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GUIDANCE DOCUMENT

on *Listeria monocytogenes* shelf-life studies for ready-to-eat foods, under Regulation (EC) No 2073/2005 of 15 November 2005 on microbiological criteria for foodstuffs

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PURPOSE OF THIS DOCUMENT

This document is mainly directed at Food Business Operators who produce ready-to-eat foods and conduct *Listeria monocytogenes* shelf-life studies for them in accordance with Article 3(2) and Annex II of Regulation (EC) No 2073/2005 of 15 November 2005 on microbiological criteria for foodstuffs.

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1. INTRODUCTION AND PURPOSE

Listeria monocytogenes may cause disease in humans and it is typically transmitted as a food-borne pathogen. *L. monocytogenes* is frequently present in the environment, in soil, vegetation and faeces of animals. The organism can be found in raw foods such as fresh meat, raw milk and fish. The ubiquitous occurrence and the increased ability to grow or survive in a chilled environment compared to most other microorganisms, makes *L. monocytogenes* a significant challenge in food production. This is especially the case for ready-to-eat (RTE) foods in which *L. monocytogenes* can grow and that will not receive a heat-treatment during production, and for foods that may be contaminated from the environment, including the production environment, during their manufacture.

It is crucial that producers of RTE foods (food intended by the producer for direct human consumption without the need for cooking or other processing effective to eliminate or reduce microorganisms of concern to an acceptable level) take actions to control contamination of *L. monocytogenes*, as well as its growth in the product until the end of shelf-life. Knowledge on, and documentation of the growth potential in a food product is needed, and must be taken into account when the producer sets the safe shelf-life for the product.

This document is intended for the producers of RTE foods. The document aims to guide RTE producers in identifying the *L. monocytogenes* risk in their RTE foods and to provide general principles for the decision on when and which shelf-life studies are needed. The document may also be used by the competent authorities verifying the implementation of shelf-life studies.

The main objective of this document is to guide food business operators (FBO) producing RTE foods:

- to demonstrate to the satisfaction of the competent authority that the products will comply with the Community Regulation until the end of the shelf-life,
- to understand the range of different approaches available to help establish a safe product shelf-life in relation to *L. monocytogenes* and to decide the appropriate approach for their products and
- to classify their products into RTE foods in which growth of *L. monocytogenes* can occur or in RTE foods in which growth of *L. monocytogenes* will not occur during their shelf-life.

This document is not intended to be prescriptive in technical details. The EU Community Reference Laboratory (CRL) for *Listeria monocytogenes*¹ has prepared a separate technical guidance document² for laboratories conducting shelf-life studies, especially durability studies and challenge tests.

¹ Agence Française de sécurité sanitaire des aliments, Laboratoire d'Etudes et de Recherches sur la Qualité des Aliments et sur les Procédés Agro-alimentaires, Maisons-Alfort, France

² Technical guidance document on shelf-life studies for *Listeria monocytogenes* in ready-to-eat foods, Community reference laboratory for *Listeria monocytogenes*.

2. EU LEGISLATION AND EXISTING NATIONAL OR INDUSTRY GUIDANCE DOCUMENTS

2.1. Microbiological food safety criteria for *Listeria monocytogenes*

The EU has established microbiological food safety criteria for *L. monocytogenes* in RTE foods in Regulation (EC) No 2073/2005³ of 15 November 2005 on microbiological criteria for foodstuffs.

Article 3 of Regulation (EC) No 2073/2005 indicates that Food Business Operators (FBO) shall ensure that foodstuffs comply with the relevant microbiological criteria and limits set out in the Regulation. Furthermore, Article 3 refers to the shelf-life studies (listed in Annex II of the Regulation), that the FBO shall conduct in order to investigate compliance with the criteria throughout the shelf-life. In particular, this applies to RTE foods that are able to support the growth of *L. monocytogenes* and that may pose a *L. monocytogenes* risk for public health.

The specific food safety criteria for *L. monocytogenes* in RTE foods are laid down in Annex I of the Regulation. Annex I of the Regulation specifies the food category, sampling plan, microbiological limits, analytical methods and stage where the criterion applies. Food safety criteria define the acceptability of a product or a batch of foodstuff applicable to products placed on the market. When testing against food safety criteria provides unsatisfactory results, the product or batch of the foodstuffs shall be withdrawn or recalled from the market. Furthermore, corrective actions at the production plant according to the hazard analysis of critical control point (HACCP) plan shall be taken.

Annex II of the Regulation describes the shelf-life studies that the FBO shall conduct, as necessary, in order to investigate compliance with the criteria throughout the shelf-life. These shelf-life studies shall always include:

- specifications of physico-chemical characteristics of the product (such as pH, a_w , salt content, concentration of preservatives and the type of packaging system) taking into account the processing steps and conditions, storage and the possibilities for contamination and the foreseen shelf-life, and
- consultation of the available scientific literature and research data regarding the survival and growth characteristics.

When the studies mentioned above are not able to give the necessary confidence in relation to the safety of the product, the FBO should conduct additional studies. These additional studies should take into account the inherent variability linked to the product and the processing and storage conditions. These studies may include:

- predictive microbiological (mathematical) modelling established for the food in question, using critical survival or growth characteristics for the micro-organisms of concern in the product, and/or
- studies to evaluate the growth or survival of the micro-organisms of concern that may be present in the product during the shelf-life under reasonably

³ OJ L 338, 22.12.2005, p. 1.

foreseeable conditions of distribution, storage and use (referred as durability studies or adequate historical data), and/or

- tests to investigate the ability of the appropriately inoculated micro-organism of concern to grow or survive in the product under different reasonably foreseeable storage conditions (referred as challenge tests).

2.2. Microbiological criteria in relation to the hygiene control measures and HACCP

The main purpose of the Community food law is to guarantee a high level of public health protection. To achieve this fundamental objective, the Community food legislation⁴ lays down general food safety requirements. These requirements, which are based on a preventive approach, include the implementation of hygiene control measures and HACCP-based procedures⁵ by FBO at any stage of the food production chain.

As microbiological hazards in foodstuffs are one of the most important sources of food-borne diseases in humans, the Community legislation states that, when implementing or adopting these hygienic procedures and measures, the food must comply with the relevant microbiological criteria (see the previous chapter).

The Regulation stipulates that for the microbiological criteria, the FBO must establish bacteriological sampling and testing programs, and that these must form an integral part of the implementation of their procedures based on the good hygiene practices (GHP) and HACCP-principles. The sampling frequencies (except where the Regulation stipulates specific minimal sampling requirements) must be based on their own risk analysis, be in accordance with the nature and the size of the food businesses and take into consideration other aspects such as characteristics of the raw materials, end-product, production process.

As food safety cannot only be based on the end-product testing (no sampling plan can ensure the absence of a particular micro-organism), the application of food safety criteria is considered one of the several management options to ensure that food produced is safe. The application of GHP in combination with HACCP should be consistently applied to control the required microbiological status of raw materials, to minimise the initial contamination at manufacturing level, and/or to reduce the potential growth of the micro-organisms. Testing against a food safety criterion after production should be only used as a method of verifying the production process and thus the correct functioning of the GHP and HACCP-based procedures.

Microbiological criteria are normally not suitable for monitoring the critical limits as defined in HACCP. Monitoring procedures must be able to detect loss of control at critical points, and should provide this information in time for corrective actions to

⁴ 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).

⁵ Articles 4 and 5 of Regulation (EC) No 853/2004 of the European Parliament and the Council of 29 April 2004 on the hygiene of foodstuffs (OJ L 226, 25.6.2004, p.3)

be taken and to regain control. Therefore, the measurement of physical and chemical parameters (such as time/temperature profiles, pH and a_w), which can be done in real time at the production plant, should be used instead of testing against microbiological criteria.

2.3. Relationship between this guidance document and other established guidance

This document is not intended to be prescriptive and does not describe in detail how to conduct each of the shelf-life studies described for a particular food product. A separate technical guidance document² for laboratories conducting shelf-life studies, especially durability studies and challenge tests, has been prepared by the EU Community Reference Laboratory (CRL) for *Listeria monocytogenes*.

The general overview provided in this document may be complemented with some more detailed shelf-life guidance documents developed by some institutes, national authorities and by the food industry and documents which may be developed in future. These guidance documents give detailed information on how to determine the product shelf-life. A reference list to some of the guidance documents can be found in Annex 5.5. This document does not supersede existing industry guidelines.

If relevant technical in-house expertise is not available, FBO should seek assistance from suitably qualified and trained personnel to ensure shelf-life studies are implemented appropriately. Laboratory shelf-life studies should be carried out in laboratories having the required expertise for such studies and demonstrating good laboratory practices.

3. PRINCIPLES AND PROCEDURES FOR SHELF-LIFE STUDIES

3.1. General

The determination of the length of the shelf life is very important for the microbial safety of RTE foods, especially of foods in which growth of *L. monocytogenes* can occur. The shelf-life is defined as a period of time for which a product remains safe and meets its quality specifications under expected storage and use. The shelf-life determines the durability date and is expressed as a "use by" or "best before" date in a product as described in Articles 9 and 10 of Directive 2000/13/EC⁶.

Shelf-life studies and review of the HACCP plan should be carried out in the following circumstances:

- new or modified product development,
- new process development or modification,
- new packaging development,
- any significant change of ingredient/s or packaging to an existing product,
- changes in the production site or production equipment, or
- no shelf-life studies have been performed previously.

The FBO is responsible for setting the shelf-life under defined conditions, which should take into account reasonably foreseen conditions of distribution, storage and use. An important part of these foreseen conditions is the storage temperature during the entire shelf-life and therefore the decision on which temperature or temperatures is used for the shelf-life setting must be justified. As a rule, if too low a storage temperature used to establish the shelf-life compared to actual temperatures during distribution and use, this may lead to the underestimation of the growth of microbes, including *L. monocytogenes*, and therefore to the overestimation of the safe shelf-life length. If the actual storage temperatures are not known for the product in question, the FBO may use e.g. 8-12 °C for the storage temperature for the shelf-life studies. However, the FBO must justify which temperatures are used for the shelf-life setting, taking into account the data from temperatures during distribution and storage by consumers.

In practice setting the shelf-life is considered part of the manufacturer's HACCP system and takes account of controls on suppliers assuring raw material quality and trend results of raw material monitoring, confidence in GHP controls applied in the manufacturing environment as reflected by results of sampling from processing areas and equipment, experience from the manufacture of similar products, the rate of microbiological spoilage and maintenance of organoleptic quality under foreseen conditions of storage and use.

⁶ OJ L 109, 6.5.2000, p. 29-42.

Shelf-life duration is integral to product safety and identification of the relevant pathogens, including *L. monocytogenes*, in the raw materials and production environment is critical for the successful assessment of a safe shelf-life. It is important to remember that deviations from normal conditions, such as high level of initial contamination of raw materials, too high temperatures during storage or transport or too long a shelf-life could have a significant impact on the safety of the product.

The purpose of the *L. monocytogenes* shelf-life studies is to demonstrate the compliance of the RTE food with the limit of the food safety criterion set for *L. monocytogenes* throughout its shelf-life. The determination of the microbiological shelf-life of foodstuffs shall always include the consideration of the different factors such as: food sector, type of product and type of process. The inherent variability of manufactured batches and the variability linked to *L. monocytogenes* species shall also be taken into account, as well as all the reasonably foreseeable conditions during the distribution, storage and use, included those applied by the consumer.

Demonstration of compliance and shelf-life studies can be done in several ways, beginning with the comparison of the product characteristics with the available scientific literature.

If the comparison of product characteristics with the available scientific or other data, is not able to provide enough data to support the shelf-life assessment, further studies are needed. This may include predictive microbiology, use of adequate historical data or special laboratory studies, such as durability or challenge tests. Each one of these tools has advantages and disadvantages and when necessary different tools can be combined.

FBO may collaborate with each other and seek advice from various food laboratories (e.g. research institutes or reference laboratories) when they conduct these shelf-life studies.

The following decision tree (Figure 1) shows a schematic approach for the steps for shelf-life studies. The decision tree also gives the FBO an indication of when additional specific studies (e.g. durability and challenge tests) are needed in order to investigate the (potential) growth of *L. monocytogenes* in the product. More information related to the decision tree can be found in Annex 5.2.

Ongoing monitoring and verification of the shelf-life is necessary to confirm maintenance of the defined shelf-life for each product.

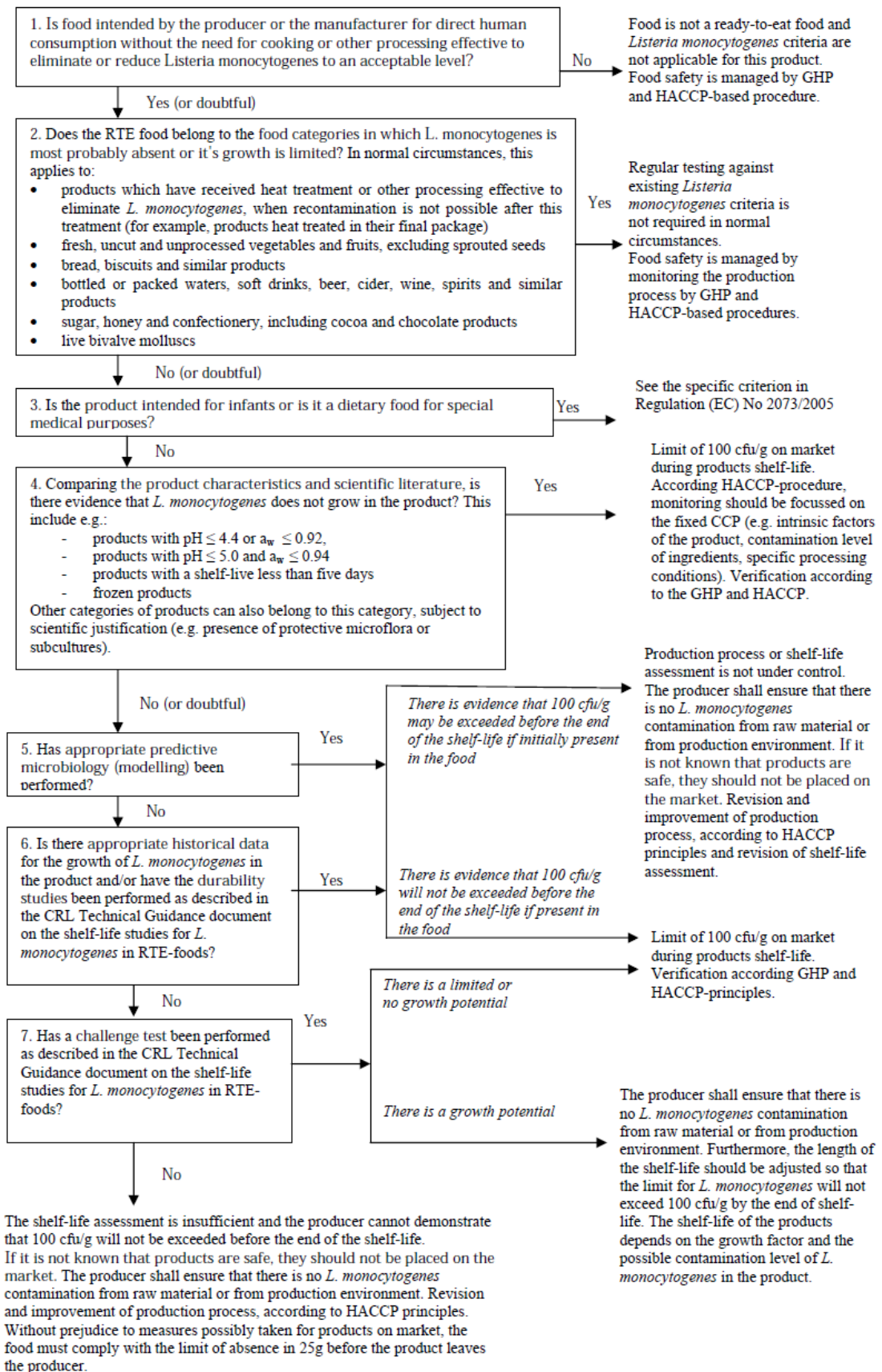


Figure 1. A decision tree showing schematically the steps for shelf-life studies.

3.2. Product characteristics and scientific literature

3.2.1. Product characteristics

When determining the shelf-life of a RTE food, it is important to consider whether the food is capable of supporting the survival or growth of *L. monocytogenes*. The survival and growth of *L. monocytogenes* in RTE foods is a function of the characteristics of the RTE food and the conditions, under which that RTE food is produced, packaged and stored. These characteristics are sometimes referred to as the intrinsic and extrinsic properties of RTE food.

The most important product characteristics influencing the survival and growth of *L. monocytogenes* in RTE foods are its pH, water activity (a_w) and the temperature and time under which the food is stored. Furthermore, the preservatives and protective microflora, including the possible starter cultures, may have a significant impact on the survival and growth of *L. monocytogenes* in the product.

By knowing the characteristics (e.g. pH, a_w , storage temperature) of a RTE food, the FBO can determine if there is a possibility that *L. monocytogenes* can survive or grow in a particular RTE food. This information may also allow food businesses to reformulate their products to prevent or minimise the survival or growth of *L. monocytogenes*.

3.2.2. Scientific literature

A wide resource of data on *L. monocytogenes* and shelf-life is available from various books, scientific journals and universities or technical institutions. In addition, many National, European (e.g. the European Food Safety Authority) and international bodies have data available.

When a FBO has established the characteristics (e.g. pH, a_w , storage temperature) of his RTE food and the conditions, under which that RTE food is produced, packaged and stored, this information should be used to compare the product with existing data on the survival and growth of *L. monocytogenes* in scientific literature. Some of the limits for the survival and growth factors of *L. monocytogenes* are given in Table 1. Other factors or combination of various factors may also be relevant, subject to scientific justification.

L. monocytogenes has specific characteristics that increase its importance as a food-borne organism. It is able to grow at 0 °C, and may thus grow well in refrigerated foods. It is able to survive harsh environments, drying and salting. Furthermore, *L. monocytogenes* is able to grow at low oxygen concentrations, and even without available oxygen, giving the organism an advantage in vacuum-packed foods.

Table 1. Selected factors having impact on the growth and survival of *L. monocytogenes*^a

Factor	Can Grow ^b			Can Survive (But No Growth) ^c
	Lower growth limit	Optimum	Upper growth limit	
Temperature (°C)	-1.5 to +3.0	30.0 to 37.0	45.0	-18.0
pH ^d	4.2 to 4.3	7.0	9.4 to 9.5	3.3 to 4.2
Water Activity (a _w)	0.90 to 0.93	0.99	> 0.99	< 0.90
Salt Concentration (%) ^e	< 0.5	0.7	12 – 16	≥ 20
Atmosphere	Facultative anaerobe (it can grow in the presence or absence of oxygen, e.g. in a vacuum or modified atmosphere package)			
Heat treatment during food processing	A temperature/time combination e.g. of 70 °C and 2 min is required for a D-6 (i.e. 10 ⁶ or 6 decimal) reduction in numbers of <i>L. monocytogenes</i> cells. Other temperature/time combinations may also provide the same reduction.			

^a The limits for growth and survival of *L. monocytogenes* presented in this table are based on research carried out primarily in laboratory media under optimum conditions and should only be used as estimates for the impact in foods.

^b Optimum indicates when the growth of *L. monocytogenes* is fastest.

^c Survival period will vary depending on nature of food and other factors.

^d Inhibition of *L. monocytogenes* is dependent on type of acid present.

^e Based on percent sodium chloride, water phase.

3.3. Historical data

Historical data is a component of records which a food business keeps as a part of its ongoing business. Some of this data will be recorded by the FBO as part of its legal obligations under the food safety legislation, such as traceability, HACCP and own-checking plans, including raw material quality, sampling from processing areas and equipment (to demonstrate the efficacy of factory hygiene and cleaning regimes) and product testing, particularly on the day of production and at the end of the shelf-life (to verify effective functioning of the HACCP system and for durability verification respectively). Historical data are useful in determining the shelf-life of RTE foods for the following reasons:

- Historical data will indicate levels of *L. monocytogenes* found in the production environment, raw materials and existing RTE foods, under the businesses current practices of GHP and HACCP,
- Historical data on levels of *L. monocytogenes* in existing RTE foods at the start and end of shelf life can be used to assess its growth potential in similar RTE foods with comparable intrinsic characteristics (pH, a_w, microflora, etc.) produced under practically identical conditions,
- Historical data on levels of *L. monocytogenes* in existing RTE foods at the start and end of shelf life is also widely used in practice to verify product durability and confirm that the allocated shelf-life remains appropriate when stored, handled and used as reasonably intended, and

- Historical data generated over a period of time for comparable RTE foods (as above) and which continues to be generated on an on-going basis can be used for trend analysis. Where levels of *L. monocytogenes* in RTE foods at the end of shelf-life are consistently low or absent and no results have been obtained which exceed 100 cfu/g, such data can be used in combination with data from sampling of processing areas and equipment, and on quality of raw materials to give a sufficient level of confidence that such RTE foods will not pose a risk to public health. The level of confidence increases with the amount of data available. The more product units that are tested the more reliable the historical data becomes.

FBO shall satisfy the Competent Authority (CA) that their historical data is sufficient to demonstrate the limit of 100 cfu/g will not be exceeded during the shelf-life. The CA may require this data to be complemented with further studies, e.g. with laboratory durability studies.

3.4. Predictive microbiology (modelling)

3.4.1. General

Predictive microbiology (modelling) aims to predict the behaviour of micro-organisms in foods during their manufacturing or storage. In recent years, significant advances have been made in the field of predictive microbiology especially for estimating the growth of *L. monocytogenes* in foods.

There are data and models available in the literature and these models have been implemented in some user-friendly software. Some of the models have been developed to predict the microbial behaviour when the physico-chemical characteristics of the food (e.g., pH, water activity, organic acids concentrations) and the storage temperature are known. Some other models have been developed to predict the behaviour of micro-organisms in particular foods whatever their storage conditions might be.

Some models are based on data obtained from liquid microbiological media and are used to describe the possible impact of several factors. Some of these models can fail to accurately describe the microbial behaviour in foods, although the more robust models of this type have been validated in foods. The food-based models can effectively describe the impact of storage conditions on a specific food but their ability to describe the impact of the variability of physico-chemical characteristics of the food or to make predictions in others foods is questionable. Some intermediate approaches have also been developed trying to overcome the limitations of these two major approaches.

In spite of the limitations, predictive models remain valuable tools for estimating the growth of *L. monocytogenes* in foods, if the limitations are known. Growth/no growth models predicting the growth probability of *L. monocytogenes* in foods can help FBO to categorise their foods.

Models predicting microbial lag times and growth rates in foods can help FBO to evaluate the growth of *L. monocytogenes* in foods during their storage taking into

account the strain variability, inherent processing and variability in foods and storage conditions.

Predictive microbiological models must be used with caution and only used by trained and experienced personnel with an understanding of the limitations and the conditions of the use. An example of a predictive microbiological modelling is given in Annex 5.3.

3.4.2. *Practical application of predictive microbiology*

Predictive microbiology may be useful for the following applications:

- to predict bacterial growth in various conditions,
- to predict the growth probability of micro-organisms in foods,
- to estimate the contamination level at a given day of the shelf-life,
- to test the variability between 2 batches,
- to optimise formulation (additives, pH, salt) to assure the best stability,
- to evaluate the impact of cold chain breaks, and to test different storage scenarios, and
- to help to identify Critical Control Points in a process.

Some widely recognized and commonly used freely available models are listed below, although this is not intended to be an exhaustive list:

- Growth Predictor
Freely available from the Institute of Food Research, UK (www.Ifr.ac.uk/safety/growthpredictor),
- Pathogen Modelling Programme
Freely available from the United States Department of Agriculture, Agriculture Research Service (<http://ars.usda.gov/Services/docs.htm?docid=6786>).

Other software is commercially available. The calculations and illustrations in Annexes 5.3 and 5.4 are done by commercial software.

3.5. Specific laboratory shelf-life studies to investigate the compliance with the criteria for *Listeria monocytogenes* in ready-to-eat foods throughout the shelf-life

3.5.1. General

This section describes microbiological procedures for determining the growth of *L. monocytogenes* using durability studies and challenge tests (Figure 2.). The challenge tests can further be divided into two categories: the assessment of the growth potential (δ) and the assessment of the maximum growth rate (μ_{\max}).

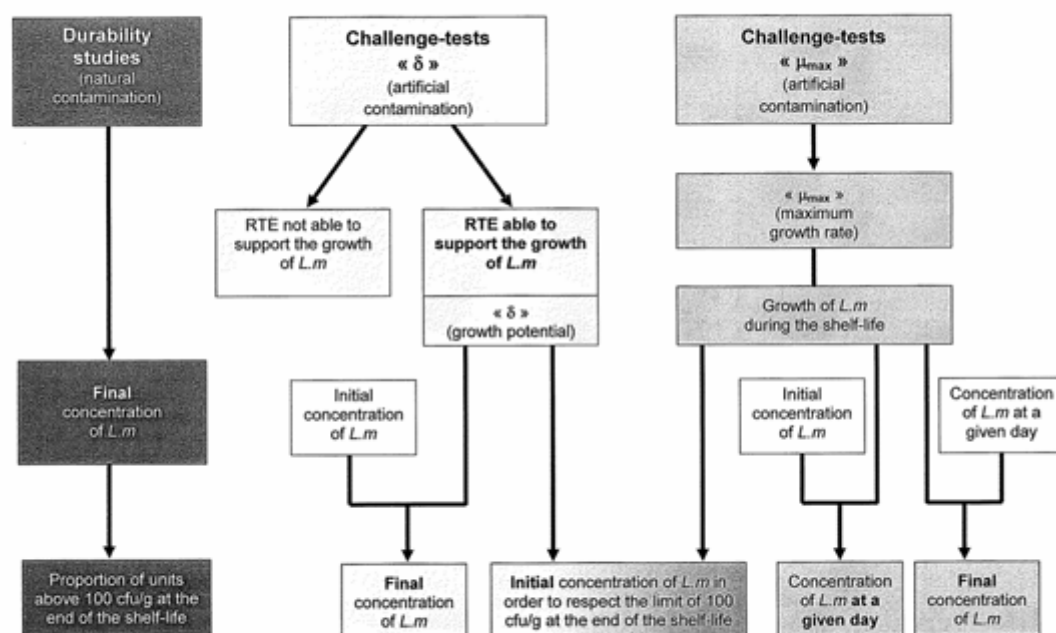


Figure 2. Description of the durability and challenge tests.

More details related to the methodology and to the calculation of the results are available in the “Technical guidance document on shelf-life studies for *Listeria monocytogenes* in ready-to-eat foods” (CRL *Listeria monocytogenes*).

3.5.2. Durability studies

3.5.2.1. Purpose and limitations of durability studies

Durability studies allow evaluation of the growth of *L. monocytogenes* in a naturally contaminated food during its storage under reasonably foreseeable conditions.

The durability studies are more realistic than challenge tests, as the contamination is natural i.e. the strain or strains, injury or stress, distribution and initial concentration of *L. monocytogenes*.

The interpretation of the results of the durability studies may be difficult as there is likely to be a relatively low prevalence of food product units contaminated with *L. monocytogenes*, very low numbers of *L. monocytogenes* often initially present and heterogeneity of the *L. monocytogenes* distribution in the food. Therefore, the use of various other tools, such as challenge tests, may be needed.

3.5.2.2. Interpretation of the results of durability studies

A history of the durability studies conducted for the same product under the same process, representative of the variability of the manufacturing conditions, will allow the levels of *L. monocytogenes* in the food to be evaluated at the end of the test. It may be used to assess the proportion (with its associated confidence interval) of units (commercial units) exceeding the limit value 100 cfu/g at the end of the shelf-life, after a storage period reflecting the foreseeable conditions of distribution and storage. The level of confidence increases with the amount of data available. The more product units that are tested the more reliable the shelf-life study becomes.

3.5.3. Challenge tests

3.5.3.1. Purpose and limitations of challenge tests

Challenge tests aim to provide information on the behaviour of *L. monocytogenes* artificially inoculated in a food before storage under given conditions. These tests can be implemented for two different purposes: to assess the growth potential or to estimate the growth parameters (e.g. maximum growth rate).

Challenge tests may take into account the variability of the foods (by using different batches), the specific contamination of the food (by inoculating strains isolated from the food), although the level of contamination, the heterogeneity of the contamination and physiological state of the bacteria are difficult to mimic.

- Microbiological challenge tests assessing growth potential (δ) allow:
 - classification of foods into “RTE foods able to support the growth of *L. monocytogenes* other than those intended for infants and for special medical purposes” or “RTE foods unable to support the growth of *L. monocytogenes* other than those intended for infants and for special medical purposes”, and
 - quantification of the behaviour of *L. monocytogenes* in a food according to defined reasonably foreseeable conditions between production and consumption (i.e. calculating the concentration at the end of the shelf-life from the initial concentration, or determining the

concentration at the beginning of the shelf-life in order to comply with the limit of 100 cfu/g at the end of the shelf-life).

- Microbiological challenge tests assessing maximum growth rate (μ_{\max}) allow:
 - determination of the concentration of *L. monocytogenes* at a given day of the shelf-life if the initial concentration is known, and
 - determination of the maximum concentration of *L. monocytogenes* that may be present at the production stage in order to comply with the limit of 100 cfu/g at the end of the shelf-life.

3.5.3.2. Interpretation of the results of challenge tests

Challenge tests assessing growth potential

A microbiological challenge test assessing growth potential (δ) is a laboratory based study that measures the growth of *L. monocytogenes* in an artificially contaminated food stored under foreseeable conditions of transportation, distribution and storage until consumption.

The growth potential (δ) is the difference between the \log_{10} cfu/g at the end of the test and the \log_{10} cfu/g at the beginning of the test. The δ depends on many factors, the most important being:

- the inoculated strain(s),
- injury or stress applied to the inoculated strain(s),
- intrinsic properties of the food (e.g. pH, NaCl content, a_w , nutritional content, associated microflora, antimicrobial constituents) and
- extrinsic properties (e.g. temperature profile, gas atmosphere).

Details relating to the calculation of the results are available in the CRL Technical Guidance Document.

With a known initial level of contamination with *L. monocytogenes*, the growth potential allows the final concentration for an identical food, an identical strain and the same storage conditions to be estimated. The growth potential can also be used to calculate the initial concentration of the food that would allow the food to comply with the 100 cfu/g limit at the end of the shelf-life.

Challenge tests assessing the maximum growth rate

A microbiological challenge test assessing the maximum growth rate (μ_{\max}) is a laboratory based study that measures the rate of growth of *L. monocytogenes* in an artificially contaminated food stored under foreseeable conditions at a fixed temperature.

For the exponential growth, plotting the natural logarithm of cell number against time produces a straight line. The slope of this line is the maximum growth rate

(μ_{\max}) of the bacteria. The maximum growth rate is an important parameter of the growth curve which depends on:

- the inoculated strain(s),
- intrinsic properties of the food (e.g. pH, NaCl content, a_w , nutritional content, associated microflora, antimicrobial constituents) and
- extrinsic properties (e.g. temperature profile, gas atmosphere).

Interpretation of challenge tests is explained in greater detail in the CRL Technical Guidance Document.

3.6. Shelf-life evaluation combining the different tools available

Two different approaches may be differentiated: a single case approach and a risk based approach (Figure 3).

The single case approach considers that, in case of contamination; the food initially contains a given number of bacterial cells, that this food has fixed characteristics and is stored under static conditions. Usually, this approach provides limited and inadequate information because these conditions do not take into account the natural variability of the parameters likely to have an impact on the contamination at the end of the shelf-life. Indeed, contamination of *L. monocytogenes* can have different evolutions according to the:

- initial contamination level (high or low initial concentration),
- physiological state of contaminating cells (bacteriological stress leading to more or less long lag time),
- the growth capacity of the bacterial strain contaminating the food,
- food characteristics (between and within-batch variability of pH, water activity), and
- the storage conditions from distribution to domestic refrigerator.

This approach can conduct to set a shelf-life:

- excessively short in a “worst case scenario”, which means that the study assumptions are too cautious (highest contaminations, fastest growing strains, absence of lag time, more growth-favourable food, highest storage temperatures), or
- alternatively, unsafely long if, e.g. all the reasonably foreseeable conditions of distribution, storage and use are not taken into account.

Nevertheless, this approach can be sufficient to demonstrate in a “worst case scenario” that the food will respect the limit at the end of the shelf-life. The FBO can thus estimate the maximum final contamination at the end of the shelf-life when the initial contamination is maximal, with a maximum growth potential and for the worst storage conditions.

If the estimated maximum contamination does not exceed the limit, the shelf-life can be considered safe. On the other hand, if this estimated maximum contamination exceeds the limit, the shelf-life must be shortened or the FBO must assess the probability of exceeding the limit for the considered shelf-life and evaluate if this probability is acceptable or not. In this case, a risk based approach must be used to estimate the distribution of the contamination at the end of the shelf-life for all the reasonably foreseeable conditions. The FBO should also consider improving the hygienic conditions of the premises and/or the microbiological status of the ingredients together with the reassessment of the shelf-life.

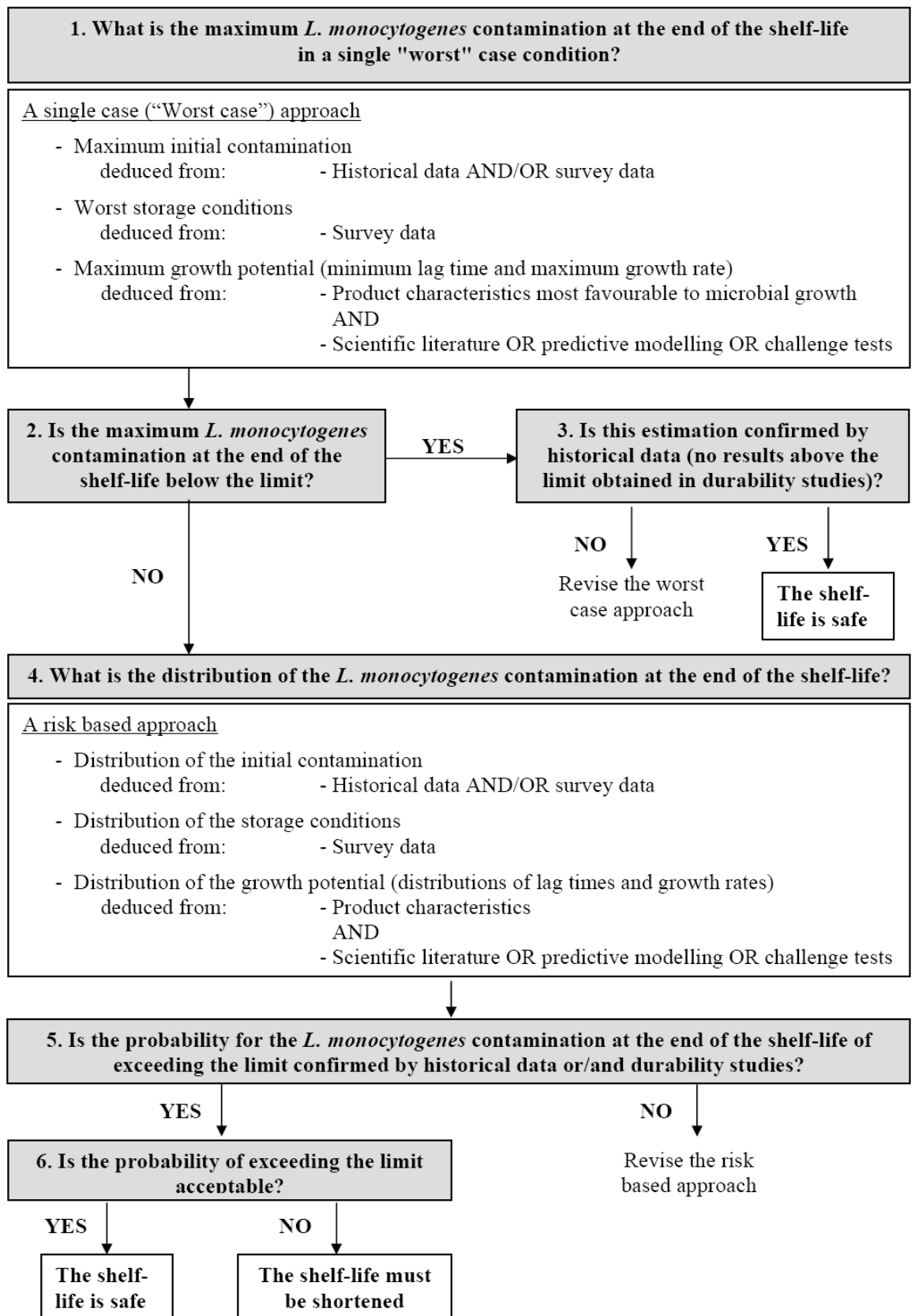


Figure 3. A decision tree describing the combination method for shelf-life decision (various examples are given in Annex 5.4.).

3.7. Collaboration between food businesses

As necessary, the FBO shall conduct studies (determination of the physico-chemical characteristics of the product, predictive mathematical modelling, historical data, durability tests or challenge tests) to investigate compliance with the criteria throughout the shelf-life. FBO may collaborate in conducting these studies. Notwithstanding this collaboration, it is important that the FBO takes account of the environment in each individual plant of manufacturer.

The FBO manufacturing similar products in similar conditions may use the results of the same studies. However, the use of the same study or studies for products produced in different food plants requires following aspects to be taken into account:

- The products should have the same characteristics (pH, a_w , salt content, concentration of preservatives, type of packaging, associated microflora or any other characteristic important for the survival and growth of *L. monocytogenes*) for these studies to be valid for the products. If one or several characteristics are different the studies cannot be used without evaluating the effect of the different characteristics on the survival and growth of *L. monocytogenes*,
- The product recipe should be same and if not the ingredients should be evaluated for their effects on the growth of *L. monocytogenes*,
- The production process of the products should be similar. The process steps should be compared in detail and the effect of the survival and growth of any differences in the processes should be evaluated. The studies shall take into account the inherent variability linked to the product,
- The storage conditions and the shelf-life should be similar, and if not, the differences should be evaluated for their effects on the growth of *L. monocytogenes*, and
- Associated microflora (starters) should be identical, and if not, have the same effect on *L. monocytogenes*.

The FBO should demonstrate to the competent authority that the products and the processing of the products are similar, and if the products are not similar, the FBO should be able to show how they are different and what effect those differences have on the survival and growth of *L. monocytogenes*. The FBO can use available scientific literature and research data as consultation.

4. DOCUMENTATION OF THE SHELF-LIFE STUDIES

The FBO should keep documentation of the shelf-life studies and their verification as a part of the GHP and HACCP procedures. The documentation should include all necessary data (product characteristics, scientific literature used and types and results of other shelf-life studies) which has been used for the shelf-life determination.

It is essential that the documentation is readily available e.g. in order that the FBO is able to demonstrate to the satisfaction of the competent authority that his products will comply with the Community Regulation until the end of the shelf-life. The format of the documentation can be decided by the FBO.

5. ANNEXES

5.1. Definitions

Batch

A group or set of identifiable products obtained from a given process under practically identical circumstances in a given place within one defined production period.

Food safety criterion

A criterion defining the acceptability of a product or a batch of foodstuff applicable to products placed on the market.

Good Hygiene Practises (GHP)

Compliance with all legal requirements and obligations and application of hygiene rules based on scientific knowledge in order to obtain safe food during the food production process and when food is placed on the market.

Hazard Analysis of Critical Control Points (HACCP)

A system which identifies, evaluates, and controls hazards which are significant for food safety. A Critical control point is a step at which control can be applied and is essential to prevent or eliminate a food safety hazard or reduce it to an acceptable level.

pH

A measure of the acidity or alkalinity of a food. The pH 7 is defined as neutral. Values of a pH less than seven are considered acidic and those with greater than seven are considered basic (alkaline).

Ready to eat (RTE) 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 an acceptable level microorganisms of concern.

Shelf life

Either period corresponding to the period preceding the 'use by' or the minimum durability date, as defined respectively in Articles 9 and 10 of Directive 2000/13/EC concerning, among others, the labelling of foodstuffs.

Water activity (a_w)

The term refers to the unbound and available water in a food and is not the same as the water content of the food. Water in food which is not bound to other molecules can support the growth of microbes. The water activity scale extends from 0 to 1.0 (pure water) but most foods have a water activity level in the range of 0.2 for very dry foods to 0.99 for moist fresh foods.

Validation

A process or a study of proving that a method or a process is acceptable for its intended purpose, also obtaining evidence that the elements of the HACCP plan are effective.

Verification

Demonstration by an experiment that an established method or process functions in the user's hands according to the specifications of the method or process determined in the validation study. Also the application of methods, procedures, tests and other evaluations, in addition to monitoring to determine compliance with the HACCP plan.

Day 0

Date of production or packaging.

End of the shelf-life

Last day of the shelf-life as defined by FBO and expressed at a product by "use by" or "best before" date.

5.2. Examples for the necessary steps for decision of the shelf-life studies

Questions below are related to the Figure 1 in the main text of this document.

Question 1:

The first question to be answered by the FBO is whether there is evidence that the product will be cooked or will be processed in a way that is effective to eliminate or reduce *L. monocytogenes* to an acceptable level before consumption. In this case, no specific criterion regarding *L. monocytogenes* is applicable to the food, as the food is not to be considered as RTE food. Food safety should be managed by implementing GHP and HACCP-based procedures, which should include the control of the microbiological status of the raw material, minimising the initial contamination at manufacturing level, control of the production process, etc.

Question 2:

The second question to be answered by the FBO is whether there is evidence that *L. monocytogenes* is likely to be absent from the food or its growth is limited. In normal circumstances, and according to footnote 4 of Annex I of Regulation (EC) No 2073/2005, the following RTE foods can be included in this group:

- products that received heat treatment or other processing effective measures to eliminate *L. monocytogenes*, and recontamination is not possible after this treatment (for example, 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 and
- live bivalve molluscs.

For these products, testing against *L. monocytogenes* is not required in normal circumstances. Food safety is managed by monitoring the production process at CCP's (for example the heat treatment). Testing against *L. monocytogenes* at the end of the shelf-life may be used as verification of the efficacy of the HACCP-plan.

Question 3:

When producing or handling products intended for infants or dietary food for special medical purposes, a specific criterion for *L. monocytogenes* (absence in 25 g, n=10, c=0) should be applied.

Question 4:

If the FBO has scientific evidence that *L. monocytogenes* does not grow in the product, limit of 100 cfu/g *L. monocytogenes* should be applied when the product is on the market.

According to footnote 8 of Annex I of Regulation (EC) No 2073/2005, the following products could be directly included in this group:

- products with $\text{pH} \leq 4.4$ or $a_w \leq 0.92$,
- products with $\text{pH} \leq 5.0$ and $a_w \leq 0.94$,
- products with a shelf-life of less than five days,
- frozen products,
- other products based on the scientific justification.

Also products as mentioned in footnote 4 of Regulation are considered unable to support the growth of *L. monocytogenes* (see question 2).

Other categories of products can be included in this group subject to scientific evidence.

According to HACCP-procedures, food safety should be managed by monitoring at fixed CCP's (e.g. monitoring of the intrinsic factors of the product, such as a_w and pH). Control of the initial level of contamination of the raw materials and ingredients and GHP (cross contamination, etc) has to guarantee that the level of *L. monocytogenes* is 100 cfu/g during the shelf life of the product.

Questions 5 and 6:

When the growth of *L. monocytogenes* in the product cannot be excluded by scientific justification or as stated in footnotes 4 and 8 in Regulation, the FBO shall conduct specific studies to investigate compliance with the criteria throughout the shelf life using historical data, predictive modelling, durability tests or challenge tests.

When these studies have been performed as described earlier in the document and there is adequate data that food will not contain *L. monocytogenes* above 100 cfu/g at the end of the shelf life, the FBO is able to demonstrate compliance with the limit of 100 cfu/g. Food safety should be managed by applying GHP and by implementing and monitoring the appropriate CCP's, and controlling the initial microbiological contamination level of the raw materials and ingredients. Testing against *L. monocytogenes* should be used as verification of GHP and HACCP-based procedures.

When there is data indicating that the limit of 100 cfu/g is likely to be exceeded at the end of the shelf-life, the FBO cannot demonstrate compliance with Regulation and according to the HACCP-principles, the production process and the original shelf-life determination must be revised and improved. This should include

controlling the microbiological quality of the raw materials and ingredients, reducing of the potential growth of *L. monocytogenes*, the adjustment of the intrinsic factors of the end product, additional heat treatment, etc.

Question 7:

When a challenge test has been conducted as described in the Technical Guidance Document and no growth potential have been found during the estimate shelf life, limit of 100 cfu/g should be applied for this product. Food safety should be managed by implementing GHP and HACCP-based procedures. The testing against *L. monocytogenes* should be used as verification of the efficacy of the control of the CCP's.

When the challenge test has shown that there is a potential for the growth of *L. monocytogenes* as described in the Technical Guidance Document, the FBO must adjust the shelf-life to guarantee compliance with the limit of 100 cfu/g during the shelf life of the product. The testing for *L. monocytogenes* against the criterion should be used as verification of the GHP and HACCP-based procedures.

When no information about the product and the possible growth of *L. monocytogenes* in the product is available, compliance with the *L. monocytogenes* criteria cannot be guaranteed and thus, the safety of the food. In this case the production process, including the requirements for the raw materials, ingredients, etc. has to be revised and improved according to the HACCP-principles. The FBO must comply with the limit of absence in 25g before the product leaves the producer.

5.3. Example on the use of predictive microbiology

From the growth parameters of *L. monocytogenes* observed in a food at a given storage temperature, the growth parameters for other storage temperatures can be predicted. For example, from a growth rate of 0.17 log₁₀ cfu/g per day and a lag time of 3.1 days obtained in a food at 8 °C, we can predict the growth parameters in the same food at 4 °C, 6 °C, and 10 °C.

- at 4 °C, the growth rate will be 0.06 log₁₀ cfu/g per day and the lag time 8.8 days,
- at 6 °C, the growth rate will be 0.11 log₁₀ cfu/g per day and the lag time 4.8 days,
- at 10 °C, the growth rate will be 0.25 log₁₀ cfu/g per day and the lag time 2.1 days.

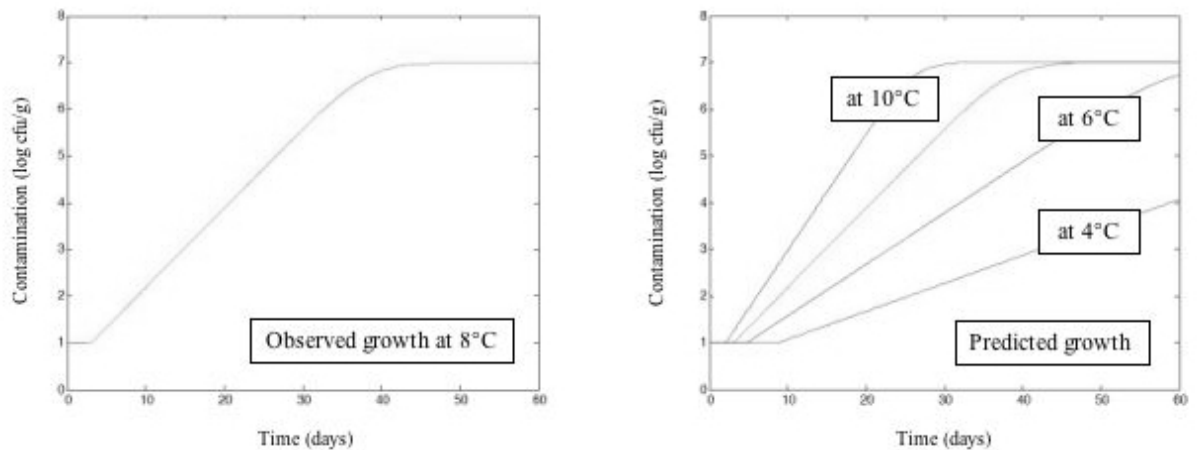


Figure 4. An example of a predictive microbiology.

5.4. Examples for the shelf-life evaluation using the approach combining different tools available

Product characteristics used for the examples 1-3 below are:

- mean pH = 5.97 ± 0.05
- mean $a_w = 0.960 \pm 0.012$
- Maximum⁷ initial contamination = 1 cfu/g

Maximum growth rate (μ_{\max}) and lag time for an identical product with pH = 6.03 and $a_w = 0.959$ at 10 °C (data given by literature or predictive modeling or challenge tests):

- $\mu_{\max} = 0.3 \log_{10}$ cfu/g per day
- lag = 4.4 days.

1. Shelf-life of 10 days at 6 °C (single case approach)

This example describes a single case approach for a shelf-life of 10 days and a storage temperature of 6 °C.

For a maximum storage temperature of 6 °C, the mean lag time will be 14 days and the mean growth rate will be $0.12 \log_{10}$ cfu/g per day. Taking into account the product physico-chemical characteristics and the behavior of the strains of *L. monocytogenes* allows evaluating:

- minimum⁷ lag time = 5.4 days
- maximum⁷ growth rate = $0.20 \log_{10}$ cfu/g per day.

For a maximum initial concentration of 1 cfu/g (i.e. $0 \log_{10}$ cfu/g) the final concentration will be:

$$0 + (10 - 5.4) \times 0.20 = 0.92 \log_{10} \text{ cfu/g i.e. } 8 \text{ cfu/g.}$$

Even for a lag time equal to zero, the final concentration would be:

$$0 + 10 \times 0.20 = 2 \log_{10} \text{ cfu/g i.e. } 100 \text{ cfu/g.}$$

In this case, the single case approach is sufficient to demonstrate that the limit of 100 cfu/g will not be exceeded at the end of the shelf life.

⁷ maximum or minimum = in this case, very low probability (<5%) to exceed the value.

2. Shelf-life of 28 days with 10 days at 4 °C and 18 days at 8 °C

Single case approach

- maximum⁷ growth rate (\log_{10} cfu/g per day) : 0.10 at 4 °C and 0.33 at 8 °C
- minimum⁷ lag time : 11 days at 4 °C, and 0.4 day at 8 °C (after 10 days at 4 °C)

Final concentration:

$$0 + (18 - 0.4) \times 0.33 = 5.8 \log_{10} \text{ cfu/g i.e. } 6.4 \times 10^5 \text{ cfu/g.}$$

In this case, a single case approach shows that the limit of 100 cfu/g is greatly exceeded at the end of the shelf-life. Using a risk based approach is necessary to take into account the inherent variability of the product and evaluate the probability to exceed the limit.

Risk based approach

Sale units = 200 g

Initial contamination is evaluated from microbiological analyses historical data: 2% of positive results in 25 g samples. If we assume a homogeneous distribution of the contamination in the product, 27% of sale units of 200 g will be contaminated with at least 1 cfu/g per unit.

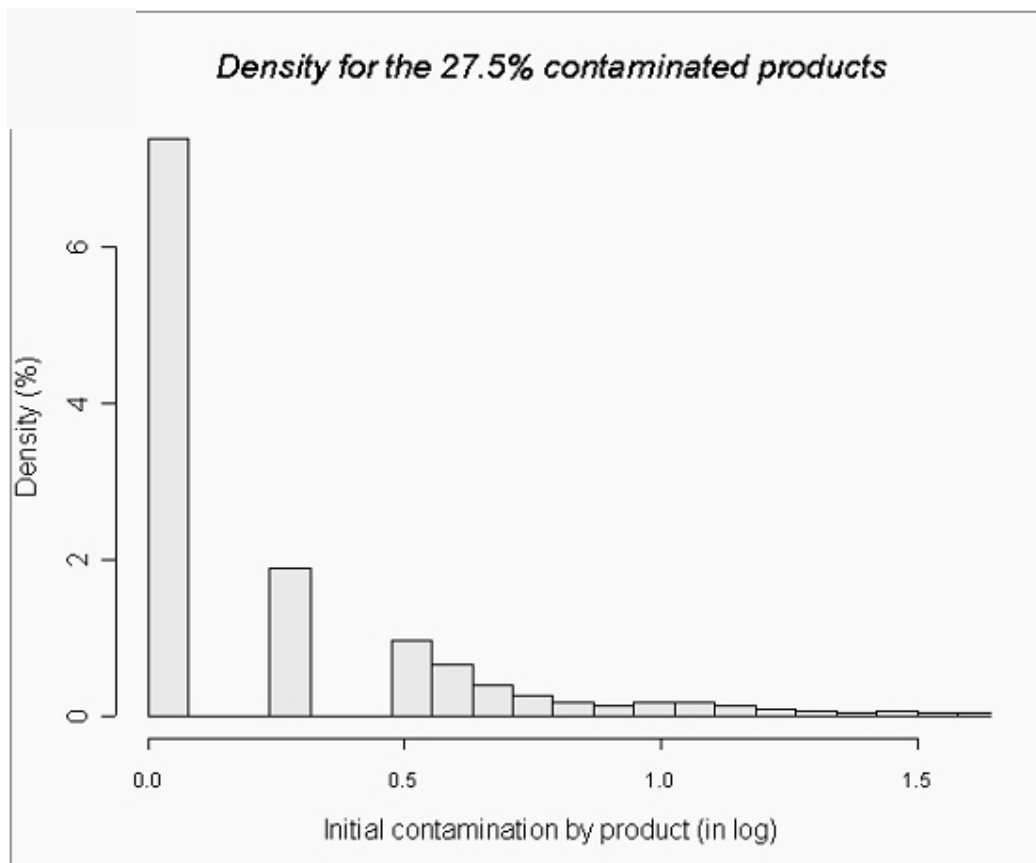


Figure 5. Evaluation of the initial contamination distribution of food products

Growth rates are included (in 95% of cases):

- at 4 °C, between 0 and 0.10 log₁₀ cfu/g per day, with a mean of 0.04
- at 8 °C, between 0 and 0.33 log₁₀ cfu/g per day, with a mean of 0.20

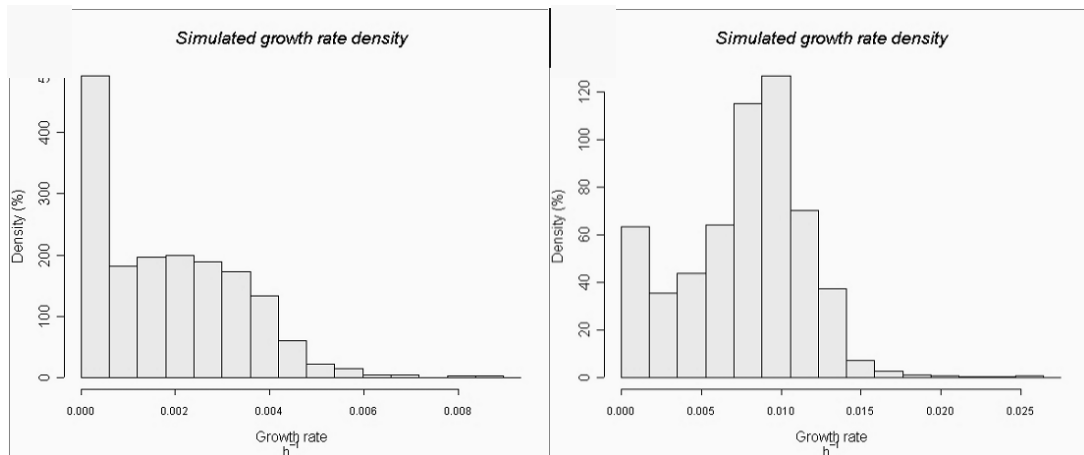


Figure 6. Growth rate variability (log₁₀ cfu/h) at 4 °C (on the left) and 8 °C (on the right)

The lag time is between 11 and more than 200 days at 4 °C with a mean of 45 days. The residual lag time at 8 °C will be included between 0.4 and more than 1000 days, with a mean of 6.6 days.

Final concentration of the contaminated units of 200 g after 10 days at 4 °C followed by 18 days at 8 °C will be included between 0.005 cfu/g and 3.10³ cfu/g, with a mean of 5 cfu/g.

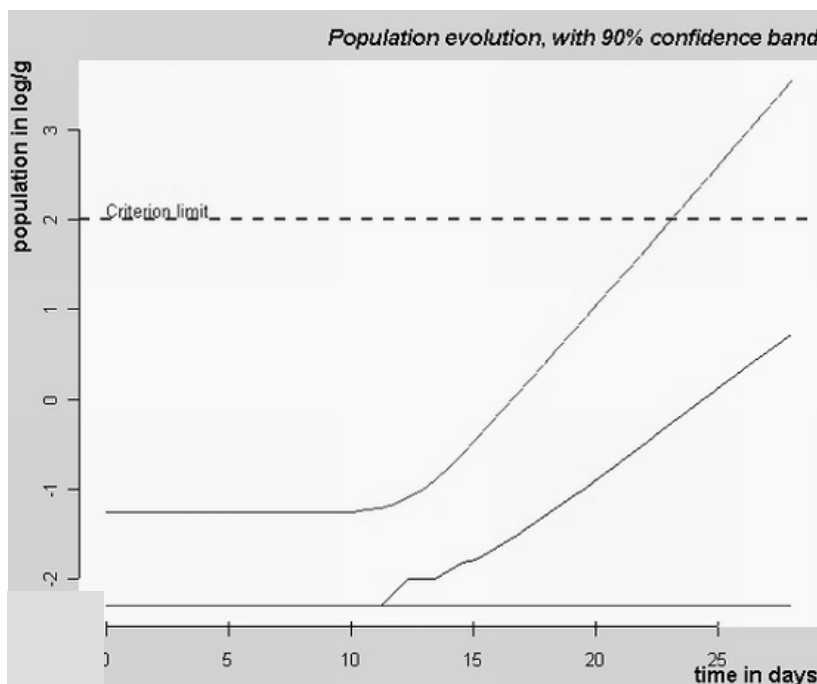


Figure 7. Growth simulation with confidence band (upper curve and lower straight lines) including variability inherent to microorganism, physico-chemical factors and initial contamination.

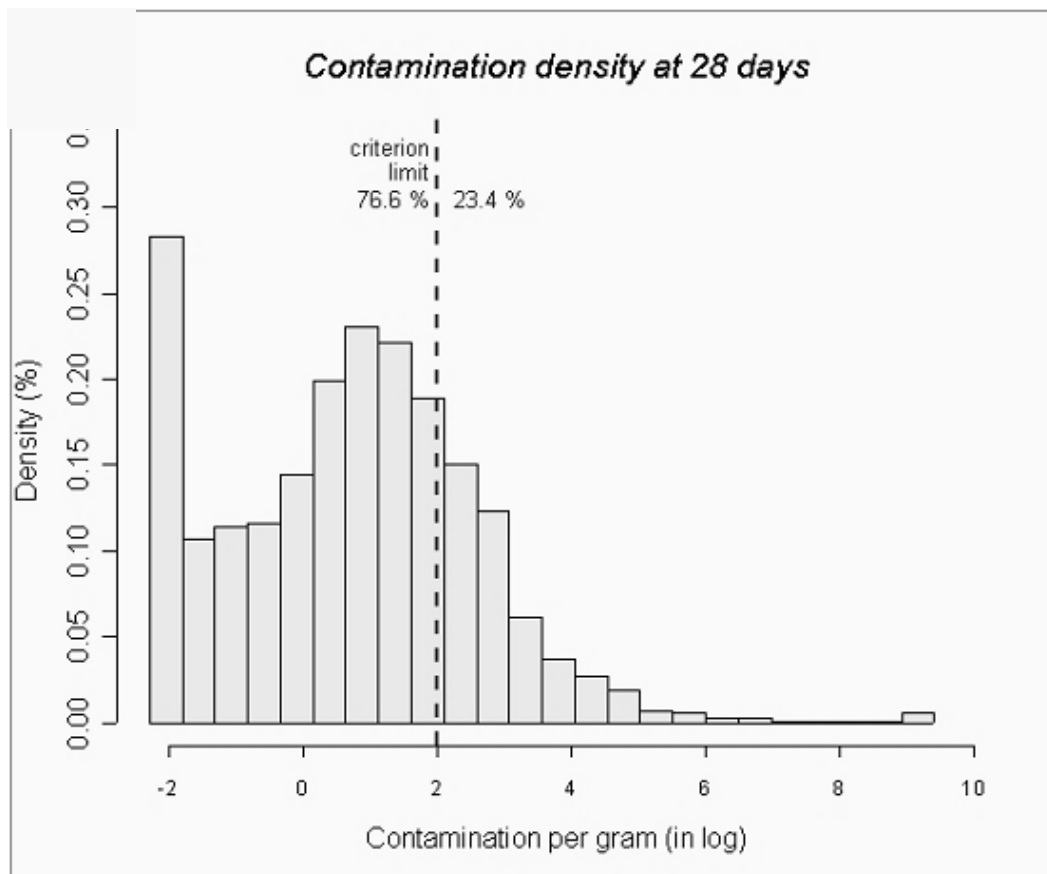


Figure 8. Population distribution at 28 days, including variability inherent to microorganism, physico-chemical factors and initial contamination.

These data allow calculating that 23.4% of the contaminated units of 200 g will exceed the limit of 100 cfu/g, i.e. 6.4% of the total of the manufactured units.

3. Shelf-life of 21 days with 1/3 at 4 °C and 2/3 at 8 °C

Single case approach

Maximum storage temperature: 7 days at 4 °C followed by 14 days at 8 °C.

- at 4 °C:
 - maximum⁷ growth rate = 0.10 log₁₀ per day
 - minimum⁹ lag time = 11 days
- at 8 °C:
 - maximum⁷ growth rate = 0.33 log₁₀ per day
 - minimum⁷ residual lag time = 1.4 days

Final concentration:

$$0 + (14 - 1.4) \times 0.33 = 4.2 \log_{10} \text{ cfu/g i.e. } 1.6 \times 10^4 \text{ cfu/g.}$$

Risk based approach

Initial contamination is evaluated from microbiological analyses historical data: 2% of positive results in 25 g samples. Only 27.5% of sale units of 200 g will be contaminated with a mean of 1 cfu/g.

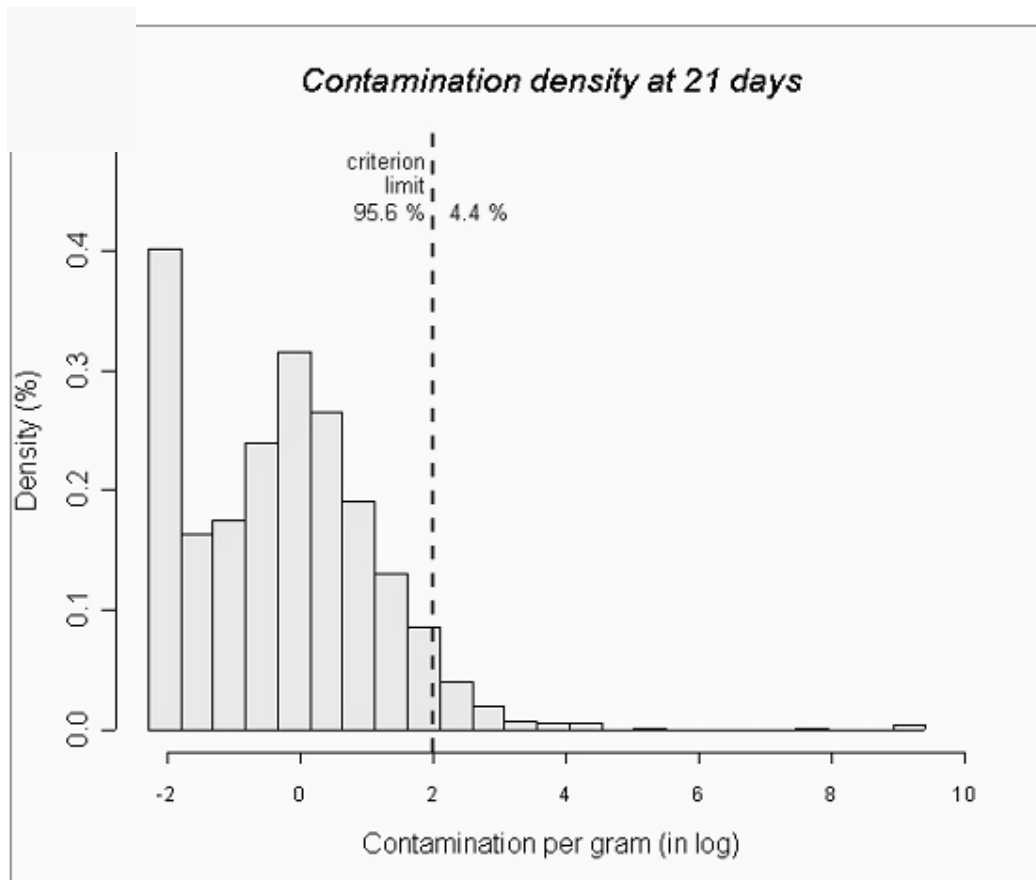


Figure 9. Population distribution at 21 days, including variability inherent to microorganism, physico-chemical factors and initial contamination.

Implementing a risk based approach as described in the chapter 3.6 allows calculating that 4.4% of the contaminated units of 200 g will exceed the limit of 100 cfu/g, i.e. 1.2% of all of the manufactured units.

5.5. Reference list to some existing guidance documents

Note: this list is only for example, other relevant documents may exist and the FBO is therefore advised to seek these (including updated) documents.

Existing national or industry guidance documents

- Evaluation of Product Shelf-life for Chilled Foods, Guideline No. 46, Campden and Chorleywood Food Research Association (CCFRA, 2004), UK.

This document was developed in conjunction with industry experts. It is intended as an outline structure for the evaluation of shelf-life of chilled foods and includes a standardised testing protocol. This approach has been widely adopted by UK food businesses in conjunction with strict control programmes based on GMP, GHP and implementation of HACCP-based procedures. It is focussed on testing on day of production and at end-of-life (durability studies) to verify shelf-life and compliance with the *Listeria monocytogenes* criteria.

Other national or industry guidance documents are generally based on one or other of these documents:

- Aide à la maîtrise de l'hygiène alimentaire des plats préparés réfrigérés, Alésial services, ISBN 2-9509242-0-4, (1995), France.
- Best Practice Guidelines for the Production of Chilled Food, Fourth Edition, Chilled Food Association, CFA, (2005), UK.
- Determination of Product Shelf-life, Guidance Note No. 18, Food Safety Authority of Ireland, FSAI, (2005), Eire.
- GBPH "Produits traiteurs", Syndicat National des Fabricants de Plats Préparés Frais, SYNAFAP, (2008), France.
- Guide to calculating the shelf life of foods, Food Safety Authority of New Zealand, NZFSA, <http://www.nzfsa.govt.nz/processed-food-retail-sale/shelf-life/shelflife1-2-2.pdf>, (2005). New Zealand.
- Protocol for the implementation of shelf life studies on *Listeria monocytogenes*, Food and Consumer Product Safety Authority, Voedsel en Waren Autoriteit, (2008), The Netherlands.
- Recommendations for the Production of Prepackaged Chilled Food, European Chilled Food Federation, ECFE, (2006).

Standards

Two AFNOR documents are available. These are French standards giving guidelines for conducting studies in order to determine the microbiological shelf-life of foodstuffs:

- Hygiène et sécurité des produits alimentaires - Lignes directrices pour l'élaboration d'un protocole de test de vieillissement pour la validation de la durée de vie microbiologique - Denrées périssables, réfrigérées (Hygiene and safety of foodstuffs: Guidelines for the design of durability studies protocols for the validation of a microbiological lifetime - Chilled perishable goods), NF V01-003 February 2004, Association française de Normalisation (AFNOR 2004), France.

Document NF V01-003 provides a methodology to design a durability study protocol for the validation of microbiological shelf-life of chilled perishable goods. This shelf-life is one of the data necessary to set the "use by" or "best before" date. It specifies the means to determine the storage conditions used for the tests (taking into account the real temperature distribution).

- Hygiène et sécurité des produits alimentaires - Lignes directrices pour la réalisation des tests de croissance microbiologiques (Hygiene and safety of foodstuffs: Guidelines for the design of microbiological challenge tests), NF V01-009 September 2007, Association française de Normalisation (AFNOR 2007), France.

Document NF V01-009 describes the laboratory protocols for implementing challenge-tests to evaluate the evolution of a bacteriological flora in artificially contaminated food, in order to evaluate either growth potential or maximum growth rate.