogenes* 0.1 ml of the primary dilution should be spread onto the surface of one plate, to allow the enumeration of up to 1.5×10^4 cfu/g. This plating shall be performed in single as provided in ISO 7218⁶ 'Microbiology of food and animal feeding stuffs – General requirements and guidance for microbiological examinations' (ISO, 2007).

D.I.7.5. Storage of isolates

It is recommended to store one confirmed *L. monocytogenes* strain (isolated from the enumeration analysis) per positive sample analysed at the end of the shelf-life for further typing studies. Isolates should be stored by NRLs using appropriate methods for culture collection as long as they ensure viability of the strains for a minimum of two years.

D.I.7.6. Determination of the pH of smoked and gravad fish

The determination of the pH of the sample shall be performed according to the International Standard EN ISO 2917:1999 – Meat and meat products – Measurement of pH – Reference method (ISO, 1999).

The analysis should be performed on the sample tested on arrival at the laboratory. The test should be performed within 24 h after arrival. The non-destructive technique listed in the ISO method is recommended to measure the pH of the sample. The result are reported to the nearest 0.05 unit of pH.

D.I.7.7. Determination of the water activity (a_w) of smoked and gravad fish

The determination of the a_w of the sample shall be performed according to the International Standard EN ISO 21807:2004 – Microbiology of food and animal feeding stuffs – Determination of water activity (ISO, 2004b).

The analysis should be performed on the sample tested on arrival at the laboratory. The test should be performed within 24 h after arrival. The method shall be capable of operating in the range 0.999 to 0.9000 and the repeatability limit shall correspond to a standard deviation of 0.002. The reported value should contain at least 2 significant figures.

D.I.8. Reporting

D.I.8.1. General provisions

The competent authority responsible for the preparation of the yearly national report on zoonoses pursuant to Article 9 of Directive 2003/99/EC shall collect and evaluate the results and report them to the Commission (EC, 2003).

The Commission shall forward the results to EFSA, which shall examine and report upon them. Any use of the data submitted by the Member States for the purposes other than the objective of this survey will be subject to prior agreement of the Member States.

National aggregated data and results will be made publicly available in a form that ensures confidentiality regarding the establishments and products sampled.

The information to be reported by Member States, as far as the information is available or accessible, is outlined in sections D.I.8.2 to D.I.8.4, and consists of two broad categories: description of the programme and individual data for each sample.

The description of the programme should provide an overview of the sampling programme in its entirety in that Member State and the overall results obtained (D.I.8.2). Member States should also provide more detailed descriptive overviews of the sampling programmes (D.I.8.2), as well as the laboratory analytical approach (D.I.8.3) in that Member State. These descriptions will be submitted once by each Member State, and should take the form of a textual account of the sampling planned, the sampling actually taken place, and the results obtained. Sections D.I.8.2 to D.I.8.3 provide some headings to indicate the type of information to be submitted in this context.

Individual data should be submitted for each sample tested as part of the sampling programme (D.I.8.4). This information shall be submitted in a form of raw data using a 'Data Dictionary' and data collection forms established and provided by the Commission.

D.I.8.2. Overview of the survey and results

- Member State name
- Date of start and end of the sampling and analysis
- Number of samples obtained and analysed from retail outlets:
 - Packaged soft and semi-soft cheeses
 - Packaged smoked and gravad fish
- Overall results:
 - Prevalence and proportion of samples exceeding the limit 100 cfu/g of *Listeria monocytogenes* in soft and semi-soft cheeses and smoked and gravad fish covered by the survey
- Description of soft and semi-soft cheeses and smoked and gravad fish markets in the Member State:

- Overall absolute market size if available
- Market share of different types of retail outlets (supermarket, small shops, speciality delicatessens, street markets including country and farmers' markets), if available
- Market share of imported (intra-Community trade and imports from third countries) and domestic production, if available
- Market share of different types of products, if available
- Retail outlets sampled:
 - Type of outlet categories covered: e.g. supermarkets, small shops etc.
 - Geographical distribution of sampling – cities/towns covered (% of human population covered)
- Description of randomisation procedure for retail sampling:
 - Month randomisation
- Comment on overall representativeness of the sampling programme
- Preparation of test sample used for pH measurement
- Analytical method used for a_w measurement

D.I.8.3. *Overview of laboratory analysis*

- For each laboratory involved in *L. monocytogenes* analysis:
 - Laboratory identifier code*
 - NRL for this organism Y/N (Yes/No)

D.I.8.4. *Detailed sample data*

- Type of sample:
 - Packaged soft and semi-soft cheeses
 - Packaged smoked and gravad fish
- Subtype of the sample:
 - Cheeses made from raw / thermised / pasteurised milk
 - Cheeses made from cow / goat / sheep / buffalo / mixed milk
 - Smear-ripened, mould-ripened, brine-matured and other ripened cheeses
 - Sliced and non-sliced products
 - Cold / hot smoked / gravad fish
 - Species of the fish
- Preservatives and acidity regulators used in smoked or gravad fish (as indicated in the label)¹⁰

¹⁰ Examples of food preservatives and acidity regulators are listed in Table 9 in Annex IV

- Cheese rind included in the sample Y/N
- Code of the laboratory involved in initial analysis*
- Date of sample collection
- Use by date
- Production / packaging date (if available)
- Surface temperature of the sample in the retail outlet
- Storage temperature in the laboratory up to the end of the shelf-life
- Analysis immediately after sampling (only for smoked and gravad fish) / end of the shelf-life
- Date of beginning of the analysis at the laboratory
- Detection of *L.monocytogenes*
 - Qualitative results (absence/presence in 25g)
- Quantification of *L. monocytogenes*
 - Quantitative results (cfu/g)
- pH (only for smoked and gravad fish)
- Water activity (a_w) (only for smoked and gravad fish)
- Code of the town*
- Code of the outlet*
- Type of retailer:
 - Supermarket
 - Small Shop /Independent retailer
 - Speciality delicatessen
 - Street market / Farmers' market
- Country of production*:
 - As ascertained with reference to the Identification Mark on packaging or commercial documentation
- Pre-packaged:
 - Modified atmosphere (MAP)
 - Vacuum-packed
 - Other package type
 - Packed at retail (only for cheeses)

* It is envisaged that Member States would adopt an approach of requiring sampling officers to record detailed information on the specific establishments on where foods are sampled or produced. For the purposes of reporting information pertaining to individual samples in this programme to the Commission, these data could be anonymised by for example the assignment of unique codes to the establishments and regions in which sampling took place, and only reporting these anonymous codes. The Identification Mark referring to the

establishment in which the food was produced, would be recorded by the sampling officer, but only the country of production referred to there would be reported to the Commission and not the establishment of production.

D.II. Proposal for a survey on *L. monocytogenes* in selected ready-to-eat food at retail in the EU, the Community-specific survey

D.II.1. Introduction

Article 5 of Directive 2003/99/EC on the monitoring of zoonoses and zoonotic agents, foresees the establishment of coordinated monitoring programmes to assess risks or to establish baseline values related to zoonoses or zoonotic agents at the Member State or EU level, especially when specific requirements are identified (EC, 2003).

In order to obtain information on *L. monocytogenes* contamination levels in RTE food categories recognised to be able to contain the pathogen at high levels, and to enable the consideration of Community measures to combat *L. monocytogenes* in these foods, there is a need to determine baseline values for this pathogen in relevant categories of RTE food products.

In these technical specifications the coordinated monitoring programme takes the form of a one year survey. The specifications, in particular those covering sample collection and analytical methods, are also recommended for undertaking continuous monitoring within the framework of Directive 2003/99/EC (EC, 2003).

D.II.2. Definitions

For the purpose of this document, the following definitions will apply:

Batch – group or set of identifiable products obtained from a given process under practically identical circumstances and produced in a given place within one defined production period (EC, 2005).

Cheeses

- **Soft cheeses** – cheeses that have a percentage moisture, on a fat-free basis, higher than 67% (CAC, 2008a).
- **Semi-soft cheeses** – cheeses that have a texture which is only slighter harder than the soft cheese category. These cheeses have a percentage moisture, on a fat-free basis, ranging from 62 to 67%. Semi-soft cheeses are characterized by their firm but elastic feel.
- **Ripened cheeses** – cheeses which are not ready for consumption shortly after manufacture but which must be held for such time, at such temperature, and under such other conditions as will result in the necessary biochemical and physical changes characterizing the cheese in question (CAC, 2008a).
- **Mould ripened cheeses** – ripened cheeses in which the ripening has been accomplished primarily by the development of characteristic mould growth throughout the interior and/or on the surface of the cheese (CAC, 2008a).
- **Smear-ripened cheeses** – ripened cheeses in which during or after ripening, the cheese rind is treated or naturally colonized with desired cultures of microorganisms, for instance *Penicillium candidum* or *Brevibacterium linens*. The resulting layer or **smear** forms a part of the rind (CAC, 2008a).
- **Brine matured cheeses** – cheeses matured and stored in brine until they are sold or

packed.

- **Unripened cheeses** including **fresh cheeses** – cheeses which are ready for consumption shortly after manufacture (CAC, 2008b).
- **Fresh cheeses** – curd-style cheeses which do not undergo any ripening, for example cottage cheese, mozzarella, ricotta, and quark. *Fresh cheeses are not included in this survey.*

Competent authority – means the central authority of a Member State competent for the organisation of official controls or any other authority to which that competence has been conferred; it shall also include, where appropriate, the corresponding authority of a third country (EC, 2004b).

Compliance with microbiological criteria – obtaining satisfactory or acceptable results set in Annex I of Regulation (EC) No 2073/2005 when testing against the values set for the criteria through the taking of samples, the conduct of analyses and the implementation of corrective action, in accordance with food law and the instructions given by the competent authority (EC, 2005).

Contamination – means the presence or introduction of a hazard (EC, 2004a).

Coordinated monitoring programme – programme referred to in Article 5 of Directive 2003/99/EC, which may be established especially when specific needs are identified to assess risks, or to establish baseline values related to zoonoses or zoonotic agents at the Member State or Community level (EC, 2003).

Country of production – the country indicated in the identification mark referred to in Article 1 of Regulation (EC) No 853/2004 (EC, 2004c).

Food safety criterion – criterion defining the acceptability of a product or a batch of foodstuff applicable to products placed on the market (EC, 2005).

Gravad fish – fish that have been cured in salt and sugar without thermal treatment.

***Listeria monocytogenes* positive food** – a food where *Listeria monocytogenes* is isolated by culture techniques from a sample taken out of it.

Meat products – processed products resulting from the processing of meat or from the further processing of such processed products, so that the cut surface shows that the product no longer has the characteristics of fresh meat (EC, 2004c).

Microbiological criterion – criterion defining the acceptability of a product, a batch of foodstuffs or a process, based on the absence, presence or number of microorganisms, and / or on the quantity of their toxins / metabolites, per unit(s) of mass, volume, area or batch (EC, 2005).

Modified atmosphere packaging (MAP) – removal of air from a food package and replacement with a strictly controlled gaseous mixture of carbon dioxide, oxygen, and/or nitrogen, and then hermetically sealed.

Packaged food – a food that has its entire surface covered in order to prevent direct contact of

the food with the environment. This would include impermeable wrapping such as hermetically sealed plastic, and also include permeable wrapping such as muslin-wrapped cheese.

Preservatives – substances which prolong the shelf-life of foods by protecting them against deterioration caused by microorganisms and/or which protect against growth of pathogenic micro-organisms (EC, 2008).

Processing – any action that substantially alters the initial product, including heating, smoking, curing, maturing, drying, marinating, extraction, extrusion or a combination of those processes (EC, 2004a).

Random sample – sample in which the characteristics of the batch from which it is drawn are maintained (CAC, 2004). It is a sample which is taken under statistical consideration to provide representative data (EC, 1998).

Ready-to-eat food – food intended by the producer or the manufacturer for direct human consumption without the need for cooking or other processing effective to eliminate or reduce to acceptable level microorganisms of concern (EC, 2005).

Retail – the handling and/or processing of food and its storage at the point of sale or delivery to the final consumer (EC, 2002). *In this document retail covers only shops, supermarkets and other similar outlets that sell directly to the final consumer. It does not include distribution terminals or centres, factory canteens, restaurants and other similar food service operations and wholesale outlets.*

Sampling frame – complete list of all units of the population, which can be sampled.

Sample size – the number of units randomly chosen from the sampling frame.

Shelf-life – the period preceding the “Use by” or the minimum durability date (EC, 2000).

Smoked fish – fish cured by smoking.

Survey – study involving a sample of units selected from a larger, well-delineated population. This (target) population is the entire set of units to which findings of the survey are to be extrapolated. The units to examine are to be selected randomly (Rothman, 1986; Noordhuizen *et al.*, 2001 and EFSA, 2008).

Vacuum packaging (VP) – evacuation of air from a food package that is then hermetically sealed.

D.II.3. Objectives

The objective of the survey is to estimate the prevalence of, and to obtain quantitative data on, the contamination with *L. monocytogenes* in selected RTE food categories at the EU level.

This survey includes three selected RTE food categories:

1. smoked and gravad fish;
2. soft and semi-soft cheeses, and
3. heat treated meat products

The survey should enable the effectiveness of the implementation of Community criteria established for *L. monocytogenes* to be assessed at the EU level. The survey should also provide an opportunity for an evaluation of the currently declared shelf-lives of these three RTE food categories in respect to the *L. monocytogenes* criteria.

The results of the survey will provide data to assess the exposure of consumers to *L. monocytogenes* via the selected RTE food categories and also on growth characteristics of *L. monocytogenes* in the smoked and gravad fish category. These data may be used in microbiological risk assessments that may further assist in the establishment of additional risk management measures aiming at reducing the incidence of human listeriosis.

D.II.4. Sampling frame

D.II.4.1. *The population to be sampled*

This survey will focus on three categories of RTE food believed to be amongst those most frequently contaminated with *L. monocytogenes*. These three categories are sampled at a retail level.

1) *Packaged, smoked or gravad fish*

Smoked fish includes both hot-smoked and cold-smoked fish. Gravad fish have been cured in salt and sugar without thermal treatment. These products must be packaged by the manufacturer and may be vacuum or modified atmosphere packed (MAP).

The fish may or may not be sliced. The package may contain a whole fish, or half or a part of a fish. The skin of the fish may be present or absent.

2) *Soft or semi-soft cheese*

This category includes cheeses made from raw, thermised or pasteurised milk of any animal species. The cheese may be, smear-ripened, mould-ripened, brine-matured or otherwise ripened. However, fresh cheeses are not included in the survey.

The cheese may be packaged including wrapped in muslin, or may be unpackaged at retail but packaged at the point of sale for the consumer.

A non-exhaustive list of examples of soft and semi-soft cheeses is included in Table 8 in Annex III.

3) *Packaged heat-treated meat products, which have been handled and vacuum or MAP packaged after heat treatment*

This category covers both exposed meat products and meat products in a permeable skin that have been sliced or otherwise handled between heat treatment and packaging. Non-

exhaustive examples of types of products within this category include:

- Consumer packs of meat products made with whole or large parts of anatomical structures e.g. cooked sliced ham and cooked chicken fillet;
- RTE sausages: meat product typically comprising fragmented/comminuted meat in a permeable skin;
- Pâtés: meat product comprising meat homogenate/paste.

This category may include meat products smoked after heat treatment. However, this category does not include:

- meat products dried after heat treatment, e.g. jerky products;
- meat products heat-treated in an impermeable package which are not handled thereafter, and
- fermented meat products including fermented sausages.

D.II.4.2. *Sampling design*

A proportionate stratified sampling scheme is used at the Community-specific survey whereby the samples are allocated to every Member State proportionally to the size of the human population in the country. The sampling plan within each Member State is further based on a multistage cluster design. The first level is composed of the major cities/towns to be sampled (in each Member State). Next at the second level are the retail outlets to be sampled. At the third level the different RTE food products within the three categories to be sampled are selected.

Ideally, the central competent authority should draw up a sampling plan following the rules described below and based on the best marketing data available. These marketing data, or assistance with how to obtain the information, may often be available from a national trade association. In the absence of marketing data, the best estimate of market shares should be used to inform the sampling plan at a central level. In the absence of any reliable marketing information it may be necessary for competent authorities to devolve the selection of the type of product to sample within a category to the sampler in the field.

Selection of the retail outlet categories to be targeted

The competent authorities are responsible for choosing the retail outlets to be visited. Typical types of retail outlets that could be included for sampling are: supermarkets, small shops, speciality delicatessens, and street markets (e.g. farmers' or country markets). The following rule shall be used to choose the types of retail outlets to be sampled and needs to be followed for each of the three categories of RTE food:

- if the biggest category of outlets (for example supermarkets) supply at least 80% of the market of a RTE food category then samples only need to be taken from those outlets. Where that is not the case, the second largest outlet category should be added and so on until at least 80% of the market is covered.
- The number of samples that should be taken from each retail outlet category included in the sampling plan should be proportionate to the market share of that outlet category within the targeted outlet categories.

Selection of the cities or towns to be sampled

The sampling shall take place in major cities/towns. The number of major cities/towns to be sampled in each Member State should be at least two and a maximum of eight. These cities/towns should cover at least 30% of the human population in the country. However, if the eight largest cities/towns are included in the plan, the human population coverage could be less than 30%.

Selection of sample timing

As it is possible that the contamination level of *L. monocytogenes* in RTE food may vary over the year, this merits further stratification. For that purpose, the 12-month period should be divided in 12 periods of 1 month. In each of those periods 1/12th of the total sample size should be taken. Taking into account the above described randomisation, it is possible to take more than one sample from each RTE food category during the same visit to the retail outlet. However, no more than five batches from each food category should be sampled at the same visit.

Selection of the RTE food within the three main categories to be sampled

The RTE food subcategories to be sampled should ideally be selected based on the marketing data and detailed in the centrally-devised sampling plan.

However, the diverse nature of products within a category may not permit estimation of market share with any degree of reliability. In this case, the competent authorities may choose to adapt an approach whereby the national sampling plan instructs samplers to select cheeses for sampling based upon an estimated contribution to market share. Such sampling may be informed by turnover details provided by local retail management, or more crudely by prominence in the marketplace, e.g. what is displayed at the retail outlet. With such an approach competent authorities should provide some direction on approximate market share of major types of food within categories to best approach a sample representative of market e.g. raw/pasteurised milk cheeses.

The retail sampling plan to be prepared by the central competent authority shall define, in line with the reporting requirements set down in section D.II.8, the following:

- the cities/towns to be included in the survey;
- the types of retail outlets covered and their share in sampling;
- the distribution of the samples throughout the year.

and where permitted by available marketing data:

- the types of products to be sampled within each of the three RTE food categories;
- the number of samples taken within each RTE food type.

D.II.5. Sample size (number of samples) calculation

Community-level sample size

The population size is the number of foodstuff units of the population. In this survey the population is considered to be infinite, as there are more than 100,000 food units constituting the population.

The sample size gives the number of foodstuffs to be tested in the Community. It is calculated on the basis of the following criteria, assuming simple random sampling:

- expected (a priori) prevalence (**p**): 5%
- the z-score ($Z_{\alpha/2}$)¹¹ corresponding to a desired (1- α)% confidence level¹²: for example a desired 95% confidence level corresponds to a $Z_{\alpha/2}$ value of 1.96¹³,
- accuracy (L) (or absolute error): 1.5%
- using these values and the formula:
$$n_{\infty} = \frac{(Z_{\alpha/2})^2 p(1-p)}{L^2}$$

An expected prevalence of 5% is based on the Community Zoonoses Summary Reports from 2007 and before, that document that the prevalence of RTE food contaminated with *L. monocytogenes*, based on quantitative (enumeration) analyses, would be low (between 1% and 10%) to very low (between 0.1% and 1%) in most of the Member States (EFSA, 2009).

The resulting sample size is 812 under the specified input values. This number should be augmented by 10% to anticipate non-response which leads to a sample size of 900. However, in the absence of knowledge on any Community-specific design effect, this sample size is multiplied by three. It results that at the Community-level a total of about 3,000 samples must be taken for each RTE food category, per analyses stage (analysing at time of sampling or at the end of the shelf-life).

Allocation of samples to Member States

For each specified RTE food category under investigation, and per analyses stage (analysing at time of sampling or at the end of the shelf-life) the Community-level number of samples to take needs to be allocated (distributed) to the Member States according to the size of their human population. This means that each Member State will need to take a subsample of each set of 3,000 samples for the targeted food categories, proportional to its human population size. The exact allocated sample size per each Member State as well as a Member State-

¹¹ In statistics, a z-score (also called z-value, standard score, normal score, and standardized variable) is a dimensionless quantity derived by subtracting the population mean from an individual raw score and then dividing the difference by the population standard deviation. This conversion process is called standardizing or normalizing.

¹² The statistical confidence level is the probability with which one wishes to be certain that an observed change of the magnitude specified did not occur by chance (that is, the level of significance). In other words it is the degree of certainty that a statistical prediction on the change in prevalence of resistance is accurate, or the chance to obtain a prevalence estimate within the desired precision.

¹³ Where $Z_{\alpha/2}$ is the upper critical value of the normal distribution which exceeded with probability $\alpha/2$.

specific harmonised allocated sample size is further detailed in Table 7, Annex I. The latter harmonized sample size results in a total Community-level sample size of 3,020 (8 Member States x 30 + 13 Member States x 60 + 2 Member States x 200 + 4 Member States x 400).

The total sample size in this Community-specific survey investigating three selected RTE food categories (smoked and gravad fish, soft and semi-soft cheeses and RTE heat-treated meat products), whereof the first one is analysed at two time periods (analysing at time of sampling and at the end of the shelf-life), is 12,080.

D.II.6. Sample collection

D.II.6.1. Type and details of sample

The objective is to collect samples from the three categories of RTE food (smoked and gravad fish, soft and semi-soft cheeses, heat-treated meat products) taken at random from the customer display e.g. refrigerator shelves in the selected retail outlets. Retail outlets that could be included for sampling are as described in section D.II.4.2, i.e. supermarkets, small shops, specialty delicatessens, and street markets (e.g. farmers' or country markets). At the same visit to a retail outlet, up to five different fish batches, five different cheese batches, and five different heat-treated meat products batches can be sampled.

Only packaged intact (sealed) packs should be collected, i.e. products packaged by the manufacturer. However, in case of cheeses, products repackaged at the retail outlet may also be sampled. The packaged samples must not show evidence of damage to packaging and necessary label information should be present. If the label on the RTE food is not clear or is otherwise damaged then the sample should not be taken. Information on the label should include details of the country of production, batch number, durability date, instructions on temperature storage conditions, and other information which is normally on the label of packaged RTE food so that this can be recorded. If this is not the case, it will be necessary to ask the proprietor or manager for the information on the required product and labelling details and/or refer to the wholesale pack for this information.

Two samples should be collected from each batch of smoked or gravad fish sampled (labelling information, such as batch numbers, sell by date, etc. should be examined to ensure that the two samples are from the same batch). One of these two samples will be analysed on the day of receipt at the laboratory and the other at the end of the shelf-life. For soft and semi-soft cheeses and heat-treated meat products, only one sample is taken from a batch that will be analysed at the end of shelf-life. The samples should be placed in a separate sampling bag and sent immediately in refrigerated containers to the laboratory for analysis.

It is essential that cross-contamination is avoided during the collection of RTE food samples. Precautions must therefore be taken at all stages to ensure that the equipment used during sampling, transport and storage are not contaminated with the pathogen *L. monocytogenes* investigated in the programme.

D.II.6.2. Sample information

All relevant information available from the sample should be recorded on a sampling form produced by the competent authority to enable the data requirements in section D.II.8 to be fulfilled. This includes for example information on type of retail outlet, the RTE food category (including the subcategory type, i.e. type of soft or semi-soft cheese, smoked fish, heat-treated meat product), traceability details (i.e. country of production, identification mark number, batch number), production or packing date (if available), durability date and storage temperature and use instructions. In the case of cheese samples repackaged at the retail outlet, it may be necessary to ask the proprietor or manager for the information on the required product and labelling details and/or refer to the wholesale pack for this information.

It is essential to identify the pack-producer codes on the RTE food (and identification marks/supplier codes when possible) for each sample so that the origin of the RTE food can be determined retrospectively as the country of production is not always apparent from examining the label of a retail pack. The co-operation of retailers may be required to permit identification of imported RTE food.

At the point of sampling, the surface temperature of the packaged samples should be taken and recorded on the sampling form. This will provide information on those sampled food products which were not stored at an appropriate refrigeration temperature at the retail premises.

Each sample and its sample form should be labelled with a unique number which should be used from sampling to analyses. The samples of smoked fish from the same batch and outlet have to be identifiable. The competent authority must arrange for the issue and use a unique numbering system.

D.II.6.3. Transport of samples

The samples must be kept at between +2 to +8°C and free from external contamination during transportation. All RTE food samples should reach the laboratory within 24 hours of sampling. In exceptional circumstances (e.g. un-avoidable long journeys) this period may be extended to 48 hours provided the sample would not exceed the use by date before testing.

D.II.7. Sampling in the laboratory and analytical methods

D.II.7.1. Laboratories

National Reference Laboratories (NRLs) for *L. monocytogenes* are the laboratories where normally all types of microbiological analyses described shall take place (detection, enumeration, identification, typing).

However the competent authorities may decide to designate other laboratories involved in official control to perform the analyses in particular the detection and enumeration of *L. monocytogenes* (typing should preferentially be still centrally performed by the NRLs). These laboratories shall have proven experience of using the required methods and shall have a quality assurance system complying with the EN/ISO standard 17025 (ISO, 2005).

NRLs may provide advice for the designation of these laboratories, based in particular on their accreditation and their participation in proficiency testing trials. In this last case, the results of these trials shall be communicated to the NRLs.

D.II.7.2. Receipt of samples

On receipt of the samples, laboratories shall check the information recorded by the sampler and complete the relevant sections of the sample form. Digital photographs or photocopies of the label of the packaging may be taken and stored electronically with the appropriate sample number to capture all product information. The photograph or photocopy should be of a high resolution so that the labeling details are clear.

All samples received shall be examined to ensure that the transport packaging is intact before storing.

For smoked and gravad fish, among the two samples:

- One sample unit is analysed (detection and enumeration of *L. monocytogenes*, pH and water activity (a_w)) directly or within 24 h of arrival at the laboratory. This sample needs to be held at $+3\text{ C}^\circ (\pm 1^\circ\text{C})$ in the laboratory before analysis;
- The other sample unit is kept under refrigeration until the end of the shelf-life, even if this represents a long period such as three months.

For soft and semi soft cheeses and heat-treated meat products:

- the sample unit is kept under refrigeration until the end of the shelf-life, even if this represents a long period such as three months

Sample units to be stored until the end of the shelf-life should be stored refrigerated:

- at the storage temperature indicated in the instructions mentioned on the package label. If these instructions mention a temperature interval (e.g. $+2 - +8^\circ\text{C}$), this sample unit must be stored at the upper limit of the temperature interval;
- if there is no specific storage temperature mentioned in the package instructions, this sample unit should be kept at the maximum refrigeration temperatures ($\pm 1^\circ\text{C}$) defined by each Member State's national legislation;

- if no national legislation exists; $+8^{\circ}\text{C} \pm 1^{\circ}\text{C}$ will be used.

If the end date of the shelf-life is during a weekend, the sample should be analysed on the last working day before the end of the shelf-life.

D.II.7.3. Sample preparation and initial suspension preparation

It is essential that laboratory handlers take care to avoid cross contamination between samples and from surrounding environment at all stages. Primary samples are discarded once laboratory analyses have been initiated. If the analysis is stopped, e.g. due to unacceptable deviations in the analysis process, new samples must be obtained.

It has been demonstrated that for heterogeneous products, the variability of microbiological quantitative results is essentially due to the sub-sampling of the test portion (see ISO/TS 19036, Annex A) (ISO, 2009a). For these conditions, it is recommended to take either the entire product or a representative 100-150 g subsample. Portions of food reflecting the proportion that would be consumed should be taken. When a product is offered in a sliced condition then take the representative sample from more than one slice of product. The obtained test portions should be cut in small pieces and placed into a stomacher bag, using a sterile instrument and aseptic technique. In accordance to the EN/ISO 6887-2 to 3 standards, the subsample should be homogenized for 1 minute (ISO, 2003a and 2003b).

From this mixture, 10 g sample test should be taken for enumeration and 25 g sample test for detection.

To the volume of the test portion (10 g or 25 g), 9 volumes (90 ml or 225 ml) of specific diluents are added and subsequently the mixture is homogenized using a stomacher or a pulsifier for 1-2 minutes. Foaming should be avoided by removing the air as much as possible from the stomacher bag. For the preparation of the initial dilution of cheese samples, a sodium citrate solution, as described in ISO 6887-5 'Microbiology of food and animal feeding stuffs – Preparation of test samples, initial suspension and decimal dilutions for microbiological examination – Part 5: Specific rules for the preparation of milk and milk products', shall be used (ISO, 2009b).

D.II.7.4. Detection and enumeration methods

Enumeration and detection shall be performed:

- for smoked and gravled fish samples: immediately after sample collection at retail and at the end of the shelf-life,
- for soft and semi-soft cheese samples and heat-treated meat product samples: only at the end of shelf-life.

D.II.7.4.1. Enumeration

The enumeration of *L. monocytogenes* shall be performed according to the International Standard ISO 11290-2/Amd 1:2004 (ISO, 2004a).

If contaminated, it is assumed that the majority of products would contain low contamination levels of *L. monocytogenes*. To enable the estimation of low numbers of bacteria in samples (between 10 and 100 cfu/g), 1 ml of the primary dilution shall be tested in duplicate as indicated in the Standard ISO 11290-2/Amd 1:2004:

- spread onto the surface of three 90-mm diameter plates;
- or spread onto the surface of one 140-mm diameter plate (ISO, 2004a).

Because of the possibility of higher contamination levels of *L. monocytogenes*, 0.1 ml of the primary dilution should be spread onto the surface of one plate to allow the enumeration of up to 1.5×10^4 cfu/g. This plating shall be performed in single as provided in ISO 7218 'Microbiology of food and animal feeding stuffs – General requirements and guidance for microbiological examinations' (ISO, 2007).

D.II.7.4.2. Detection

Detection of *L. monocytogenes* shall be performed according to the Standard EN ISO 11290-1 (ISO, 2004c).

D.II.7.5. Storage of isolates

It is recommended to store one confirmed *L. monocytogenes* strain per positive sample analysed at the end of the shelf-life for further typing studies. If *L. monocytogenes* strains are recovered both from the detection and enumeration methods, only the isolates from the enumeration method should be stored.

Isolates should be stored by NRLs using appropriate methods for culture collection as long as they ensure viability of the strains for a minimum of two years.

D.II.7.6. Determination of the pH of smoked and gravad fish

The determination of the pH of the sample shall be performed according to the International Standard EN ISO 2917:1999 – Meat and meat products – Measurement of pH – Reference method (ISO, 1999).

The analysis should be performed on the sample tested on arrival at the laboratory. The test should be performed within 24 h after arrival. The non-destructive technique listed in the ISO method is recommended to measure the pH of the sample. The result are reported to the nearest 0.05 unit of pH.

D.II.7.7. Determination of the water activity (a_w) of smoked and gravad fish

The determination of the a_w of the sample shall be performed according to the International Standard EN ISO 21807:2004 – Microbiology of food and animal feeding stuffs – Determination of water activity (ISO, 2004b).

The analysis should be performed on the sample tested on arrival at the laboratory. The test should be performed within 24 h after arrival. The method shall be capable of operating in the range 0.999 to 0.9000 and the repeatability limit shall correspond to a standard deviation of 0.002. The reported value should contain at least 2 significant figures.

D.II.8. Reporting

D.II.8.1. General provisions

The competent authority responsible for the preparation of the yearly national report on zoonoses pursuant to Article 9 of Directive 2003/99/EC shall collect and evaluate the results and report them to the Commission (EC, 2003).

The Commission shall forward the results to EFSA, which shall examine and report upon them. Any use of the data submitted by the Member States for the purposes other than the objective of this survey will be subject to prior agreement of the Member States.

National aggregated data and results will be made publicly available in a form that ensures confidentiality regarding the establishments and products sampled.

The information to be reported by Member States, as far as the information is available or accessible, is outlined in sections D.II.8.2 to D.II.8.4, and consists of two broad categories; description of the programme, and individual data for each sample.

The description of the programme should provide an overview of the sampling programme in its entirety in that Member State and the overall results obtained (D.II.8.2). Member States should also provide more detailed descriptive overviews of the sampling programmes (D.II.8.2), as well as the laboratory analytical approach (D.II.8.3) in that Member State. These descriptions will be submitted once by each Member State, and should take the form of a textual account of the sampling planned, the sampling actually taken place, and the results obtained. Sections D.II.8.2 to D.II.8.3 provide some headings to indicate the type of information to be submitted in this context.

Individual data should be submitted for each sample tested as part of the sampling programme (D.II.8.4). This information shall be submitted in a form of raw data using a 'Data Dictionary' and data collection forms established and provided by the Commission.

D.II.8.2. Overview of the survey and results

- Member State name
- Date of start and end of the sampling and analysis
- Number of ready-to-eat (RTE) food samples obtained and analysed from retail outlets:
 - Packaged soft and semi-soft cheeses
 - Packaged smoked and gravad fish
 - Packaged heat-treated meat products

- Overall results:
 - Prevalence and proportion of samples exceeding the limit of 100 cfu/g of *Listeria monocytogenes* in soft and semi-soft cheeses, smoked and gravad fish as well as in heat-treated products covered by the survey
- Description of soft and semi-soft cheeses, smoked and gravad fish as well as in heat-treated meat products markets in the Member State:
 - Overall absolute market size if available
 - Market share of different types of retail outlets (supermarket, small shops, speciality delicatessens, street markets including country and farmers' markets), if available
 - Market share of imported (intra-Community trade and imports from third countries) and domestic production, if available
 - Market share of different types of products, if available
- Retail outlets sampled:
 - Type of outlet categories covered: e.g. supermarkets, small shops etc.
 - Geographical distribution of sampling – cities/towns covered (% of human population covered)
- Description of randomisation procedure for retail sampling:
 - Month randomisation
- Comment on overall representativeness of the sampling programme
- Preparation of test sample used used for pH measurement and
- Analytical method used for a_w measurement

D.II.8.3. Overview of laboratory analysis

- For each laboratory involved in *L. monocytogenes* analysis:
 - Laboratory identifier code*
 - NRL for this organism Y/N (Yes/No)

D.II.8.4. Detailed sample data

- Type of sample:
 - Packaged soft and semi-soft cheeses
 - Packaged smoked and gravad fish
 - Packaged heat-treated meat products
- Subtype of the sample:
 - Cheeses made from raw / thermised / pasteurised milk
 - Cheeses made from cow / goat / sheep / buffalo / mixed milk
 - Smear-ripened, mould-ripened, brine-matured or other ripened cheeses
 - Sliced and non-sliced products
 - Cold / hot smoked / gravad fish

- Species of the fish
- Type of the meat product
- Preservatives and acidity regulators used in smoked or gravad fish (as indicated in the label)¹⁴
- Cheese rind included in the specimen analyses Y/N
- Code of the laboratory involved in initial analysis*
- Date of sample collection
- Use by date
- Production / packaging date (if available)
- Surface temperature of the sample in the retail outlet
- Storage temperature in the laboratory up to the end of the shelf-life
- Analysis immediately after sampling (only for smoked and gravad fish) / end of the shelf-life
- Date of beginning of the analysis at the laboratory
- Detection of *L.monocytogenes*
 - Qualitative results (absence/presence in 25g)
- Quantification of *L. monocytogenes*
 - Quantitative results (cfu/g)
- pH (only smoked and gravad fish)
- Water activity (a_w) (only smoked and gravad fish)
- Code of the town*
- Code of the outlet*
- Type of retailer:
 - Supermarket
 - Small Shop /Independent retailer
 - Speciality delicatessen
 - Street market / Farmers' market
- Country of production*:
 - As ascertained with reference to the Identification Mark on packaging or commercial documentation
- Pre-packaged:
 - Modified atmosphere (MAP)
 - Vacuum-packed
 - Other package type

¹⁴ Examples of food preservatives and acidity regulators are listed in Table 9 in Annex IV

- Packed at retail (only for cheeses)

* It is envisaged that Member States would adopt an approach of requiring sampling officers to record detailed information on the specific establishments on where foods are sampled or produced. For the purposes of reporting information pertaining to individual samples in this programme to the Commission, these data could be anonymised by for example the assignment of unique codes to the establishments and regions in which sampling took place, and only reporting these anonymous codes. The Identification Mark referring to the establishment in which the food was produced, would be recorded by the sampling officer, but only the country of production referred to there would be reported to the Commission and not the establishment of production.

E. Task Force on Zoonoses Data Collection members

Andrea Ammon, Alenka Babusek, Marta Bedriova, Karin Camilleri, Marianne Chriel, Georgi Chobanov, Ingrid Dan, Jürg Danuser, Kris De Smet, Marie Edan, Matthias Hartung, Birgitte Helwich, Merete Hofshagen, Patrícia Inácio, Sarolta Idei, Elina Lahti, Lesley Larkin, Peter Much, Edith Nagy, Lisa O'Connor, Rob Van Oosterom, Jacek Osek, José Luis Paramio Lucas, Antonio Petrini, Christodoulos Pipis, Saara Raulo, Antonia Ricci, Petr Šatrán, Joseph Schon, Jelena Sögel, Snieguole Sceponaviciene, Ana María Troncoso González, Kilian Unger, Luc Vanholme, Francois Veillet, Dimitris Vourvidis, Nicole Werner-Keišs.

F. References

CAC (Codex Alimentarius Commission), 1997. Principles for the establishment and application of microbiological criteria for foods. CAC/GL 21.

CAC (Codex Alimentarius Commission), 2004. General guidelines on sampling, CAC/GL 50-2004.

CAC (Codex Alimentarius Commission), 2008a. Codex general standard for cheese, Codex Stan 283-1978, Adopted 1973, Revision 1999, Amendment 2008.

CAC (Codex Alimentarius Commission), 2008b. Codex group standard for unripened cheese including fresh cheese, Codex Stan 221-2001, Adopted 2001, Amendment 2008.

EC (European Community), 1995. European Parliament and Council Directive No 95/2/EC of 20 February 1995 on food additives other than colours and sweeteners, OJ L 61, 18.3.1995, p.1.

EC (European Community), 1998. Commission Decision 98/179/EC of 23 February 1998 laying down detailed rules on official sampling for the monitoring of certain substances and residues thereof in live animals and animal products, OJ L 65, 5.3.1998, p. 31.

EC (European Community), 2000. Directive 2000/13/EC of the European Parliament and of the Council of 20 March 2000 on the approximation of the laws of the Member States relating to labelling, presentation and advertising of foodstuffs, OJ L 109, 6.5.2000, p. 29.

EC (European Community), 2002. Regulation (EC) No 178/2002 of the European Parliament and of the Council of 28 January 2002 laying down the general principles and requirements of food law, establishing the European Food Safety Authority and laying down procedures in matters of food safety, OJ L 31, 1.2.2002, p. 1.

EC (European Community), 2003. Directive 2003/99/EC of the European Parliament and of the Council of 17 November 2003 on the monitoring of zoonoses and zoonotic agents, amending Council Decision 90/424/EEC and repealing Council directive 92/117/EEC, OJ L 325, 12.12.2003, p. 31.

EC (European Community), 2004a. Regulation (EC) No 852/2004 of the European Parliament and of the Council of 29 April 2004 on the hygiene of foodstuffs, OJ L 139,

30.4.2004, p.1.

EC (European Community), 2004b. Regulation (EC) No 882/2004 of the European Parliament and of the Council of 29 April 2004 on official controls performed to ensure the verification of compliance with feed and food law, animal health and animal welfare rules, OJ L 165, 30.4.2004, p.1.

EC (European Community), 2004c. Regulation (EC) No 853/2004 of the European Parliament and of the Council of 29 April 2004 laying down specific hygiene rules for food of animal origin, OJ L 139, 30.4.2004. p.55.

EC (European Community), 2005. Commission Regulation (EC) No. 2073/2005 of 15 November 2005 on microbiological criteria for foodstuffs, OJ L 338, 22.12.2005, p. 1.

EC (European Community), 2007. Commission Regulation (EC) No. 1441/2007 of 5 December 2007 amending Regulation (EC) No 2073/2005 on microbiological criteria for foodstuffs, OJ L 322, 7.12.2007, p. 12.

EC (European Community), 2008. Regulation (EC) No 1333/2008 of the European Parliament and of the Council of 16 December 2008 on food additives, OJ L 354, 31.12.2008, p.16.

EFSA (European Food Safety Authority), 2006. Report of Task Force on Zoonoses Data Collection on Guidance Document on Good Practices for Design of Field Surveys, The EFSA Journal (2006), 93, 1-24.

EFSA (European Food Safety Authority), 2007a. The Community summary report on trends and sources of zoonoses, zoonotic agents, antimicrobial resistance and foodborne outbreaks in the European Union in 2006, The EFSA Journal, 130.

EFSA (European Food Safety Authority), 2007b. Scientific Opinion of the Panel on Biological Hazards on a request from the European Commission on Request for updating the former SCVPH opinion on *Listeria monocytogenes* risk related to ready-to-eat foods and scientific advice on different levels of *Listeria monocytogenes* in ready-to-eat foods and the related risk for human illness. The EFSA Journal 599, 1-42.

EFSA (European Food Safety Authority), 2007c. Opinion of the Scientific Panel on Biological Hazards on microbiological criteria and targets based on risk analysis, The EFSA Journal (2007) 462, 1-29.

EFSA (European Food Safety Authority), 2008. Report of the Task Force on Zoonoses Data Collection - Manual for reporting on zoonoses, zoonotic agents and antimicrobial resistance in the framework of Directive 2003/99/EC and of some other pathogenic microbiological agents for information derived from the reporting year 2007, The EFSA Journal, 142:1-84.

EFSA (European Food Safety Authority), 2009. The Community summary report on trends and sources of zoonoses and zoonotic agents in the European Union in 2007, The EFSA Journal, 223.

- Graves L.M., Swaminathan B. 2001. PulseNet standardized protocol for subtyping *Listeria monocytogenes* by macrorestriction and pulsed-field gel electrophoresis. *Int J Food Microbiol.* 65, 55-62.
- ISO (International Organisation for Standardization), 1996. ISO 11290-1:1996. Microbiology of food and animal feeding stuffs – Horizontal method for the detection and enumeration of *Listeria monocytogenes* - Part 2: Detection method, Geneva.
- ISO (International Organisation for Standardization), 1999. ISO 2917:1999 – Meat and meat products – Measurement of pH – Reference method, Geneva.
- ISO (International Organisation for Standardization), 2003a. ISO 6887-2:2003 – Microbiology of food and animal feeding stuffs – Preparation of test samples, initial suspension and decimal dilutions for microbiological examinations – Part 2: Specific rules for the preparation of meat and meat products, Geneva.
- ISO (International Organisation for Standardization), 2003b. ISO 6887-3:2003 – Microbiology of food and animal feeding stuffs – Preparation of test samples, initial suspension and decimal dilutions for microbiological examinations – Part 3: Specific rules for the preparation of fish and fishery products, Geneva.
- ISO (International Organisation for Standardization), 2004a. ISO 11290-2:1998/Amd 1:2004. Microbiology of food and animal feeding stuffs – Horizontal method for the detection and enumeration of *Listeria monocytogenes* - Part 2: Enumeration method – Amendment 1: Modification of the enumeration method, Geneva.
- ISO (International Organisation for Standardization), 2004b. ISO 21807:2004 – Microbiology of food and animal feeding stuffs – Determination of water activity, Geneva.
- ISO (International Organisation for Standardization), 2004c. ISO 11290-1:1996/Amd 1:2004. Modification of the isolation media and haemolysis test, and inclusion of precision data, Geneva.
- ISO (International Organisation for Standardization), 2005. ISO/IEC 17025:2005. General requirements for the competence of testing and calibration laboratories, Geneva.
- ISO (International Organisation for Standardization), 2007. ISO 7218:2007. Microbiology of food and animal feeding stuffs – General requirements and guidance for microbiological examinations, Geneva.
- ISO (International Organisation for Standardization), 2009a. ISO/TS 19036:2006/Amd 1:2009. Microbiology of food and animal feeding stuffs – Guidelines for the estimation of measurement uncertainty for quantitative determinations – Amendment 1: Measurement uncertainty for low counts, Geneva.
- ISO (International Organisation for Standardization), 2009b. ISO/DIS 6887-5:2009. Microbiology of food and animal feeding stuffs – Preparation of test samples, initial suspension and decimal dilutions for microbiological examinations – Part 5: Specific rules for the preparation of milk and milk products, Geneva.

Lanzieri, G., 2008. Population in Europe 2007: first results. Statistics in focus. Issue number 81/2008. Eurostat.

Noordhuizen, J.P.T.M., Frankena, K., Thrusfield, M.V. and Graat, E.A.M., 2001. Application of quantitative methods in veterinary epidemiology. Wageningen Pers, Wageningen, The Netherlands.

Rothman, K.J., 1986. Modern epidemiology. Little, Brown and Company, Boston.

WHO/FAO 2004. Risk assessment of *Listeria monocytogenes* in ready-to-eat foods: interpretative summary. Microbiological risk assessment series no. 4, Geneva, World Health Organisation.

ANNEX I – SAMPLE SIZE (NUMBER OF SAMPLES) CALCULATION

I. Member States' specific surveys

Sample size calculation

The population size is the number of foodstuff units of the population. In this survey the population is considered to be infinite, as there are more than 100,000 units constituting the population.

The sample size gives the number of foodstuffs to be tested, in every Member State. It is calculated on the basis of the following criteria, assuming simple random sampling:

- expected (a priori) prevalence (**p**): 5%
- the z-score ($Z_{\alpha/2}$)¹⁵ corresponding to a desired (1- α)% confidence level¹⁶: for example a desired 95% confidence level corresponds to a $Z_{\alpha/2}$ value of 1.96¹⁷,
- accuracy (**L**) (or absolute error): 1.5%
- using these values and the formula:
$$n_{\infty} = \frac{(Z_{\alpha/2})^2 p(1-p)}{L^2}$$

The resulting sample size is 812 under the specified input values.

In Annex I, table 6 presents sample sizes calculated for combinations of expected prevalences ranging from 0.1% to 50%; accuracy levels ranging from 1% to 5% and confidence levels ranging from 90% to 95%.

Justification for the input values

According to the Community Zoonoses Summary Reports from 2007 (EFSA, 2009) and before, the prevalence of packaged smoked fish at retail contaminated with *L. monocytogenes*, based on quantitative (enumeration) analyses, would be 5% at maximum, which is low. This would even be lower for packaged soft cheeses at retail, but not very much, which is the reason why a 5% expected prevalence is also assumed, which leads to a high(est) sample size.

In case of an expected 5% prevalence of contaminated foodstuffs, an appropriate (relative) level of accuracy is 1.5%, which is the absolute error. A desired higher level of accuracy of 1% leads to a calculated sample size (1,825) which is more than twice as high compared to the 1.5% accuracy level (812). On the contrary, when the accuracy is lowered to 2.5%, the sample size (292) is diminished by about two thirds.

¹⁵ In statistics, a z-score (also called z-value, standard score, normal score, and standardized variable) is a dimensionless quantity derived by subtracting the population mean from an individual raw score and then dividing the difference by the population standard deviation. This conversion process is called standardizing or normalizing.

¹⁶ The statistical confidence level is the probability with which one wishes to be certain that an observed change of the magnitude specified did not occur by chance (that is, the level of significance). In other words it is the degree of certainty that a statistical prediction on the change in prevalence of resistance is accurate, or the chance to obtain a prevalence estimate within the desired precision.

¹⁷ Where $Z_{\alpha/2}$ is the upper critical value of the normal distribution which exceeded with probability $\alpha/2$.

A desired confidence level of 95% is assumed to provide sufficient data for the prevalence estimates at the Member State level.

Table 6. Number of samples to be taken per RTE food category in the Member States as a function of assumed varying levels of expected prevalence, accuracy and of confidence level, Member States' surveys

Expected prevalence	Level of confidence											
	90 %				95%				99%			
	Desired accuracy				Desired accuracy				Desired accuracy			
	5%	2.5%	1.5%	1%	5%	2.5%	1.5%	1%	5%	2.5%	1.5%	1%
0.1%	2	5	13	28	2	7	18	39	3	11	30	67
0.5%	6	22	60	135	8	31	85	192	14	53	147	331
1%	11	43	120	268	16	61	170	381	27	106	292	657
2%	22	85	236	531	31	121	335	753	53	209	578	1,301
5%	52	206	572	1,286	73	292	812	1,825	127	505	1,401	3,152
10%	98	390	1,083	2,435	139	554	1,537	3,458	239	956	2,654	5,635
15%	138	552	1,534	3,450	196	784	2,177	4,899	339	1,354	3,760	7,800
20%	174	693	1,924	4,329	246	984	2,732	5,791	425	1,699	4,719	9,597
30%	228	910	2,526	5,376	323	1,291	3,586	7,466	558	2,230	5,832	12,230
50%	271	1,083	3,006	6,335	384	1,537	4,269	8,763	664	2,654	6,866	14,228

II. Community-specific survey sample size

In the proposal of a Community-specific survey, the resulting sample size is 900 under the same specified input values as the Member States specific surveys. However, in the absence of knowledge on any Community-specific design effect, this sample size is multiplied by three. This results in that at the Community-level a total of about 3,000 samples must be taken for every specified food category, per analyses stage (analysing at time of sampling or at the end of the shelf-life). This number of samples to take needs to be allocated (distributed) to the Members States according to the size of their human population. This means that the Members States will need to take a subsample from each of these sets of 3,000 samples, proportional to the population sizes for every targeted foodstuff in the countries. Since the stratification variable, the population size, is the same for every targeted foodstuff, the allocation scheme is also the same for every investigated foodstuff. The exact Member State-specific sample size is detailed in Table 7, Annex I. However, a harmonized Member State-specific sample size is proposed the reasons of which are allowing minimal meaningful data

collection at the Member State-level, thus a minimum Member State-specific sample size of 30. Also this scheme may render the sampling robust against possible non-optimal specification of the stratification variable. It results that 3,020 samples must be taken for every specified food category, per analyses stage (analysing at time of sampling or at the end of the shelf-life), at the Community-level.

Table 7. Number of samples to be taken per RTE food category in the Member States according to the size of their human population, Community-level survey.

Member State	EUROSTAT population 1.1.2008 (Lanzieri, 2008)		Exact stratified sample size	Harmonized stratified sample size
	N (Mio)	%		
Austria	8,332	1.7	50	60
Belgium	10,667	2.1	64	60
Bulgaria	7,640	1.5	46	60
Cyprus	795	0.2	5	30
Czech Republic	10,381	2.1	63	60
Denmark	5,476	1.1	33	60
Estonia	1,341	0.3	8	30
Finland	5,301	1.1	32	60
France	63,753	12.8	384	400
Germany	82,222	16.5	496	400
Greece	11,215	2.3	68	60
Hungary	10,045	2.0	61	60
Ireland	4,420	0.9	27	30
Italy	59,618	12.0	360	400
Latvia	2,271	0.5	14	30
Lithuania	3,366	0.7	20	30
Luxembourg	484	0.1	3	30
Malta	411	0.1	2	30
Poland	38,116	7.7	230	200
Portugal	10,618	2.1	64	60
Romania	21,529	4.3	130	60
Slovenia	2,026	0.4	12	30
Slovakia	5,401	1.1	33	60
Spain	45,283	9.1	273	200
Sweden	9,183	1.8	55	60
Netherlands	16,404	3.3	99	60
United Kingdom	61,186	12.3	369	400
EU	497,482	100.0	3,000	3,020

ANNEX II – TYPICAL ASSUMPTIONS UNDERLYING THE MODELS

As previously described, the details of the model features will be specific to *L. monocytogenes* in RTE food. It will be developed in a dedicated EFSA working group and then fitted to the data obtained. However, some general characteristics and points to consider can already be listed. Any production-to-retail model can be regarded as a step-wise model following the food chain flow. Specifically for each production type, model assumptions will be made at each production stage, such as:

At production:

- all batches at production within a country are not inter-changeable; some batches are more likely to be contaminated than other. This can be taken into account using a population model (i.e. using random effect on batches);
- batch sizes are assumed to be large and the batches to be homogeneous;
- compliance with microbiological criteria assumes that all batches should meet the criteria, therefore it is required to assume that the proportion of batches tested (regardless of what is done in practice) is $\pi = 1$. However, to better interpret and discuss the final results, other values of π could also be investigated;
- temperature and storage scenarios need to be defined;
- possible growth over storage time until the sampling time needs to be accounted for, by either a deterministic or probabilistic approach;
- under the assumption of compliance with *L. monocytogenes* criteria, the controlled batches in which contamination is above a given threshold are assumed to be removed from the production.

From production to retail:

- equi-distribution from production to retail: the batches produced are assumed to be dispatched randomly over all retailers.

At retail:

- the production is assumed to be distributed randomly over all retailers of a country and/or of the EU;
- no batch can be defined at retail. It is possible that different production batches are mixed at retail. This may result in an increased sensitivity of tests at retail to be accounted for. Various scenarios could be investigated;
- sizes of the retail outlets are assumed to be large with respect to the number and sizes of samples taken;
- the sampling at retail is assumed to follow a sampling model based on the survey data observed;
- temperature and storage scenarios need to be defined.

On top of mechanistic assumptions to be made on growth models, a number of classical but necessary statistical assumptions need to be made to allow the calculations to be conducted (e.g. independent observations from batch to batch). Those assumptions are usually the simplest ones, similarly to those underlying the binomial model used to calculate the minimum sample sizes by country.

Another critical point to consider within the estimation is the difference of test sensitivity between production and retail. The models should take that into account. This difference of sensitivity is due, for example, to the different size of samples, but also to cross-contamination or dispersion of contamination after retail packaging. The best and most robust way to quantify such a difference is to use quantitative results of the *L. monocytogenes* contaminated samples from the survey, and possibly from samples at production stage. A Poisson model could then be used to describe the *L. monocytogenes* contamination.

ANNEX III – EXAMPLES OF SOFT, SEMI-SOFT AND FRESH CHEESES

Table 8. List of examples of soft and/or semi-soft cheeses and fresh cheeses

Names of soft and/or semi-soft cheeses (excluding fresh cheeses)
<p>Abbaye de Citeaux; Adelist; Afuega'l Pitu; Aisy Cendre; Altenburger Ziegenkäse; Ambert; Ami du Chambertin; Anari; Anevato; Anneau du Vic-Bilh; Ardahan; Aromes au Gene de Marc; Arzúa Ulloa; Asiago cheese; Autun; Banon; Banon à la feuille; Bath Cheese; Bavaria Blu; Beenleigh Blue; Bel Paese; Bergader; Bierkäse; Bishop Kennedy; Blue cheese; Bleu de Bresse; Bleu d'Auvergne; Bleu de Gex; Blue Stilton; Blue veined cheese; Bonchester; Boschetto al Tartufo; Bosworth; Bougon; Boulette d'Avesnes; Boursin; Bra; Brick cheese; Brie; Brie de Meaux; Brillat-Savarin; Brin; Bryndza; Buchette d'Anjou; Bouyssou; Brânză topită; Bresse Bleu; Brie au lait thermisé; Brie de Meaux; Brie de Melun; Brie au Poivre; Broccio; Bruder Basil; Brunost; Butte; Butterkäse; Buxton Blue; Cabécou; Caboc; Cabrales cheese; Cacciottina; Cachaille; Caciocavallo; Calenzana; Cambozola; Camembert; Camembert de Normandie; Cancoillotte; Caprice des Dieux; Capricorn Goat; Carré de l'Est; Casatella Trevigiana; Casciotta d'Urbino; Cashel Blue; Cashel Irish Blue; Cathelain; Cendre d'Olivet; Celtic Promise; Cerney banon; Chabichou; Chabichou du Poitou; Chabis de Gatine; Chaource; Charolais; Chaumes cheese; Chavignol; Chaource; Chevre, Chevrotin; Civray; Coeur de Chevre; Cooleney; Cornish pepper; Coulommiers; Crescenza; Croghan; Crottin; Crottin de Chavignol; Cure Nantais; Damski; Danbo; Danish Blue cheese; Danish Fontina; Danish Port Salut; Dauphin; Delice des Fiouves; Doppelrhamstufel; Dorset Blue; Dovedale; Dreux a la Feuille; Dunbarra; Durrus; Édél de Cléron; Emlett; Époisses de Bourgogne; Esrom; Exmoor Jersey Blue; Explorateur; Feta; Figue; Fin-de-Siecle; Fine Fettle Yorkshire; Finn; Flower Marie; Fontainebleau; Fontina; Fontina Val d'Aosta; Formaggio di capra; Fougerus; Fourme d'Ambert; Fourme de Montbrison; French Neufchâtel; Fresh caciotti; Frinault; Fromage Corse; Fromage de Montagne de Savoie; Fromage Frais; Fromager d'Affinois; Galette Lyonnaise; Galette du Paludier; Galician cheese; Galotyri; Gaperon; Golden cross; Gorgonzola; Graddost; Grand Vatel; Grataron d' Areches; Gratte-Paille; Grevé; Gris de Lille; Gubbeen; Guerbigny; Halloumi; Handkäse; Havarti; Höfðingi; Hushallsost; Innes Button; Italian cheese Bra; Italico; Jerome; Kalathaki Lymnou; Katiki Domokou; Kernhem; Klosterkäse; Lajta; Langres; Lappi; La Vache Qui Rit; L'Aveyronnai; La</p>

Taupiniere; Le Brin; L'Ecir de l'Aubrac; Le Fium Orbo; Le Roule; Liederkranz; Limburger; Lingot Saint Bousquet d'Orb; Little Rydings; Livarot; Loddiswell Avondale; Lymeswold cheese; Lyonnais; Mâconnais; Margotin; Maroilles; Metton Cancoillotte; Milleens; Mine-Gabhar; Mizithra; Mont d'Or; Morbier; Morbier Cru de Montagne; Morski; Mothais a la Feuille; Munster; Munster-géromé; Murazzano; Mycella; Myzithra; Nantais; Neufchâtel; Normana; Norzola; Odenwälder Frühstückskäse; Olivet Cendre; Olivet au Foin; Olomoucké syrečky; Oltermanni; Pannerone Lodigiano; Pantys Gawn; Pate de Fromage; Pave d'Affinois; Pave d'Auge; Pave de Chirac; Pélardon; Pencarreg; Petit; Petit-Suisse; Pichtogalo Chanion; Picodon; Picón-Bejes-Treviso; Pierre; Pont-l'Évêque; Port-Salut; Poivre d'Ane; Pouligny-Saint-Palet de Babligny; Pouligny-Saint-Pierre; Pourly; P'tit Berrichon; Pultost; Pyramide; Quartirolo Lombardo; Quatre-Vents; Queijo Amarelo da Beira Baixa; Queijo de Azeitão; Queijo de Castelo Branco; Queijo Mestiço de Tolosa; Queijo Serpa; Queijo Terrincho; Queso de Murcia; Queso de l'Alt Urgell y la Cerdanya; Queso del Montsec (Cendrat); Queso de Valdéron; Queijo do Pico; Raschera; Rauchkase; Reblochon; Regal de la Dombes; Robbiola di Roccaverano; Rocamadour; Rocamadour goat's cheese; Rochebarron; Rollot; Romadur; Romans Part Dieu; Roquefort; Rouleau De Beaulieu; Rouy; Rubens; S.A.F.R Port Salut; Saga; Saint Agur; Saint-André cheese; Saint-Félicien; Saint-Marcellin; Sainte-Maure de Touraine; Sainte Maure goat's cheese; Saint-Nectaire; Saint Paulin; Scamorza; Selles-sur-Cher goat's cheese; Serra da Estrela Cheese; Sharpam; Sirene; Slovenska bryndza; Snøfrisk; Somerset brie; Sottocenere al Tartufo; Soumaintrain; Sourire Lozerien; Spanish queso de Cabrales; Ste-Maure; Stracchino; St Olof; String cheese; Sussex Slipcote; Taleggio; Tamie; Taupiniere; Telemea; Tetilla; Tilsit cheese; Tomme des Chouans; Tomme de Savoie; Tomme mountain; Torta del Casar; Trois Cornes De Vendee; Tronchon; Truffe; Tymsboro; Urdä; Vacherin cheese; Valençay; Velveeta; Venaco; Vendomois; Vignotte; Vulskombe; Waterloo; Weichkäse; White Stilton; Wigmore; Wilstermarsch.

Names of fresh cheeses

Cottage cheese (farmer's cheese); Mozzarella; Mozzarella di Buffala Campana; Quark; Ricotta.

ANNEX IV – EXAMPLES OF FOOD ADDITIVES (PRESERVATIVES AND ACIDITY REGULATORS)

Table 9. Examples of food preservatives and acidity regulators and their E numbers (EC, 1995)

Code	Name
E200	Sorbic acid
E202	Potassium sorbate
E203	Calcium sorbate
E210	Benzoic acid
E211	Sodium benzoate
E212	Potassium benzoate
E213	Calcium benzoate
E214	Ethyl p-hydroxybenzoate
E215	Sodium ethyl p-hydroxybenzoate
E218	Methyl p-hydroxybenzoate
E219	Sodium methyl p-hydroxybenzoate
E220	Sulphur dioxide
E221	Sodium sulphite
E222	Sodium hydrogen sulphite
E223	Sodium metabisulphite
E224	Potassium metabisulphite
E226	Calcium sulphite
E227	Calcium hydrogen sulphite
E228	Potassium hydrogen sulphite
E230	Biphenyl; diphenyl
E231	Orthophenyl phenol
E232	Sodium orthophenyl phenol
E234	Nisin
E235	Natamycin
E239	Hexamethylene tetramine
E242	Dimethyl dicarbonate
E249	Potassium nitrite
E250	Sodium nitrite
E251	Sodium nitrate
E252	Potassium nitrate
E280	Propionic acid
E281	Sodium propionate
E282	Calcium propionate
E283	Potassium propionate
E284	Boric acid
E285	Sodium tetraborate; borax
E1105	Lysozyme
E260	Acetic acid
E263	Calcium acetate
E270	Lactic acid

E296	Malic acid
E297	Fumaric acid
E325	Sodium lactate
E330	Citric acid
E334	Tartaric acid