Report of the Scientific Committee of the Spanish Agency for Food Safety and Nutrition (AESAN) in relation to shelf-life studies for *Listeria monocytogenes* in certain food products

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Abstract

Commission Regulation (EC) No 2073/2005 on microbiological criteria for foodstuffs establishes that for Ready-to-Eat (RTE) foods on which are able to support the growth of *Listeria monocytogenes*, the limit is absence in 25 g. However, a limit of 100 cfu/g is established for foodstuffs other than those intended for infants and for special medical purposes, given the manufacturer is able to demonstrate, to the satisfaction of the competent authority, that the product will not exceed this limit throughout the shelf-life.

When necessary, food business operators will conduct studies in accordance with annex II of Regulation, especially on RTE foods that may support the growth of *L. monocytogenes* and may pose a *Listeria monocytogenes* risk for public health. Evidence proving that the limit of 100 cfu/g will not be exceeded throughout the product shelf-life shall be based on shelf-life studies, initially consisting of information on the product specific composition (intrinsic and extrinsic characteristics) and of comparison with data from relevant scientific literature on the pathogen growth and survival characteristics. The repository of data of manufactured products, the predictive microbiology and the specific shelf-life studies conducted in laboratory (durability and challenge studies) are additional tools to be used in case the studies on foodstuff composition, the process conditions and comparison to published data on scientific journals are not enough to prevent any doubt on *L. monocytogenes* ability to grow. Nevertheless, challenge studies on foodstuffs should be avoided and be used with caution. It is recommended these studies be conducted by food business operators only if they count on the means, training and experience on these microbiological techniques. In this document some guidelines are given for the implementation of the stipulations in annex II of Regulation (information search and complementary studies that allow the establishment of products shelf-life) and expect to help the

manufacturer to choose the best way to determine the shelf-life of his products and the competent authorities to verify, when necessary, that the manufacturer proves that the limit of 100 cfu/g will not be exceeded during the product shelf-life.

Key words

Listeria monocytogenes, shelf life, challenge test, durability studies, Ready-to-Eat Foods.

Introduction

Listeria monocytogenes is a micro-organism that has become one of the main pathogenic agents transmitted by Ready-to-Eat (RTE) foods affecting at-risk populations. *L. monocytogenes* has some unique and distinctive properties (FSAI, 2005b) (Luber et al., 2011). It grows adequately at refrigeration temperatures, at a minimum of between 1.5 ºC and 3 ºC and a maximum of 45 ºC. This allows it to remain viable inside or on the surfaces of foods that are generally stored at low temperatures. It is widespread throughout the environment, and is highly present in food processing plants, contaminating the surfaces that come into contact with food products in addition to the products themselves. This bacterium can enter food processing plants via soil on the shoes and clothing of factory staff, the vehicles used to transport the food, animals that excrete the bacterium or whose skin is contaminated with it, or contaminated raw vegetables. Thus, *Listeria monocytogenes* represents a major problem in food production. This is especially true for RTE foods (foods that are destined for direct human consumption and do not require any cooking or any other preparation that would eliminate micro-organisms that pose a threat to public health or to reduce their concentration to an acceptable level), which support the growth of *Listeria monocytogenes* and will not receive any heat treatment during production, and for foods that may become contaminated by the factory environment (EU, 2008).

It is crucial that manufacturers of RTE foods take the appropriate measures to control both contamination by *Listeria monocytogenes* and its growth in a product until the end of its shelf-life. They therefore need to have access to information and documents regarding the potential growth of this micro-organism in a particular food, and should take this into account when calculating a product's shelf-life (EU, 2008).

On 15 November 2005, the European Commission published Regulation (EC) No 2073/2005 (EU, 2005) on microbiological criteria for foodstuffs, which addressed both RTE foods and *Listeria monocytogenes*.

Article 3 of this Regulation states that food business operators must ensure that food products meet with the relevant microbiological criteria limits set out in such Regulation. Furthermore, article 3 details the tests (listed in annex II of the Regulation) that a food business operator may carry out, if necessary, to investigate whether a food product meets such criteria throughout its shelf-life. This particularly applies to RTE foods that may support the growth of *Listeria monocytogenes* and thus pose a risk to public health with regard to this bacterium.

In 2008 the SANCO/1628/2008 report, version 9.3 (26112008), was published (EU, 2008). This report is a guide to undertaking shelf-life tests on RTE foods in order to detect the presence of *Listeria monocytogenes* under Regulation (EC) No 2073/2005 of 15 November 2005 on microbiological criteria for foodstuffs, and is aimed at food business operators. However, it does not describe in detail how to conduct a shelf-life test for a particular food, and states that it may be supplemented by more detailed guidelines drawn up by institutes, national authorities and the food industry. Therefore, the European Union Reference Laboratory for *Listeria monocytogenes* has prepared a separate technical document aimed at laboratories that conduct shelf-life studies, in particular, durability studies and challenge tests (AFSSA, 2008).

In addition, some food industry sector associations (Chilled Food Association Ltd.) and food standards agencies (Food Safety Authority of Ireland) have drawn up documents and guidelines to assist

food businesses to carry out shelf-life studies on RTE foods with regard to *Listeria monocytogenes*, as there is apparently some confusion among the various industry sectors in how they should conduct these studies on their own products in such a way that the results have the necessary scientific and statistical basis to be accepted by health authorities.

In order to clarify the situation, a Report has been requested from the Scientific Committee of the Spanish Agency for Food Safety and Nutrition (AESAN) on the types of studies than can be used to determine the shelf-life of RTE foods that may support the growth of *Listeria monocytogenes.*

Terms of reference: The Scientific Committee is required to conduct an assessment and comparison of the types of shelf-life studies than can be conducted on RTE foods that may support the growth of *Listeria monocytogenes*.

This report would help food manufacturers to design shelf-life studies for their products and could act as a guide to support any relevant authorities that may wish to use it (on a voluntary basis) to assess, where appropriate, whether the microbiological criteria established for *Listeria monocytogenes* in Regulation (EC) No 2073/2005 are being met.

Regulation

Article 3, paragraph 2 of Regulation (EC) No 2073/2005 of 15 November 2005 provides that, where necessary, food business operators responsible for the manufacturing of food products will conduct studies in accordance with the provisions set out in annex II, in order to check that said product complies with the criteria outlined therein throughout its shelf-life. This applies in particular to RTE foods that may support the growth of *Listeria monocytogenes* and thus may pose a risk to public health with regard to this bacterium. Food businesses may collaborate on such studies.

The studies referred to in article 3, paragraph 2, consist of the following:

- specifying the physicochemical properties of the product, such as pH, a_w, salt content, concentration of preservatives, type of packaging system, taking into account storage and processing conditions, the possibility of contamination and expected shelf-life; and
- consulting the available scientific literature and research data regarding the survival and growth characteristics of the micro-organisms in question.

When necessary, based on the aforementioned studies, a food business operator should perform additional studies, which may include:

- applying predictive mathematical models set up for the food in question, using critical growth or survival factors applicable to the specific micro-organisms present in the product;
- conducting tests to investigate the ability of the micro-organism in question, when properly inoculated, to grow or survive in the product under different and reasonably foreseeable storage conditions;
- carrying out studies to evaluate the growth or survival of the micro-organisms in question that may be present in the product during its shelf-life under reasonably foreseeable conditions of distribution, storage and use.

The abovementioned studies should take into account the inherent variability of the product, the micro-organisms in question and the processing and storage conditions.

Purpose and scope of this document

The purpose of this document is to provide guidelines on how to implement the provisions detailed in annex II of the Regulation (EC) No 2073/2005 on microbiological criteria for foodstuffs.

As the European Union Reference Laboratory for *Listeria monocytogenes* has already published a comprehensive guide to challenge test and durability study methodologies, the scope of this document is limited to establishing criteria to enable food manufacturers to choose the most appropriate methodology in each individual case (food or formulation) so that the results will allow the relevant Authorities to check if the safety of the products in question can be duly guaranteed in line with the regulation on microbiological criteria applicable in this case.

Tools to establish shelf-life

1. Introduction

Regulation (EC) No 2073/2005 sets out three types of RTE foods with regard to the control of *Listeria monocytogenes*.

The first group consists of RTE foods intended for infants and for special medical purposes. *L. monocytogenes* should be absent in such products on the market (absence in 25 grams of the product after the analysis of 10 samples).

The second group includes RTE foods that can support the growth of *L. monocytogenes.* Manufacturers must guarantee that, throughout the shelf-life of these foods, the concentration of *L. monocytogenes* will not exceed 100 cfu/g (analysis of 5 samples, c=0) or, if the operator cannot demonstrate that the concentration in the product will not exceed 100 cfu/g that, in the manufacturing control, *Listeria* is absent in 25 grams (analysis of 5 samples, c=0).

The third group consists of those foods that do not support the growth of *L. monocytogenes* or that have a shelf-life of less than 5 days. The limit for these foods is set at 100 cfu/g after the analysis of 5 samples.

The evidence for these requirements should be based on shelf-life studies. These studies should begin by obtaining all of the necessary information on the composition of the food in question (chemical and physical properties, for example) and by reviewing the relevant scientific literature on said composition. If the results are sufficiently conclusive to state that *Listeria monocytogenes* cannot grow in the food in question, then it is not necessary to conduct further studies. However, if the results raise doubt on the possibility of the growth of this micro-organism, it will be necessary to conduct a series of additional studies that may include one or more of the following (CFA, 2010):

- Historical data on the products manufactured.
- Predictive microbiology.
- Specific shelf-life tests conducted in a laboratory (durability studies, challenge tests).

Shelf-life is defined as the period of time during which a food remains safe and meets its quality specifications as per its storage and expected use. A product's shelf-life indicates the durability date and

is expressed on the product as a "use by" or "best before" date, as outlined in article 11 of the Royal Decree 1334/1999, of 31 July (Real Decreto, 1999), which approves the General Standard for the labelling, presentation and advertising of food products.

Shelf-life studies should be carried out in the following circumstances (EU, 2008):

- If food businesses develop new products or modify existing ones.
- If food businesses develop new processes or modify existing ones.
- If food businesses develop new packaging or packaging procedures.
- If there is any significant change to the ingredients or packaging of an existing product.
- If there are changes to the production location or equipment.
- If no previous shelf-life studies exist.

2. Information obtained from a product's properties and the scientific literature

Product properties

Shelf-life studies should always include specific information on the composition of the food being studied and a comparison with the relevant scientific literature on the growth and survival characteristics of the pathogenic micro-organism in question (FSAI, 2005a).

A product's intrinsic properties, such as its pH, a_w (water activity), salt concentration, and concentration of preservatives, in addition to its extrinsic properties, affect the ability of *Listeria monocytogenes* to survive and grow in it, and determine how it should be packaged, the length of time it should be stored for and the temperature at which it should be stored. Therefore, these physicochemical properties should be clearly identified. They should be determined under the product's normal manufacturing, packaging and storage conditions, and both the possibility that the food may be re-contaminated and its expected shelf-life must be taken into account. Table 1 illustrates some intrinsic and extrinsic properties that should be taken into consideration in shelf-life studies.

Table 1. Intrinsic and extrinsic properties of food

Intrinsic properties	Extrinsic properties
Microbiological quality of raw material	Good hygiene and manufacturing practices
Historical data on raw material	HACCP[1]
Food composition and formulation	The food's processing
Food structure and assembly	Storage temperature
pH	Gas composition
Type of acid present	Relative humidity
Water activity (a_w)	Packaging
Redox Potential (Eh)	Retail practices
Biological structures	Consumer practices
Oxygen availability	–
Nutritional content and availability	–
Antimicrobial constituents	–
Natural or artificial microbiota present in the food	–

[1]HACCP: Hazard Analysis Critical Control Point. **Adapted from** (Jay, 1992) (Mc Donald, 1999).

If a food manufacturer does not have the experience to identify these properties, it should contact a research institution or specialised laboratory that will assist it to understand and obtain the necessary information (CFA, 2010).

Identifying the properties of a food will allow a food manufacturer to determine whether or not it supports the growth of *Listeria monocytogenes*. According to information published in the Regulation on microbiological criteria (EU, 2005), if a food has the following intrinsic properties it can be considered not to support the growth of *Listeria monocytogenes*.

- pH of less than or equal to 4.4.
- a_w of less than or equal to 0.92.
- pH of less than or equal to 5.0 with a_w of less than or equal to 0.94.

However, other documents and guidelines consider pH values of less than or equal to 4.2 or 4.3 and water activity of less than or equal to 0.90 to be the conditions under which *Listeria monocytogenes* cannot grow in food (FSAI, 2003) (EU, 2004) (EU, 2008) (CFA, 2010). These intrinsic properties are somewhat more restrictive than those published in the Regulation on microbiological criteria (EU, 2005).

If these parameters demonstrate that *Listeria monocytogenes* cannot grow in a particular food, then they should be treated as Critical Control Points within the relevant HACCP system, and subsequent shelf-life studies in relation to *Listeria monocytogenes* are not necessary. If there is scientific evidence that *Listeria monocytogenes* cannot grow in a food, due to its physicochemical (intrinsic) properties, a limit of 100 cfu/g of *Listeria monocytogenes* throughout the food's shelf-life should be applied as per the legislation.

Scientific literature

There is currently a large amount of data on *Listeria monocytogenes* available in books and scientific publications, and from universities and public and private research institutes. Data is also available from the various national, European and international agencies (for example European Food Safety Authority).

When a food manufacturer has established its product's properties and the conditions under which it will be packaged and stored, it should compare these with the scientific literature on the survival and growth capabilities of *Listeria monocytogenes* (or any other pathogen). Table 2 shows some factors that limit the survival and growth of *Listeria monocytogenes*.

Table 2. Factors that affect the growth and survival of *Listeria monocytogenes*

Factor	Can grow			Can survive but not grow
	Lower limit	Optimum	Upper limit	
Temperature (ºC)	-1.5 to +3.0	30.0 to 37.0	45.0	-18.0
pH	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 (%)	<0.5	0.7	12 to 16	≥20
Atmosphere	LM is a facultative anaerobe that can grow without oxygen, for example, when vacuum packed or in a modified atmosphere			

Adapted from (FSAI, 2003) (EU, 2004, 2008).

3. Additional studies

If, after a product's properties have been compared with the relevant scientific literature, or other data, it cannot be guaranteed that such a product does not support the growth of *Listeria monocytogenes*, it will be necessary to conduct additional studies. These may include the analysis and use of historical data, the use of predictive microbiology or the conducting of durability studies or challenge tests. All of these studies should take into account the inherent variability of the food, of the micro-organism in question and of the processing and storage conditions.

Historical data

All companies must keep records, including those relating to the safety of foods that are already on the market. This information is called historical data and consists of specific records on manufacturing locations and foods that are compiled over time.

This data (including tests conducted on the finished product on the day it is produced and at the end of its shelf-life) can be used as evidence that the concentration of *Listeria monocytogenes* in a food does not exceed the limit of 100 cfu/g. Data on the levels of *Listeria monocytogenes* in existing RTE foods can be used to evaluate the possibility of potential growth and to confirm that the shelf-life assigned to a food is appropriate. It can also be applied to similar RTE foods with comparable intrinsic properties that are manufactured under processing conditions that are fundamentally the same.

The data to be used should be (CFA, 2010):

- Derived from the HACCP and monitoring controls and should include the following:
  - Validation of processes, checking and monitoring (temperature, pH, a_w).
  - Traceability of the ingredients and testing of their microbiological quality.
  - Sampling of species of *Listeria* (including non-pathogenic species) and of appropriate hygiene indicator organisms from the different areas of the manufacturing plant and from the equipment.
  - Checking for the presence and concentration of *Listeria monocytogenes* in the finished product, for example, on the day of its production and at the end of its shelf-life. This will establish whether the HACCP has functioned effectively and will verify durability.

- Derived from shelf-life studies.

The level of confidence increases with the amount of data available from a company, therefore the more units of a product that are checked, the greater the reliability of the historical data (EU, 2008). However, it is not possible to recommend a specific amount of data since it is, in essence, an approximation based on risk, and depends on variations in the manufacturing process and the nature of the food (CFA, 2010).

Predictive microbiology

Predictive microbiology is a tool to consider using when additional studies are required to confirm the shelf-life that has been established for a food.

Predictive microbiology aims to predict the behaviour of micro-organisms in products during their manufacturing or storage. In recent years significant advances have been made in this field, especially with regard to estimating the growth of *Listeria monocytogenes* in food. In fact, the microbial growth prediction program *Combase* (2010) is a readily available source for all businesses and can assist in determining growth. There are also several other tools that can be combined with *Combase* to help small businesses to determine what process controls are necessary (Dalgaard, 2009). Other programs of interest include the *Growth Predictor*, which can be obtained for free at the following web address: www.Ifr.ac.uk/safety/growthpredictor, and the *Pathogen Modelling Programme*, also available for free, at this address: http://ars.usda.gov/Services/docs.htm?docid=6786.

In general, by entering a food's key intrinsic factors (pH, a_w, or salt concentration) and processing data into a predictive microbiological model (a computer program) it is possible to obtain an indication of the potential growth of the particular micro-organism that the model is designed to predict. However, it should be mentioned that the data used to construct mathematical models are usually obtained from experiments conducted in a laboratory, therefore these models should be validated against survival and growth data obtained from the food itself prior to their use.

Predictive mathematical models can be useful for the following (EU, 2008):

- In order to predict bacterial growth under various conditions.
- In order to predict the probability of the growth of micro-organisms in foods.
- In order to estimate the level of contamination at a given moment in a food's shelf-life.
- In order to estimate variability between two batches.
- In order to optimize the formulation of a food in order to ensure maximum stability.
- In order to assess the impact of the breakage of the cold chain and to test different storage conditions.
- In order to help define the Critical Control Points in a process.

Regarding the use of mathematical models, it should be taken into account that many are obtained in liquid media, the majority of which are homogeneous, in order to determine the impact of certain environmental factors on micro-organisms. These liquid-based models may not accurately predict the behaviour of micro-organisms in food, even if the model has been validated in the food. Other mo-

dels are obtained from the food itself and may quite accurately predict the behaviour of the micro-organism in a specific food during storage, however, their ability to predict the impact of variability in the physicochemical properties of the food or to make predictions for other different foods is questionable.

Sometimes inexact predictions are the result of inconsistent responses from the micro-organisms or slight variations in the culture medium. Some research has shown that these are the reasons behind some predictive models' lack of accuracy in determining the survival and growth of micro-organisms (FDA, 2001).

In conclusion, although predictive mathematical models provide an inexpensive means to minimise the microbiological samples used in shelf-life studies, they should be used with caution and only by trained, experienced personnel that are aware of their limitations and conditions of use.

Specific shelf-life laboratory studies

It is also possible to conduct specific laboratory studies to determine the growth of *Listeria monocytogenes*. These studies are known as challenge tests (the experimental inoculation of food) and durability studies.

Challenge tests

In general, these tests are only used when other methods for evaluating the safety or stability of a food cannot be carried out or have not made it sufficiently clear that *Listeria monocytogenes* cannot grow in a food, or where there are doubts about the suitability of the shelf-life established for such food. Challenge tests provide information about how micro-organisms (*Listeria monocytogenes*) –inoculated in a food that is then stored– behave under certain environmental conditions in a laboratory setting (Betts, 2010). The European Union has established protocols to conduct challenge tests (EU, 2008). These essentially consist of inoculating a food with a specific concentration of a mixture of strains of *Listeria monocytogenes* and measuring the changes in the micro-organism's population rates during storage, taking into account the worst case scenario. This type of test should account for the variability in food (the use of different batches) and specific instances of contamination (the inoculation of strains isolated in the food). However, the level of contamination, the heterogeneity of the contamination and the physiological state of the bacteria are difficult to replicate (AFSSA, 2008).

Experimental inoculation challenge tests are used to evaluate the potential growth of a micro-organism, i.e. whether or not it can grow in a specific food (δ), and to estimate growth parameters such as the maximum growth rate (μmax).

Studies should simulate the temperature abuse that might reasonably occur while the RTE food is on sale and during its storage by the consumer (Scott et al., 2005).

It is recommended that inocula with low concentrations of different strains of *Listeria monocytogenes* be used in order to introduce variability into the results (Metris et al., 2008). The strains used should be those typically found in the product being tested but should also include clinical strains and those that are resistant to the environmental stresses experienced during the product's shelf-life (Luber et al., 2011).

Studies to establish growth potential

A challenge test (experimental inoculation) to establish growth potential is a laboratory test in which *Listeria monocytogenes* is artificially inoculated into a food and its growth measured during the product's storage under foreseeable transport, distribution and storage conditions. Conditions must be realistic, meaning that they could occur during the cold chain, and must include all the storage conditions experienced from after production up until consumption (AFSSA, 2008).

The potential growth of a micro-organism (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. This parameter depends on several factors, the most notable being the following (EU, 2008):

- The inoculated strain or strains.
- Damage or stress (physiological condition) to the inoculated strains.
- The food's intrinsic properties (pH, a_w, salt concentration, associated microbiota, antimicrobial components).
- Extrinsic properties (temperature profile, gas composition).

Of all these factors, it is temperature that may have the greatest influence on the growth of *Listeria monocytogenes* in a given food (EFSA, 2007).

The evaluation of growth potential enables (AFSSA, 2008):

- The classification of foods as RTE.
  - When $\delta > 0.5$ $\log_{10}$ cfu/g, a food is classified as an RTE food in which the growth of *Listeria monocytogenes* can occur, not intended for breast-fed babies or special medical purposes.
  - When $\delta \leq 0.5$ $\log_{10}$ cfu/g, a food is classified as an RTE food in which the growth of *Listeria monocytogenes* cannot occur, not intended for breast-fed babies or special medical purposes.
- The quantifying of the behaviour of *Listeria monocytogenes* in a food, under the conditions that it may reasonably be expected to experience between production and consumption (for example, by calculating the difference between the concentration at the end of its shelf-life with its initial concentration).
- The determination of the concentration of *Listeria monocytogenes* upon production that will enable compliance with the limit of 100 cfu/g at the end of its shelf-life.

The main advantages of this method are that it is relatively simple to carry out and that the results can be used directly, as outlined above. The disadvantage is that it is relatively inflexible in its interpretation: the results are specific to the food studied under the particular conditions, so that every time a product or process changes, additional studies must be conducted, with sufficient levels of replication and repetition for the result to be statistically significant.

Studies to establish the maximum growth rate

Growth potential studies' disadvantages can be mitigated by combining predictive microbiology models with growth rate tests (for example μmax) (AFSSA, 2008). These tests are more expensive and time-consuming that the challenge tests used to assess growth potential. They are restricted to those

cases in which predictive microbiology can be applied and should be carried out in laboratories that are experienced in the use and application of predictive mathematical models.

A challenge test to assess growth rate is essentially a study conducted in a laboratory that measures *Listeria monocytogenes'* growth rate in an artificially contaminated food stored at an appropriate temperature. The temperature used for the test needs not be the same as that used to carry out the predictions, as it is possible to predict what will happen at different temperatures to those used in the study if a secondary predictive model is available (for example, a polynomial model) or along a temperature-time profile chosen to represent foreseeable transport, distribution and storage conditions.

Technically, once the test has been completed at the temperature in question, *Listeria monocytogenes'* maximum growth rate can be calculated from the resulting growth curve. In order to do this, the natural logarithm of the number of cells in the exponential phase is plotted against the storage time. Linear regression is carried out and the slope of the regression curve represents the maximum growth rate (μmax). The result can be expressed in hours^{-1} or days^{-1}.

The maximum growth rate depends on the following factors:

- The inoculated strain or strains.
- The intrinsic properties of the food (pH, a_w, salt concentration, associated microbiota, antimicrobial components).
- Extrinsic properties (temperature profile, gas composition).

If more than one study is carried out at different temperatures, it is possible to construct a secondary mathematical model that enables the maximum growth rate at different temperatures to be deduced, but always in the same food.

This type of challenge test enables the following information to be obtained (AFSSA, 2008):

- An estimate of the concentration of *Listeria monocytogenes* at a given moment in a food's shelf-life if the initial concentration immediately after its manufacture is known.
- An estimate of the maximum allowable concentration of *Listeria monocytogenes* in a food on the day it is produced if it is to comply with the limit of 100 cfu/g at the end of its shelf-life.

Durability studies

Durability studies allow for the assessment of the growth of *Listeria monocytogenes* in a food during storage under reasonably foreseeable conditions without the use of artificial inoculation by examining only the natural contamination of such a food. These studies are more realistic than challenge tests as they examine the progression of natural contamination in a product. However, interpreting durability studies can prove difficult, as there will likely be a very low number of units contaminated with *Listeria monocytogenes*, a low concentration of *Listeria monocytogenes* in the product under study, and heterogeneity of the distribution of *Listeria monocytogenes* in the food. Therefore additional tests, such as challenge tests, may have to be conducted.

The record of durability studies conducted in the same product under the same processing conditions –representing variability in manufacturing conditions– enables the levels of *Listeria monocyto-*

genes in the food being tested to be assessed at the end of study. The results may be used to assess the proportion (with its associated confidence interval) of units (commercial units) that would exceed the limit of 100 cfu/g at the end of their shelf-life after being stored under foreseeable conditions of storage and distribution. In these types of studies the level of confidence increases with the amount of available data. Therefore, the more units of a product that are evaluated, the more reliable the shelf-life test (AFSSA, 2008) (EU, 2008).

Scientific Committee Conclusions

1. Evidence that the limit of 100 cfu/g of *Listeria monocytogenes* will not be exceeded in a RTE product during its shelf-life (Regulation (EC) No 2073/2005 of 15 November 2005), should be obtained from shelf-life studies. These studies should firstly determine whether or not the food in question supports the growth of *L. monocytogenes,* using information on its specific composition (intrinsic and extrinsic properties) and by comparison with data from the relevant scientific literature on the growth and survival characteristics of the pathogen in question.
2. Shelf-life studies should be conducted by food businesses in the following circumstances:
   - If they develop new products or modify existing ones.
   - If they develop new processes or substantially modify existing ones.
   - If they develop new packaging or packaging processes.
   - If there is any significant change in the ingredients or in the packaging of an existing product.
   - If there is any change in the production location or equipment.
   - If there are no previous shelf-life studies.
3. Historical data on the products manufactured, predictive microbiology and specific shelf-life tests conducted in a laboratory (durability studies and challenge tests) are additional tools that can be used in the event that studies on a food's composition and processing conditions, and their comparison with data published in scientific journals, raise doubts as to the possibility of the growth of *Listeria monocytogenes*.
4. Predictive mathematical models are an inexpensive means of minimising the microbiological samples used in shelf-life studies when the product in question is subject to a minor formulation or change process in its early stages of development. However, these must be used with caution and only by trained, experienced personnel that understand their limitations and conditions of use. Consultation with a relevant organisation prior to use is recommended.
5. In general, challenge tests are only used when other methods for evaluating the safety or stability of a food cannot be carried out or have not made it sufficiently clear that *Listeria monocytogenes* cannot grow in a food, or where there are doubts about the suitability of the shelf-life established for said food. They should be used with great caution and it is recommended that a relevant organisation with experience in this type of study is contacted prior to use.
6. The main advantages of a challenge test in determining growth potential are that it is relatively simple to carry out and that the results can be used directly. Its main disadvantages are that it is inflexible in its interpretation and that its results are specific to the food studied under the particular conditions, so that every time a product or process changes, additional studies must be conducted.

7. Studies to identify maximum growth rate can estimate the concentration of *Listeria monocytogenes* at given moment in a food's shelf-life –if the initial concentration immediately after production is known. They also provide an estimate of the maximum allowable concentration of *Listeria monocytogenes* in a food on the day it is produced in order to comply with the limit of 100 cfu/g at the end of its shelf-life.
8. Durability studies on foods are more realistic than challenge tests because they examine the progression of natural contamination. However, interpreting durability studies can prove difficult, as there will likely be a very low number of units contaminated with *Listeria monocytogenes*, a low concentration of *Listeria monocytogenes* in the product under study, and heterogeneity of the distribution of *Listeria monocytogenes* in the food.
9. Food businesses should establish their own protocols and operating procedures or data acquisition procedures in order to ensure that a consistent and precise shelf-life is determined for specific products. If a food business does not have sufficient resources to determine the shelf-life of a product, it is recommended that it seeks the advice of an institution or laboratory with adequate experience and the recognized accreditation to conduct microbiological testing and food shelf-life assessments.
10. It is recommended that food businesses apply a safety margin in shelf-life studies, in order to take into account the foreseeable conditions of use that may affect the food's safety and shelf-life. In applying this safety margin, food businesses must take into account any foreseeable variation during the production, transportation, storage and use of the food.
11. Regardless of whether these shelf-life studies are performed by the food business themselves or are conducted by a recognized laboratory, standard or internationally recognised microbiological methods (for example ISO) must be used. If in-house methods are used, they must be validated and documented in order to demonstrate equivalence with the standard methods.

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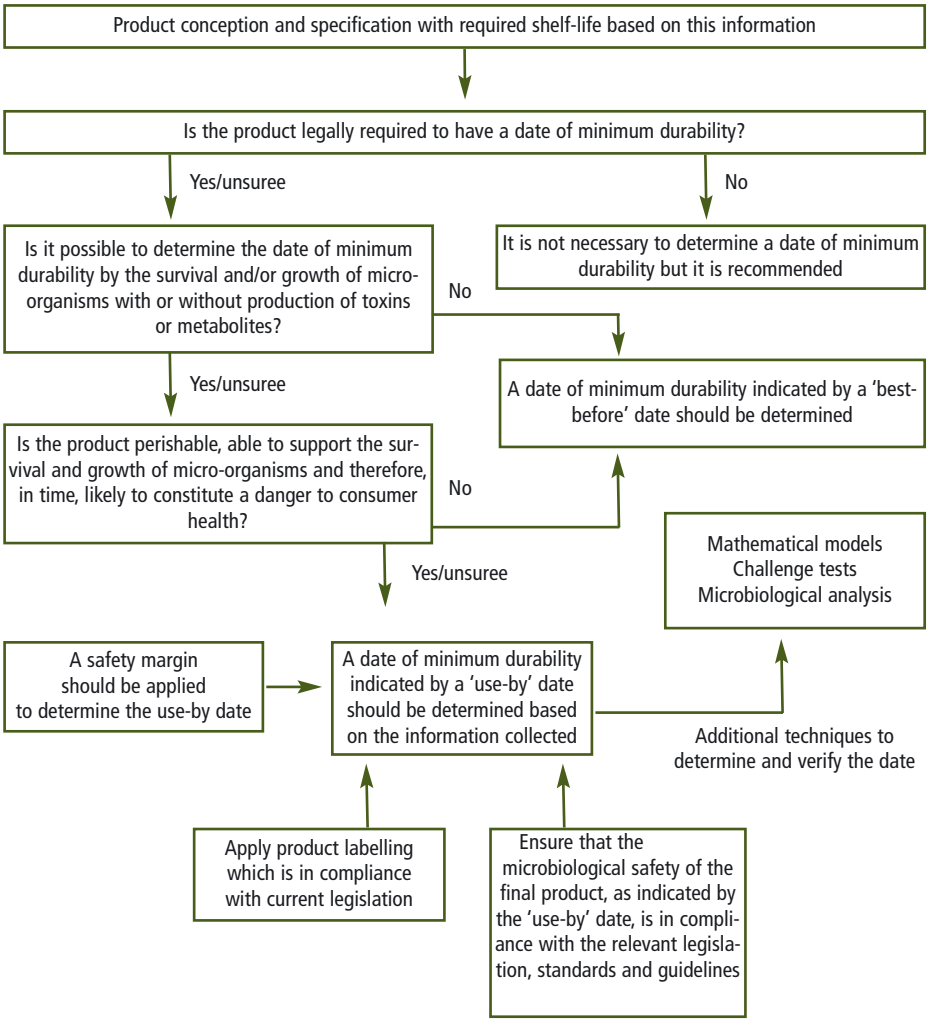
Appendix 1

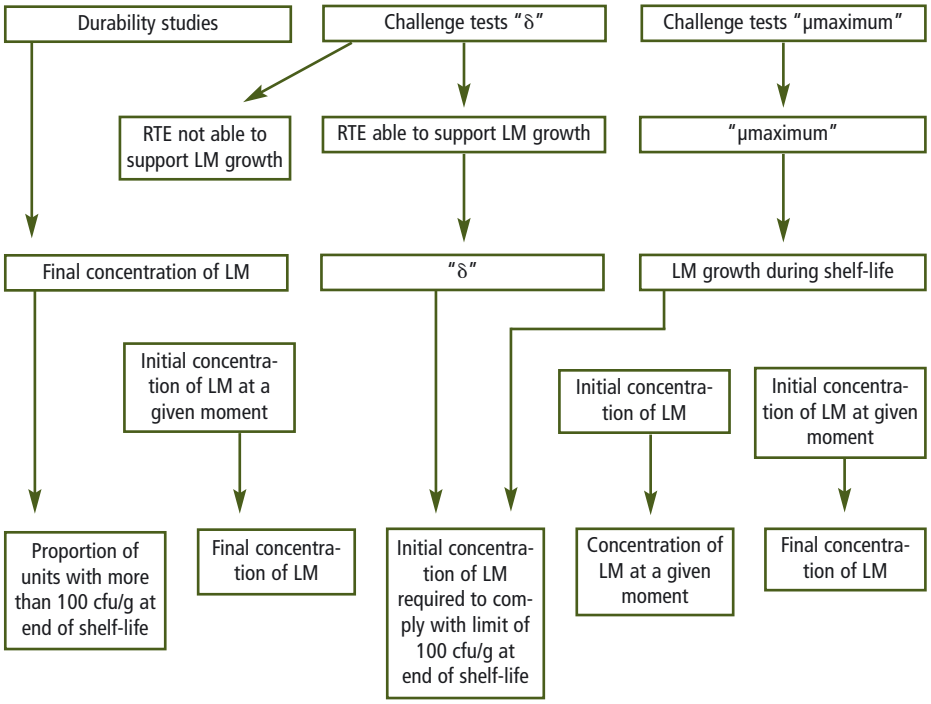
International data sources	
Athlone Institute of Technology	www.ait.ie/
An Bord Bia	www.bordbia.ie/
Carlow Institute of Technology	www.itcarlow.ie/news_events/index.php
Codex Alimentarius	www.codexalimentarius.net
Consumers Association of Ireland	www.consumerassociation.ie/
Dept. of Agriculture and Food	www.irlgov.ie/daff/
Excellence Ireland Hygiene	www.hygienemark.com/
Cork Institute of Technology	www.cit.ie/
Dublin Institute of Technology	www.dit.ie/DIT/Homepage/index.html
Dundalk Institute of Technology	www.dkit.ie/
European Committee for Standardisation	www.cenorm.be/cenorm/index.htm
European Food Safety Authority	www.efsa.eu.int
European Legislation	http://europa.eu.int/eur-lex/en/index.html
European Union Risk Analysis Information Network	www.eu-rain.com/
Food and Agriculture Organisation	www.fao.org/
Food and Drug Administration	www.fda.gov/default.htm
Institute of Food Research	www.fsai.ie
Galway-Mayo Institute of Technology	www.gmit.ie/
Food Safety Authority of Ireland	www.ifrn.bbsrc.ac.uk/
Institute of Food Science and Technology	www.ifst.org/
Institute of Food Technologists	www.ift.org/
International Journal of Food Microbiology	www.elsevier.com/
International Life Sciences Institute	www.ilsi.org/
International Standards Organisation	www.iso.org/iso/
Irish Legislation	www.irishstatutebook.ie/front.html
Irish National Accreditation Board	www.inab.ie/
Journal of Food Protection	www.foodprotection.org/
Journal of Food Safety	www.foodscipress.com/
Limerick Institute of Technology	www.lit.ie/
Microbial Risk Assessment of Meat Products	http://smas.chemeng.ntua.gr/miram/
Physical Properties of Food Database	www.nelfood.com
Relay (Research for the Food Industry)	www.relayresearch.ie/
Teagasc	www.teagasc.ie/
National University of Ireland	www.nui.ie/
University of Limerick	www.ul.ie/
Waterford Institute of Technology	www.wit.ie/

Appendix 2

1. Determination of Product Shelf-Life



2. Data obtained from a challenge test and durability studies (AFSSA, 2008)



Appendix 3

Definitions

Colony-forming Units (cfu): Microbial cells that form a single colony in a plate with an appropriate culture medium.

pH: A measurement of the acidity or alkalinity of a food.

Shelf-life: Shelf-life is defined as the period of time during which a product remains safe and meets its quality specifications under the expected storage conditions. A product's shelf-life determines the use-by date printed on the label as per law.

Shelf-life studies: Shelf-life studies must demonstrate that a food complies with the safe limit (maximum of 100 cfu/g) established for Listeria monocytogenes throughout its shelf-life.

Water activity (a_w): A measurement of the water available for a micro-organism metabolic activity and growth.

Growth potential (δ): The difference between the $\log_{10}$ cfu/g at the end of the challenge test and the $\log_{10}$ cfu/g at the beginning of the test.

Maximum growth rate (μmax): This refers to the slope of the regression line depicting exponential growth during a challenge test.