EUROPEAN
COMMISSION

SANCO/11510/2013, **revision of 18 December 2025**

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

This document is provided for information purposes only and does not create any legal rights or obligations. It has not been adopted, approved or endorsed by the European Commission. Its content does not prejudge any position the European Commission may take before the Court of Justice of the European Union (CJEU) as regards provisions contained in Commission Regulation (EC) No 2073/2005. The authoritative interpretation of EU rules remains within the sole remit of the CJEU.

The European Commission does not guarantee the accuracy or the completeness of the information provided, nor does it accept liability for any use made thereof. Users are advised to exercise their own judgment and assume all risks associated with the application of the information contained herein.

PURPOSE OF THIS DOCUMENT

This guidance document is primarily directed at Food Business Operators (FBOs) producing ready-to-eat (RTE) foods and conducting related *Listeria monocytogenes* shelf-life studies in accordance with Article 3(2) and Annex II of Regulation (EC) No 2073/2005 of 15 November 2005 on microbiological criteria for foodstuffs. It can also assist competent authorities (CAs) conducting official controls on these FBOs and serve as a resource for third parties involved in developing *Listeria monocytogenes* shelf-life studies.

Table of contents

1. Introduction, purpose and scope

Listeria monocytogenes (*Lm*) is a bacterium that can cause severe illness in humans, primarily through contaminated food. Infection (listeriosis) poses a particularly high risk to vulnerable populations, including infants, pregnant women, individuals over 65, and immunocompromised persons (e.g., cancer patients and transplant recipients). Scientific evidence indicates that the median infectious dose in these groups is significantly lower than in healthy individuals. Since no safe threshold exists to protect all consumer groups, vulnerable individuals should avoid exposure to *Lm* in food at any concentration as a precaution. For healthy individuals, scientific consensus generally considers *Lm* concentration not exceeding 100 cfu/g to present a low health risk, though no absolute safe threshold exists. These principles are reflected in the food safety criteria for *Lm* laid down in Regulation (EC) No 2073/2005.

Lm is frequently present in the environment, in soil, vegetation and faeces of animals, and can also be found in raw food (e.g. fresh meat, raw milk and fish) and food derived therefrom. The ubiquitous occurrence of *Lm* and its ability to survive and even grow in difficult environments (e.g. low temperature, low oxygen concentrations, high salt concentration, low water activity [a_w]) compared to most other food pathogens, makes *Lm* a significant challenge when producing ready-to-eat (RTE) foods i.e. foods intended by the producer for direct human consumption without the need for cooking or other processing effective to eliminate or reduce pathogenic microorganisms to an acceptable level. *Lm* is one of the most relevant pathogenic microorganisms associated with persistence in food processing environments in the meat, fish & seafood, dairy and fruit & vegetable sectors.

RTE foods in which *Lm* can grow and that will not receive a heat-treatment during the production process, or other treatment able to eliminate *Lm*, are of particular concern. It is therefore crucial that producers of such foods take actions to control initial contamination by *Lm* and understand the growth behaviour of *Lm* in the RTE foods they produce to set a safe shelf-life for their products. They must be able to demonstrate that their products will comply with the provisions of Commission Regulation (EC) No 2073/2005 throughout the shelf-life.

This document is primarily intended for food business operators (FBOs) producing RTE foods and aims to provide them with guidance to meet the requirements of Commission Regulation (EC) No 2073/2005 as regards *Lm.*

In particular, this document aims to guide FBOs:

- to classify and adequately label their food products as RTE or non-RTE foods,

- to determine which *Lm* food safety criterion applies to their food products,

- to decide on when and which shelf-life studies are needed with respect to *Lm* to demonstrate that their food products will comply with the *Lm* criteria until the end of the shelf-life,

- to validate, verify (initial verification and regular verification) and document that such shelf-life studies are adequate to respect the applicable *Lm* food safety criterion,

- on the options to collaborate in conducting such shelf-life studies.

This document can also assist competent authorities (CAs) when performing official controls on these FBOs. It can also be useful for third parties involved in the development of such shelf-life

studies.

This document is not intended to be prescriptive and does not describe in detail how to conduct each of the shelf-life studies with respect to *Lm* for a particular food product. A separate Technical Guidance Document (TGD) for laboratories conducting shelf-life studies for assessing shelf-life of RTE foods related to *Lm*, especially durability studies and challenge tests, has been prepared by the EU Reference Laboratory (EURL) for *Lm* (EURL *Lm*, 2021). An EURL TGD for CAs to evaluate the competence of laboratories implementing challenge tests and durability studies related to *Lm* in RTE foods is also available (EURL *Lm*, 2023c).

This document should be read in conjunction with relevant EU and national legislation or guidance and other similar documents developed by food safety authorities/institutes/agencies and food industry organizations. It does not supersede any applicable legal requirements or official guidance.

Determining the shelf-life of RTE products for food safety requirements other than those related to *Lm* is outside the scope of this document. For more guidance on this topic, guidelines from EFSA on date marking (European Food Safety Authority, 2020b, 2021) and national best practice guidelines on determining shelf-life (e.g. Direction générale de l'alimentation, 2024; Food Safety Authority of Ireland, 2022) should be consulted.

2. European Union food hygiene legislation

2.1. General provisions of EU hygiene legislation

The main purpose of the European Union (EU) food hygiene legislation is to ensure a safe food supply and a high level of consumer protection. Under Regulation (EC) No 178/2002, it is the FBO's legal responsibility to make sure that their food business produces safe food. FBOs should consult any European and national food hygiene legislation in their own Member States that may be applicable to their food business activities, along with the websites of the CAs for further information and guidance.

Figure 1 shows the relevant legislation to be complied with by FBOs producing RTE foods when determining their shelf-life, to produce safe food. Amendments are made to food law occasionally and it is FBO's responsibility to ensure they are complying with the most up-to-date version of the relevant legislation.

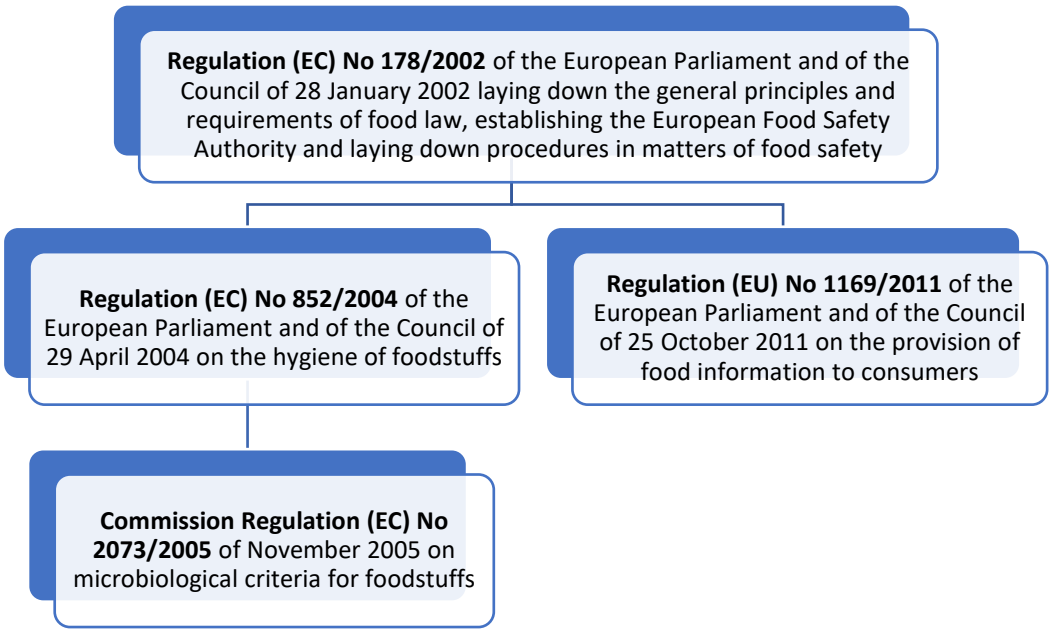

Figure 1. EU food legislation linked to determining the shelf-life of foods

The EU food hygiene legislation is based on a preventive approach, which includes the implementation of hygiene control measures termed Prerequisite Programmes (PRPs) and Hazard Analysis and Critical Control Point (HACCP)-based procedures by the FBO at any stage of the food production chain. This control system used by the FBO is called the food safety management system (FSMS).

Regulation (EC) No 178/2002 sets out the basic principles of food law to protect human health and consumer interests. It applies to all stages of production, processing and distribution of food and feed. The main purpose of this Regulation, also known as the General Food Law, is to guarantee a high level of public health protection. Under Article 14 of Regulation (EC) No 178/2002, food must not be placed on the market if it is unsafe.

Regulation (EC) No 852/2004 on the hygiene of foodstuffs sets in its Article 5 the steps of the HACCP principles to identify and control food safety hazards in the FBOs premises. As microbiological hazards in foodstuffs are one of the most important sources of foodborne diseases in humans, Article 4.3 (a) of Regulation (EC) No 852/2004 provides that, when implementing or adopting these hygienic procedures and measures, the food must comply with the relevant microbiological criteria. FBOs, as appropriate, must establish sampling and analysis programmes to demonstrate compliance with microbiological criteria for foodstuffs set down in **Commission Regulation (EC) No 2073/2005**. These programmes must form an integral part of the implementation of the FBO's FSMS procedures based on good hygiene practices (GHP) and HACCP principles.

Regulation (EU) No 1169/2011, known as Food Information to Consumers (FIC), sets out a list of compulsory information that must appear on pre-packaged foods, which includes:

- the date of minimum durability (i.e. the “best before”) or the “use by” date;
- any special storage conditions and/or conditions of use where applicable;
- instructions for use (e.g. cooking instructions) where it would be difficult to make appropriate use of the food in the absence of such instructions.

2.2. Commission Regulation (EC) No 2073/2005 for microbiological criteria of foodstuffs

2.2.1. Microbiological food safety criteria for *Lm* in RTE foods

Commission Regulation (EC) No 2073/2005 sets the microbiological criteria for foodstuffs placed on the market. The specific food safety criteria for *Lm* in RTE foods are laid down in food categories 1.1, 1.2 and 1.3 of Annex I, Chapter 1. The food category for RTE with regard to *Lm* is chosen by its ability to support or not support the growth of *Lm* and its intended use (i.e. for infants or for special medicinal purposes /see also section 5).

Food safety criteria define the acceptability of a product or a batch of foodstuff applicable to products placed on the market. When testing reveals unsatisfactory results and products do not comply with microbiological food safety criteria, FBOs should take corrective measures as defined in their HACCP based procedures and should initiate procedures to withdraw or recall unsafe food from the market as appropriate. When relevant, products that are not yet at retail level may be submitted to further processing to eliminate the hazard. Additionally, FBOs should take measures to find the root cause of the unsatisfactory results and modify the HACCP-based procedures accordingly.

Due to the unequal distribution and the possible low prevalence of a pathogen such as *Lm* in a batch of food, no microbiological sampling and testing plan can completely guarantee its absence in a batch. It is therefore not sufficient to base food safety management solely on end product testing yielding a not detected result for the pathogen. In fact, applying the food safety criteria set out in Commission Regulation (EC) No 2073/2005 is considered as one of several management options to ensure that RTE foods are safe.

The application of GHP in combination with adequate control of raw materials, when relevant, should be consistently used to minimise the initial contamination directly after production. The application of HACCP and the use of validated processing steps will inactivate the hazards of concern or reduce their potential growth. The formulation of safe by design foods would limit growth in the event of contamination. A validated shelf-life is the last control measure to be considered in combination with the previously described measures to ensure food safety.

In addition, microbiological criteria are normally not suitable for the regular monitoring of the critical limits as defined in HACCP. Regular monitoring procedures should be able to detect loss of control at critical control points (CCPs) and should provide this information in time for corrective actions to be taken 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 during production, should be used to complement end product testing to check for compliance with microbiological criteria.

2.2.2. Studies listed in Annex II of the Commission Regulation (EC) No 2073/2005

Annex II of the Commission Regulation (EC) No 2073/2005 describes the shelf-life studies that the FBO shall conduct, as necessary, in order to ensure compliance with the relevant *Lm* criterion for RTE products throughout their shelf-life. The studies should be carried out under reasonably foreseeable conditions of distribution, storage and use.

These shelf-life studies include:

- determination 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 storage conditions, the possibilities for re-contamination, the foreseen shelf-life, and
- consultation of available scientific literature and research data regarding the growth and survival characteristics of the micro-organisms of concern in the product of interest.

When the studies mentioned above are not able to give the necessary confidence to **validate** the shelf-life and comply with the relevant criterion, the FBO should conduct additional studies. These additional studies consider the inherent variability linked to the product, processing and storage conditions and may include one or more of the following:

- studies to investigate the ability of the micro-organism of concern to grow or survive in the product using predictive mathematical modelling established for the food in question, using parameters that are representative of microbial behaviour (survival or growth) in the product under reasonably foreseeable conditions of distribution, storage and use, and/or;
- studies to investigate the ability of the appropriately inoculated micro-organism of concern to grow or survive in the product under reasonably foreseeable conditions of distribution, storage and use (referred to as challenge tests), and/or;
- studies to evaluate the growth or survival of the micro-organisms of concern that may be naturally present in the product during the shelf-life under reasonably foreseeable conditions of distribution, storage and use (referred to as durability studies).

2.2.3. Commission Regulation (EU) 2024/2895

Commission Regulation (EU) 2024/2895 amended Commission Regulation (EC) No 2073/2005 in December 2024 and is applicable from 1st July 2026. The amendment to food criterion 1.2 related to "*ready-to-eat foods able to support the growth of L. monocytogenes, other than those intended for infants and for special medical purposes"* in Commission Regulation (EC) No 2073/2005 has been introduced to ensure a consistent level of public health protection from

production through to distribution for RTE foods. To support this objective, the food safety criterion of '*Lm* not detected in 25 g' (referred to throughout this guidance document as criterion 1.2b) will apply to all situations where those foods are placed on the market during their shelf-life, unless the producing FBO can demonstrate, to the satisfaction of the CA, that the level of *Lm* will remain below the limit of 100 cfu/g throughout their shelf-life. To demonstrate this, the FBO responsible for the manufacture of the product shall conduct studies in accordance with Annex II in order to investigate compliance with the 100 cfu/g *Lm* criterion limit throughout the shelf-life (referred to throughout this guidance document as criterion 1.2a).

This guidance document provides relevant information to the FBO regarding the evaluation of whether a RTE food supports the growth of *Lm*, and if applicable, whether criterion 1.2a or 1.2b applies. If no studies are available, by default it will fall into criterion 1.2.b. FBOs should thoroughly investigate several factors as described in this document to fully understand the risk of *Lm* in relation to the food they produce. Understanding how these factors interrelate is crucial for forming a comprehensive assessment of the associated risks.

2.2.4. Environmental monitoring of *Lm* in food business operations

As *Lm* are widely distributed in the environment and can persist in food production environment, implementing a robust *Lm* environmental monitoring programme should be a key component of the FSMS.

While Article 5.2 of Commission Regulation (EC) No 2073/2005 specifies the FBO manufacturing RTE foods, which may pose a *Lm* risk for public health, shall sample the processing areas and equipment for *Lm* as part of their sampling scheme*,* it does not provide a microbiological criterion to assess the test results, nor does it provide information on what corrective actions to take.

Specific guidance on sampling surfaces and equipment used in food business operations is not in the scope of this document. However, information on designing a *Lm* environmental monitoring programme in a RTE food operation and implementing appropriate corrective actions is available in various documents (FSSC 22000, 2023; Campden BRI, 2022; Spanu and Jordan, 2020; Codex CXG 61-2007, Rev. 2009; Food Safety Authority of Ireland, 2005; Tompkin, 2002). Guidance on implementing a robust hygiene programme to remove *Lm* contamination in the environment of a food business operation is also available (Campden BRI 2020; 1999). For further guidance on surface sampling methods, food business operators are recommended to consult ISO 18593:2018. This information is further complemented by specific guidance published by the EURL *Lm* on where, how and when to sample RTE food processing areas and equipment (EURL *Lm,* 2023a).

The Actia Chlean Joint Technological Network guidance (2021) also provides useful guidance on specific considerations for swabbing of surfaces (e.g. protocols for use of stick swabs and

sponge/gauze swabs). The EURL *Lm* (2023b) have created three short videos[1] which visually show how to implement sampling techniques for stick, sponge and cloth swabbing of surfaces.

2.2.5. Testing of food products for *Lm*

Article 4 of Commission Regulation (EC) No 2073/2005 states that FBOs need, as appropriate, to perform testing against microbiological criteria set out in Annex I, Chapter 1 of the Regulation. End-product testing alone cannot be used as a method to guarantee food safety but should be used within a relevant FSMS including HACCP based procedures and GHP (see section 2.2.1). The aim of testing against the microbiological criteria is primarily to verify the correct functioning of these procedures. According to Commission Regulation (EC) No 2073/2005, testing shall be performed in accordance with the relevant *Lm* criterion considering reasonable conditions of distribution, storage and use.

Commission Regulation (EC) No 2073/2005 does not set a sampling frequency for testing *Lm* in food products. The FBO should determine the appropriate sampling frequency based on risk assessment. For example, when determining the risk, FBOs should take the following points into consideration:

- a possible increased vulnerability of the intended consumer (e.g. food targeting children);
- the method of production;
- the size of production;
- the probability of initial contamination of the food product. For example, this could be due to the nature of a product, contamination of raw ingredients, historical test results for environmental monitoring and product testing;
- a known history of listeriosis outbreaks related to a specific food or ingredient.

Microbiological contamination is often heterogeneously spread throughout a batch of food. Therefore, discarding a first positive result by retesting a second sample from the batch or a new test portion of the original sample should not be undertaken as these new results cannot overrule the previous obtained results.

3. Responsibilities and role of FBOs, third parties and CAs

3.1. Responsibilities of the FBO producing RTE foods

Under Regulation (EC) 178/2002, FBOs have primary legal responsibility for the safety of the food which they produce, transport, store and / or sell. FBOs should ensure that food is not placed on the market if it is unsafe (i.e. injurious to health or unfit for human consumption).

[1] https://www.youtube.com/watch?v=8Gy2f8LiQuU
https://www.youtube.com/watch?v=0tbaqvX0HRU&t=2s
https://www.youtube.com/shorts/AtEJGw3sK8A

To meet this requirement, the FBO is notably responsible for setting the shelf-life of the food he/she produces/packs under defined conditions, which should consider reasonably foreseeable conditions of distribution, storage and use. FBOs opening, slicing, cutting, portioning and repacking RTE food products should also establish an adequate product shelf-life by following good practice guidelines to estimate, set and verify the safety of food over its shelf-life (Food Safety Authority of Ireland, 2022, European Food Safety Authority Panel on Biological Hazards, 2021, 2020).

Some FBOs may not have the necessary expertise employed within their business to validate the shelf-life of food they produce. They may decide to employ additional external third-party support (e.g. laboratories, consultants) to facilitate certain aspects of the validation. However, FBOs still remain legally responsible for issuing and updating the documentation that justifies the safety of their products. Determining a shelf-life of a food is a control measure considered to be part of the producer's HACCP-based procedures. 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 should demonstrate the compliance of the product with the food safety criteria for *Lm* throughout its shelf-life considering the reasonably foreseeable conditions of distribution, storage and use. The responsibilities of the FBO are to:

- Determine if the foodstuffs they produce are "RTE" or "non-RTE";
- Determine if the RTE food supports or does not support the growth of *Lm*;
- Determine the *Lm* criterion that applies to their product;
- Set and validate the shelf-life of the product, followed by regular verification of the shelf-life (see Fig. 5 and FBO checklist in Appendix 1). For RTE food supporting the growth of *Lm,* and until their shelf-life has been fully validated with respect to *Lm*, the FBO should carry out more extensive sampling and testing than would be required for routine verification to ensure compliance with criterion 1.2b of Regulation (EC) No 2073/2005 (i.e. *Lm* not detected in 25 g).
- Conduct an environmental monitoring programme of the processing areas and equipment for *Lm* (see section 2.2).

FBOs may collaborate with each other and seek expertise from various other organisations (e.g. research organisations or reference laboratories) when they conduct shelf-life studies (see section 7).

3.2. Role of third parties

Where it is necessary for a FBO to engage external expertise, it is essential that they source a suitable third party to support them. The third party should have the necessary knowledge and expertise (e.g. food microbiology, food sciences, food processing, statistics, predictive microbiology models and tools).

In this regard, third parties and FBOs should be aware of the following key points:

- For microbiological testing of food samples and swabs, it is strongly recommended to engage a laboratory able to test under accreditation (accreditation based on the most recent version of EN ISO/IEC 17025) for the detection and/or enumeration of *Lm.* In the context of challenge tests and durability studies, all analytical results, and more specifically the enumeration and detection of *Lm*, should be obtained under a quality assurance system. This is achieved either through laboratory accreditation according to EN ISO/IEC 17025 or through documented good laboratory practices, quality control of measurement instruments and participation in proficiency tests. This is also recommended for other analysis such as physico-chemical characteristics and indigenous micro-organisms.
- Validated predictive microbiology models and tools should be employed by trained and competent personnel who are thoroughly familiar with their limitations and appropriate conditions of use, particularly when considering the intrinsic variability and range of factors associated with specific food. The choice of the model and tool, the nature and range of the factors to be considered, and the values of the model inputs should be clearly justified. When available, models based on data from food matrices should be preferred over broth-based models. Currently, lag time predictions can be less robust than maximum growth rates or probability of growth predictions. Thus, unless justified, a worst-case scenario considering no lag time should be preferred. When predictive microbiology tools are used, the user should clearly identify the model used, the input parameters, the simulation hypothesis that were considered to obtain the results and any additional information required to reproduce the results.
- Challenge tests and durability studies are specific examinations that can be part of a shelf-life study and should be carried out to the satisfaction of the CA.
- Challenge tests should be carried out according to EN ISO 20976-1 and to the EURL *Lm* Technical Guidance Document (TGD) on challenge tests and durability studies for assessing shelf-life of ready-to-eat foods related to *Listeria monocytogenes* (EURL *Lm*, 2021).
- Durability studies should be carried out according to the EURL *Lm* TGD (EURL *Lm*, 2021).
- Laboratories implementing challenge tests and durability studies should have specific competences that are described in the EURL *Lm* Guidance Document to evaluate the competence of laboratories implementing challenge tests & durability studies related to *Listeria monocytogenes* in ready-to-eat foods (EURL *Lm*, 2023c).
- There are several ways in which the competency in performing challenge tests can be evaluated. In some Member States laboratories can be part of specific networks recognized by the authorities to conduct such studies and participation in proficiency testing can be part of

that. In other Member States the possibility to obtain the ISO/IEC 17025 accreditation for performing challenge tests in accordance with the EURL *Lm* TGD (EURL *Lm*, 2021) and EN ISO 20976-1 is available via the national accreditation body.
- A challenge test report or a durability study report including the goal of the study, the results and conclusion should be provided to the FBO.
- The results of the studies should be integrated in the FBO internal shelf-life study documents. The acceptability of the completed study (including a challenge test and/or durability study) is evaluated by the CA.

3.3. Role of CAs

Article 17 (2) of Regulation (EC) 178/2002 establishes a general duty for the CAs in the Member States to monitor and control that food law requirements have comprehensively and effectively been enforced at all stages of the food chain. This includes verifying that any legal requirements relating to the setting and validation of shelf-life duration for food products are met to the satisfaction of the CA.

During official controls CAs should evaluate the studies that are conducted under the responsibility of the FBO, and more specifically:

- whether or not the product is correctly classified and labelled by the FBO (RTE or non-RTE),
- if it is RTE, whether the product is correctly categorised as supporting or not supporting the growth of *Lm*,
- if it is RTE, whether the shelf-life in relation to *Lm* was properly validated (with justification of the choice of the type of study) including compliance of the product with respect to *Lm.* For RTE food supporting the growth of *Lm,* and until their shelf-life has been fully validated with respect to *Lm*, the FBO should carry out more extensive sampling and testing than would be required for routine verification to ensure compliance with criterion 1.2b of Regulation (EC) No 2073/2005 (i.e. *Lm* not detected in 25 g).
- if environmental monitoring for *Lm* is properly performed, in FBOs producing RTE foods, which may pose a *Lm* risk for public health,
- if proper corrective measures and actions are documented by the FBO in their FSMS after identification of non-compliances (including environmental monitoring).

4. Classification and labelling of food products as RTE or non-RTE

RTE foods are defined in Commission Regulation (EC) No 2073/2005 - Article 2 as a "*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 micro-organisms of concern*".

It is the responsibility of the FBO to determine if a food is RTE or not and to ensure the presence and accuracy of mandatory food information to consumers through an appropriate labelling of the

packaging. The CA should verify that the classification and the labelling of the food has been done correctly.

Pursuant to Article 3.1(b) of Regulation (EC) No 2073/2005, FBOs must implement measures to ensure that their products meet food safety criteria throughout their shelf life under reasonably foreseeable conditions of distribution, storage, and use. When considering **reasonably foreseeable conditions of use**, FBOs should assess the potential for their non-RTE products to be consumed in ways that deviate from their intended use taking into account variations in consumption patterns that may arise from e.g. cultural factors and thus lead to food safety risk. This evaluation should be based on analysis of available information that suggests a likelihood of such deviations occurring, such as differences in food preferences or preparation methods between countries or populations. The outcome of this assessment will inform the development of **adequate information** to the consumer to minimise the risk of foodborne illness (as detailed below) and will also guide FBOs in determining whether **reclassification** of the product as RTE should be considered. Overall, labels identifying foods as RTE or non-RTE should always be consistent and free of conflicting messages to prevent consumer confusion and ensure safe consumption.

Pursuant to Regulation (EC) No 1169/2011, food product packaging must indicate:

1. **special conditions of use**, as outlined in Article 9.1(g) and Article 25, if the food products require them. For most non RTE-foods, and without prejudice to other specific legal provisions, packaging should normally indicate conditions related to cooking or reheating the product before consumption. These conditions may not require the same level of details as instructions for use (see next point), particularly when the nature of the product is such that the risk of inappropriate use by consumers is expected to be low or when the safety risk associated with *Lm* is negligible such as for foods that have undergone a validated heat treatment to eliminate *Lm* within their final packaging with no possibility of recontamination after the treatment. In such cases, a concise and straightforward statement of the special conditions of use may be deemed sufficient.

2. **instructions for use**, as outlined in Article 9.1(j) and Article 27, where it would be difficult to make appropriate use of the food in the absence of such instructions. This may apply to situations where the food's appearance may be misleading, such as non-RTE food that resemble cooked or RTE products, or where consumption patterns are deemed likely to vary after consideration of the reasonably foreseeable conditions of use. These instructions should be prominently displayed and articulated with greater detail and specificity than special conditions of use, providing clear and unambiguous guidance to consumers. Any specific heating instructions based on specific time/temperature combinations provided on the packaging should be validated by FBOs (preferably in accordance with requirements of ISO 20976-2:2022) to ensure their effectiveness in achieving food safety and the results of such validation should be documented as part of the FBO's FSMS. Since *Lm* is one of the most heat-resistant, among foodborne pathogens that do not form spores, any heat treatment that is effective against *Lm* should be sufficient to destroy other non-spore forming vegetative pathogens that may be present in the food. Furthermore, any serving

suggestions, whether presented in pictures or text on the packaging or disseminated through other channels (websites, social media, etc.), should be consistent with the instructions and not provide conflicting information.

5. Determination of the criterion to apply and the possible studies used

Commission Regulation No 2073/2005 sets food safety criteria with respect to *Lm* for three different categories of RTE food. The determination of the appropriate food category is needed to identify the correct microbiological criterion for *Lm*. To substantiate that the food belongs to one of the three specified categories, it is necessary to identify the target consumers population and to investigate and characterise the growth potential of *Lm* throughout the shelf-life based on:

- the physico-chemical characterisation of the food,
- scientific literature,
- historical data,
- predictive microbiology predictions,
- challenge tests,
- durability studies.

The three categories, their sampling plans, stages of application and criteria limits are:

- category 1.1 covers RTE foods **intended for infants and special medical purposes**. The sampling plan involves testing n=10 samples of which none should exceed the limit of "not detected in 25 g" throughout the shelf-life;

- category 1.2 covers RTE foods other than those covered by category 1.1 and which are **able** to support the growth of *Lm* with two subcategories:

    - category 1.2a is reserved to RTE foods that support *Lm* growth but for which evidence is provided, to the satisfaction of the CA, that *Lm* will not exceed 100 cfu/g throughout the shelf-life. The sampling plan involves n=5 samples of which none should exceed the limit of 100 cfu/g throughout the shelf-life. In this situation, an intermediate limit during the process can be used by the FBO. The limits must be low enough to guarantee that the limit of 100 cfu/g is not exceeded at the end of shelf-life.

    - category 1.2b is reserved to RTE foods that support *Lm* growth but for which there is none or no satisfactory evidence provided that the limit of 100 cfu/g will not be exceeded throughout the shelf life. The sampling plan involves testing n=5 samples of which none should exceed the limit of "not detected in 25 g" throughout the shelf-life (applicable from 1st July 2026).

- category 1.3 covers RTE foods other than those covered by category 1.1 and which are **unable** to support the growth of *Lm*. Products with pH ≤ 4,4 or $a_w \leq 0,92$, or pH ≤ 5,0 and $a_w \leq 0,94$, or with a shelf-life of less than five days belong automatically to this category (footnote 8 of Commission Regulation (EC) No 2073/2005). Scientific literature and other studies (e.g. predictive microbiology, challenge tests) can also be used to justify the classification of the food under category 1.3. The sampling plan of this category involves testing n=5 samples of which none should exceed the limit of 100 cfu/g throughout the shelf-life.

Figure 2 proposes a simplified approach to the process of the determination of the appropriate food safety criterion for a RTE food as regards *Lm*.

It should be noted that footnote 4 of Chapter 1 of Annex I to Commission Regulation (EC) No 2073/2005 provides that regular *Lm* testing is not required in normal circumstances for the following RTE products:

- products which have received processing effective to eliminate *Lm*, when recontamination is not possible after this treatment;
- fresh, uncut and unprocessed vegetables and fruits;
- bread, biscuits and similar products;
- bottled or packet waters, soft drinks, beer, cider, wine, spirits and similar products;
- sugar, honey and confectionery, including cacao and chocolate products;
- live bivalve molluscs;
- food grade salt.

As it is the responsibility of the FBO to produce safe foods and to ensure that its overall FSMS is working well, the frequency of the monitoring might need to be adapted depending on other circumstances (e.g. previously documented outbreaks, recalls related to the specific food).

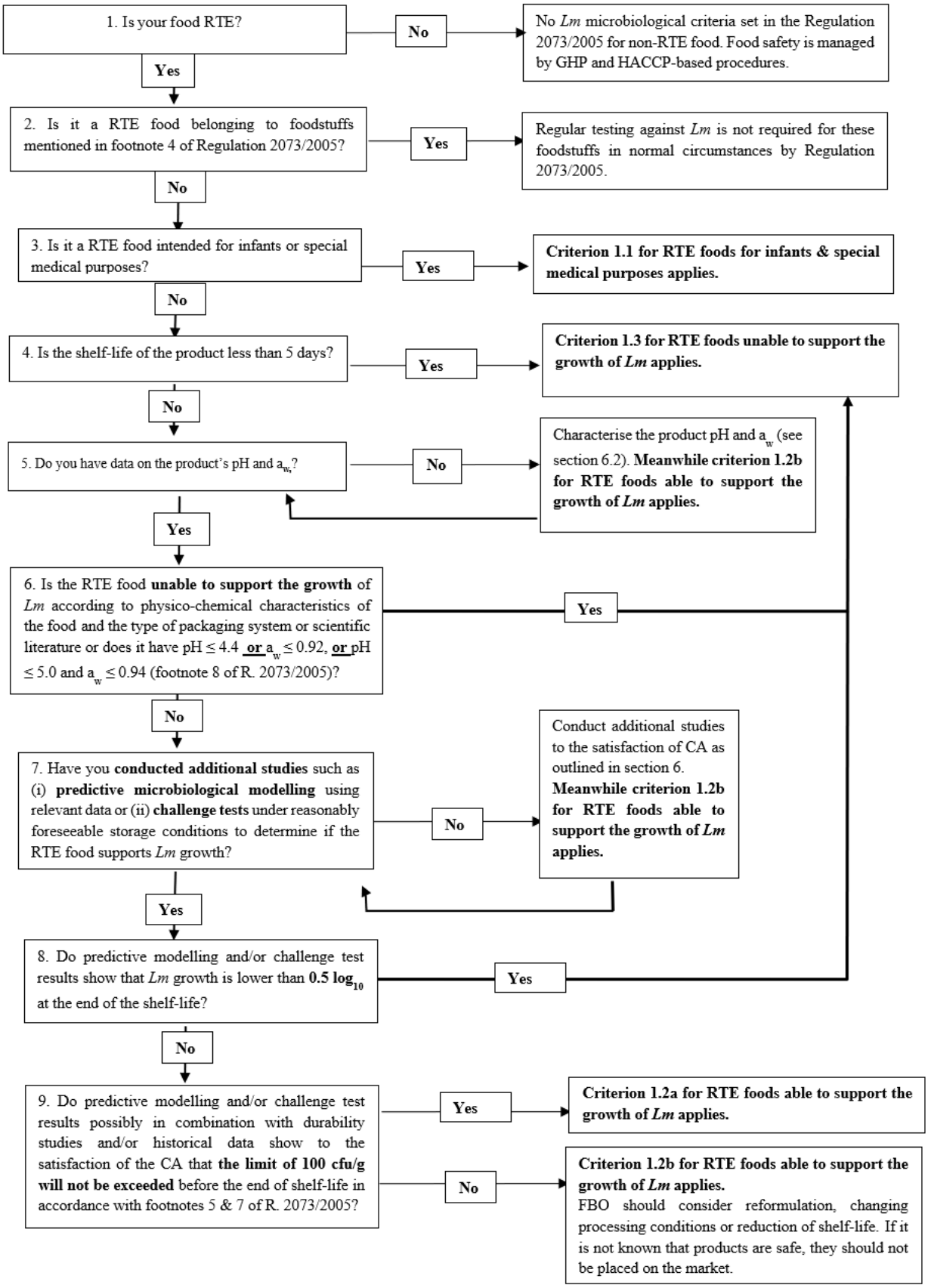

Figure 2: Simplified decision tree to determine the appropriate Lm food safety criterion for a RTE food according to Commission Regulation (EC) No 2073/2005

6. Assessing the growth behaviour of *Lm* in RTE food products

6.1. Product description

Before assessing the growth behaviour of *Lm* in RTE food products, suitable shelf-life for the food products should be established by describing the food in detail along with its intrinsic and extrinsic characteristics (Food Safety Authority of Ireland, 2022). Appendix 1 has a documentation **checklist** to help FBOs ensure they carry out all the steps necessary to investigate the growth behaviour of *Lm* in the RTE food products they produce.

The first step to establishing a reasonable shelf-life for a food product should be to prepare a detailed product description. This should be documented for each product and detail all product related information. This should include (but is not limited to) the following:

- List of ingredients and specifications for each ingredient;
- RTE status of the food;
- Processing parameters applied in production;
- Good manufacturing and hygiene practices;
- Product specific procedures based on HACCP;
- Quality control parameters and measures;
- Selection of the applicable *Lm* food safety criterion, along with details of limits to assess compliance (for correct *Lm* criterion to apply to RTE food see Fig. 2);
- Packaging details and specifications for all packaging;
- Labelling considerations (e.g. allergens, best before or use by date etc.);
- Storage, distribution and retail display conditions;
- Instructions for use as applicable.

6.2. Gather information before performing shelf-life studies with respect to *Lm*

The next step is to determine the physico-chemical characteristics for each food product (such as pH, water activity (a_w), salt content and concentration of preservatives) considering the type of packaging, the storage and processing conditions, the possibilities for contamination and the foreseen shelf-life.
The measurement methods (especially pH and a_w) should be internationally recognised or fit for purpose to reflect the intrinsic characteristics of the food relative to microbial growth. Blending, mixing and dilution of the product is not recommended, especially for composite foods where the individual components should be studied independently but after the preparation of the composite food in order to allow the different ingredients to interact with each other ones The worst-case scenario is then selected from one component. The information collected should be documented and presented in a format that is accessible for the FBO to use when needed. For example, this information should be provided to a third-party laboratory if the FBO decides to employ their services to conduct a challenge test (EURL *Lm*, 2023c).

6.2.1. Intrinsic and extrinsic characteristics

All food products have their own unique intrinsic and extrinsic characteristics which will affect food safety and shelf-life duration. Some characteristics prolong shelf-life while others decrease it. Intrinsic characteristics are those inherent to the composition of the food (e.g. pH, a_w, indigenous micro-organisms). Extrinsic characteristics are those which relate to the external processing environment which impact on the food (e.g. storage temperature and packaging). Table 1 provides information on typical intrinsic and extrinsic characteristics that can influence the growth behaviour of *Lm* in food.

Table 1. Growth /survival characteristics of Lm (strain-specific) in broth medium

Factor[a,b]	**Can grow**			**Survival (but not growth)** [d]
	Min. (lower growth limit)	**Growth Optimum**[c] **(fastest growth)**	**Max. (upper growth limit)**	
Temperature (°C)	-2	30 - 37	45	-18
pH[e]	4.0 - 4.3	7.0	9.6	3.3 - 4.2
a_w	0.92 (0.90 with glycerol)	0.99	/	<0.90
Salt (NaCl) content[f]	/	/	12	≥20
Gas atmosphere	Facultative anaerobic and microaerophilic (able to grow in presence/absence of O_2) (e.g. under vacuum or modified gas atmosphere)			
Heat treatment during food processing	A temperature/time combination e.g. of 70°C x 2 min is required for a D-6 (i.e. 10^6 or 6 decimal) reduction in numbers of *Lm* cells. Other temperature/time combinations may also provide the same reduction.			

[a] The limits for growth and survival of *Lm* 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] Note that these numbers are set based on different models and practical approaches. See section 6.4.

[c] Optimum indicates when the growth of *Lm* is fastest.

[d] Survival period will vary depending on nature of food and other factors.

[e] Inhibition of *Lm* is dependent on type of acid present.

[f] Based on percent NaCl, water phase.

Understanding, measuring and describing the intrinsic and extrinsic characteristics will help to identify characteristics that will:

i. allow microbial survival and growth in/on the food, and;
ii. act, alone or in combination, as hurdles or barriers to microbial survival and/or growth in/on the food.

Products with pH ≤ 4,4 or aw ≤ 0,92, or pH ≤ 5,0 and aw ≤ 0,94, or with a shelf-life of less than five days belong to the category to foods **unable** to support the growth of *Lm* according to footnote 8 of Commission Regulation (EC) No 2073/2005.

Once the food product has been described in detail, the FBO should use this information to compare with existing published data (e.g. scientific journals, books, industry guides, etc.) on the survival and growth of micro-organisms and, if relevant, food safety issues and incidents issued in similar foods in the past (see section 6.3 on scientific literature for more details).

The survival and growth of *Lm* in RTE foods is a function of their characteristics and the conditions, under which they are produced, packaged and stored (i.e. the intrinsic and extrinsic properties of the food). The most important product characteristics influencing the survival and growth of *Lm* in RTE foods are its pH, a_w and the temperature and time under which the food is stored. Furthermore, the preservatives and protective indigenous micro-organisms, including starter cultures, if present, may have a significant impact on the survival and growth of *Lm* 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 *Lm* can survive or grow in a particular RTE food. This information may also allow the FBO to reformulate their products to prevent or minimise the survival or growth of *Lm*.

Determination of the food product characteristics should also include a determination of the variability between batches (called inter-batch variability) and within individual batches themselves (called intra-batch variability). To estimate the inter-batch variability and the intra-batch variability, the FBO should collect data on a minimum of five samples from three different batches produced on three different occasions to reflect the possible variability that could reasonably be expected to occur for certain characteristics in the food (e.g. pH, a_w, concentration of preservatives etc.) (EURL *Lm,* 2021; EN ISO 20976-1). See section 6.2.3 for guidance on how to use this data to select the worst-case scenario when carrying out shelf-life studies to assess the growth behaviour of *Lm.*

The reasonably foreseeable conditions of use of the food product by the consumer should also be considered when determining whether *Lm* can survive or grow in a particular RTE food. In particular, consideration should be given to the product information on the label (e.g. storage conditions after opening, cooking instructions (if any), serving suggestions providing recommendations for the consumption of the product etc.).

6.2.2. Historical data

Historical data is a component of records which a food business keeps as a part of its ongoing food business operation. Historical data plays an important role in verifying the shelf life of RTE foods concerning *Listeria monocytogenes*, as they offer valuable insights into product behaviour under actual production and storage conditions. This information helps ensure that the safety criteria established during the validation process are consistently maintained. However, due to the lack of controlled environments typical in validation studies, historical data often exhibit significant variability in factors such as temperature fluctuations, handling practices, storage conditions, and sample sizes. As a result, relying on historical data for shelf-life validation should be approached with caution. In most cases, additional tools or methodologies will be necessary to enhance the reliability of the validation process.

Some of this data will be recorded by the FBO as part of its legal obligations under the food safety legislation, such as traceability records, and verification records to demonstrate the FBO's FSMS is working as it should be to ensure the production of safe food.

The following is a non-exhaustive list of potential sources of historical data:

- Certificates of Analysis (CoA) from ingredient suppliers;
- Routine FBO regular monitoring checks (e.g. temperatures, pH, a_w etc.);
- Microbiological laboratory testing of supplied ingredients;
- Microbiological laboratory testing of finished product throughout shelf-life;
- Microbiological laboratory testing of water and environmental samples;
- Records of cleaning and disinfection procedures;
- Records of corrective actions linked to non-conforming results;
- Records of complaints;
- Records of recalls and withdrawals;
- Records of official controls.

Examples of where historical data might be useful:

- Where levels of *Lm* in RTE food at the end of shelf-life are consistently low or absent and no results have been obtained which exceed the legal microbiological criterion limits set in Commission Regulation (EC) No 2073/2005. These data could be used in combination with data from environmental sampling and quality of ingredients to give the FBO 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 (EURL *Lm,* 2021).
- Historical data on levels of *Lm* in existing RTE foods at the start and end of shelf-life can be used to help verify product shelf-life under reasonably foreseeable conditions of processing, storage, distribution and use.
- Historical data on levels of *Lm* in existing RTE foods at the start and end of shelf-life can also be used to verify its potential for growth in similar RTE foods with comparable

characteristics (pH, a_w, indigenous micro-organisms, etc.) produced under practically identical conditions.

- Historical data will indicate levels of *Lm* found in the production environment, raw materials and existing RTE foods, under the food business operation's current practices of GHP and HACCP.
- It is also important to gather historical data to understand the potential inter- and intra-batch variability of the critical parameters that control *Lm*.

FBOs should demonstrate to the relevant CA that the historical data they use are sufficient to verify that the growth of *Lm* in the food throughout the shelf-life will not exceed 100 cfu/g. If the historical dataset is evaluated as insufficient by the CA to verify that the limit of 100 cfu/g will not be exceeded at the end of the shelf-life, the CA may require this data to be complemented with other studies, and a product reformulation or a reduction of the shelf-life should be considered.

6.2.3. Selecting the worst-case scenario

When the operator has a wide range of food products, it may be acceptable from a scientific point of view to categorise these products into groups with similar characteristics before carrying out shelf-life studies, for example to group similar products together for testing and/or to determine the worst-case product. The FBO should have a justifiable rationale to categorise the food products together.

Key characteristics such as pH, a_w, preservatives, packaging (gas composition and packaging material), indigenous micro-organisms etc. are recommended to be used to determine the similarity of products. Documented supporting evidence of these characteristics will be required by the CA to justify their grouping together as a single category for further study.

The following steps should be carried out when grouping products together:

- Make an inventory of products, describe their production processes and determine their intrinsic (e.g. physico-chemical, microbiological, preservatives and any other additives, etc.) and extrinsic (e.g. temperature, modified atmosphere packaging, etc.) characteristics.
- Predictive microbiology (see section 6.4) could be used to group products and establish the worst-case product within a group based on physico-chemical characteristics (e.g. probability of growth of *Lm* according to pH and a_w for a given storage temperature).

Based on the steps described above, FBOs can classify their products into groups of finished products that are similar in terms of their key physico-chemical characteristics. Then, the FBO can reasonably use the worst-case product of the group to carry out additional shelf-life studies according to the EURL *Lm* TGD (EURL *Lm*, 2021).

The FBO should confirm that the inter-batch variability is representative of its production based on historical data and that the worst-case scenario is taken into account.

The principle of inter-batch worst-case selection for conducting subsequent shelf-life studies is illustrated in Figure 3.

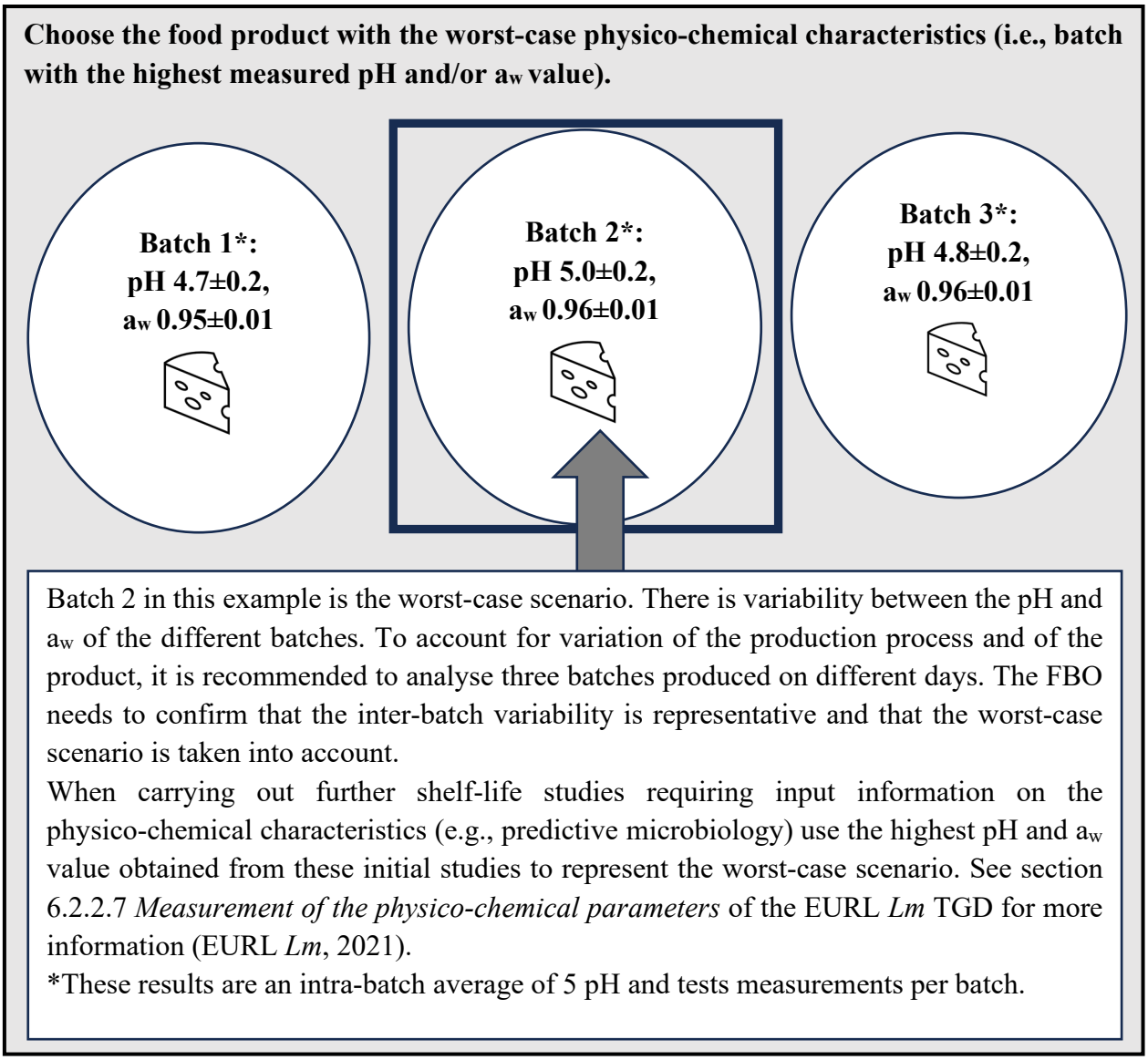

Figure 3. Example of how to select, among several batches produced, the food product with the most unfavourable combination of physico-chemical characteristics (e.g. highest pH and highest a_w).

6.3. Scientific literature

A wide range of scientific data on *Lm* in foods is available in literature. Examples of literature are scientific papers from journals, reports from ECDC and EFSA about foodborne outbreaks and surveillance studies, from research organisations, studies performed by other companies or summarised on national level in grey literature being available in, for instance, trade standards, guidance documents from competent authorities (e.g. Food Safety Authority of Ireland, 2021a). Scientific literature can be searched for from platforms like PubMed, Google Scholar, ScienceDirect, etc., and also in the predictive model IT tool ComBase.

The goal of consulting scientific literature is to get information on *Lm* survival and/or growth in various products and environmental conditions. The FBO can use scientific literature to:

- give an overview of available data for similar products;
- help in identifying the factors that affect *Lm* growth / survival in their own product;
- guide in determining the microbiological shelf-life of their products;
- provide evidence to determine if the product supports or does not support *Lm* growth;
- give information on whether additional studies are required or not.

It should be noted that FBOs need to have sufficient knowledge of microbiological studies to critically assess whether the reported study has been carried out in a context that is relevant for the product under investigation. In some cases, it may be difficult to find suitable information that matches exactly with the requirements of the product and the cold chain. Scientific literature is normally written in a style and language that is not easily understandable, and advice from qualified experts with experience in reading such literature may be useful if in house competence is not available. On the other hand, it is important that FBOs inform any third parties they may use to carry out the literature study about the intrinsic and extrinsic characteristics of their products, the realistic variations, and the reasonably foreseeable conditions of distribution, storage and use.

There are at least four categories of data presented in the scientific literature, outlined as follows:

1. Reported challenge and durability studies for specific products.

Challenge tests or durability studies performed according to official protocols have been reported for some products. It should be noted that specific challenge studies reported in the grey literature often follow the EURL *Lm* TGD (EURL *Lm*, 2021), while scientific papers may have other purposes than only to perform a challenge test. Therefore, there may be deviations to the EURL *Lm* TGD in one or more steps.

Some points to consider when interpreting the reported results include:

- checking that the product composition corresponds to the products that the FBO is investigating;

- what is the inoculation concentration? If too high (> 1000 cfu/g) it can lead to different results;
- what are the time-temperature combinations used? Have temperature scenarios relevant for reasonably foreseeable conditions of distribution, storage and use been taken into account? Do the time-temperature combinations used only represent ideal conditions and not the worst-case scenario?
- is the information describing the product in the scientific literature sufficient? Does it match exactly with the requirements of the FBO's product which is under evaluation? Does the information describing *Lm* growth behaviour in the product described in the scientific literature take the worst-case scenarios into account?

2. Other studies linked to the growth and survival of Lm in foods

Numerous scientific studies on growth or survival of *Lm* in response to use of preservatives, processes, raw materials, etc have been published in scientific literature. Such studies are also useful background information for judgment of growth and survival in specific products, but the results need to be considered more carefully considering the specific product under evaluation. Food matrices with less variation than in a real product may have been used in order to more clearly show the effects of the parameters tested.

For example, standardised forms of foods (e.g. cubes, thin slices, minced or grated food) may have been used to ensure a homogenous treatment, or a broth may be used to prevent the presence of non-homogenous food parts with varying growth conditions. While such studies provide valuable insights, the elimination or reduction of variations in real food decreases the likelihood of detecting the effects of these variations under actual conditions.

In order to ensure that information from scientific literature is applicable to the relevant food it is recommended to check at least these points if:

- the food matrix in the study is representative for the product. For instance, check if the food has been cut, minced, heat treated or in other ways been made more homogenous than in real products. Studies carried out with broth as test matrix can give useful information about relative differences of storage temperatures for example, but they may not provide accurate information about the growth behaviour in the real product.
- the concentration and volume of the inoculum of *Lm* is in an appropriate range for their purpose (EURL *Lm*, 2021). The concentration in the published study should be in the same range as in the EURL *Lm* TGD so growth can be observed. The inoculum should not change the pH, a_w or increase the buffering capacity of the product, etc. The study can be used to consider reasonably foreseeable conditions of distribution, storage and use of a real product.
- the inoculum has been exposed to treatments that lead to stress or adaptation (for example, exposure to cold chain temperatures) and will have an impact on the recovery period (lag phase) after the inoculum has been added to the product.

3. Reports of foodborne outbreaks of listeriosis

Analysis of reports of foodborne outbreaks of listeriosis associated with similar product types are among the most relevant studies to consider. They are useful to determine the root cause of the outbreak and to identify if the particular food product under investigation could be high risk in terms of becoming contaminated with *Lm* and causing foodborne listeriosis.

There is usually more than one reason for an outbreak of foodborne listeriosis. The contamination level can, in some cases, be high and/or may not be evenly distributed. Lower levels of contamination may cause illness in individuals at high risk of infection, e.g. pregnant women, infants, older adults and those who are immunocompromised (Food and Agriculture Organization/World Health Organization, 2004). An outbreak may be caused by the growth of the pathogen in the early stages of processing, or by intermittent contact of the food with a highly contaminated surface (cross-contamination from the environment). In such cases, illness can occur even if there is no growth during the final stage in the farm-to-fork chain as growth occurred earlier during processing. In other cases, outbreaks occur due to the growth of the pathogen over prolonged storage or where there are temperature abuse conditions.

4. Literature summaries obtained by using artificial intelligence

Artificial intelligence (AI) has developed largely during the last few years as a tool to summarise knowledge. Even though it is a useful tool to give a rapid overview it is still the responsibility of the FBO to check that the information is reliable and that it covers the worst-case scenario for the product under investigation. If AI is applied, it is required that the user can justify the outcome. It should also be noted that the AI tools may apply their algorithms based on biased databases and therefore may omit important information or give misleading results even if the generated report appears convincing. Use of AI is expected to become more common and also more regulated in the near future. AI cannot serve as a replacement for the sequence of studies required to validate and verify the shelf-life, but it can be used to find relevant sources of information to be critically evaluated by qualified experts.

6.4. Predictive microbiology

6.4.1. Introduction

Predictive microbiology aims to predict the behaviour of micro-organisms in raw materials, semi-finished and finished products during their production and/or storage. To do so, mathematical models (primary and secondary models) are developed in broth and/or in food to predict the impact of environmental factors (e.g. temperature) and intrinsic factors (such as pH, water activity (a_w)), and/or inhibitors (e.g. organic acids and CO_2) on *Lm* behaviour. In recent years, significant advances have been made in this field especially in acquiring data and developing models to predict *Lm* growth in foods. Today, there are data and models available in the literature and some of them have been implemented in user-friendly software to make their use accessible to a larger audience.

6.4.2. Modelling approaches

In order to predict microbial behaviour two main modelling approaches can be used (1) growth/no growth models and (2) kinetic type models:

- (1) Growth/no growth models are used to predict the probability of growth of the target organism in specific conditions. Unlike kinetic models, growth/no growth models do not predict microbial concentration over time. Based on the intrinsic characteristics of the food (e.g. pH/a_w) and the storage temperature, this approach is used to predict the growth probability of *Lm* in foods which is compared to pre-defined thresholds (Augustin *et al*. 2005):
    - If a calculated growth probability is lower than 10%, the product is qualified as not supporting growth;
    - If a calculated growth probability is between 10% and 90%, additional studies are required to conclude (kinetic model and/or challenge test);
    - If the calculated growth probability is higher than 90% the product is qualified as supporting growth.

These models can help the FBO categorise their foods into the categories defined in Regulation (EC) No 2073/2005, those allowing growth (category 1.2) and those not allowing growth (category 1.3).

- (2) Kinetic type models combine primary and secondary models to predict the concentration of the organisms over time, considering different environmental factors, during growth. The objective of using such models is to calculate, considering an initial contamination level (e.g. end of production), the time required to reach a threshold to respect the legal microbiological limit.

Usually, it is good practice to start with growth/no growth for product design (to formulate the product so that it does not support growth) and then use kinetic models to prove *Lm* remains below 100 cfu/g at the end of the shelf-life considering the initial contamination level.

For products supporting growth, it is possible to use kinetic models to demonstrate that the limit of 100 cfu/g will not be exceeded at the end of the shelf-life. Kinetic models predicting microbial lag times and growth rates in foods can help the FBO evaluate the growth of *Lm* in foods during their storage. This can be done in (i) a deterministic way, considering unique values for the input factors or (ii) in a stochastic way, considering the variability associated with the input factors:

i. In the deterministic approach, a unique value of pH, a_w and temperature profile is combined with the initial concentration to predict the concentration of *Lm* over time until the end of the shelf-life. A unique value is obtained for the concentration of *Lm* at the end of the shelf-life and can easily be compared to the limit of 100 cfu/g.
ii. In the stochastic approach, the factors are described by their distributions reflecting the different sources of variabilities that can be included in the prediction. In this case, by

taking into account the strain variability, inherent processing, inter-batch and intra-batch variability in the food products under investigation, and in reasonably foreseeable conditions of distribution, storage and use, a distribution is obtained to characterise the concentration of *Lm* at the end of the shelf-life. In this case, a high percentile of the output distribution could be compared to the limit of 100 cfu/g to evaluate the compliance with the criterion.

6.4.3. Pre-requisites before using predictive microbiology models to validate the shelf-life

The use of predictive microbiology models should be reserved to appropriately trained and proficient users. Before using predictive microbiology models, the FBO should collect information such as the physico-chemical characteristics of the food, the targeted shelf-life, a justified initial contamination level (see 6.1, 6.2 and Appendix 2). FBOs should have an understanding of the models to be used for the prediction and their underlying hypothesis. Models should be validated for the intended use. To ensure that models are validated, it is possible to use published models and code them in programming software or use predictive microbiology software, some of which were evaluated by Food Safety Agencies or Competent Authorities (see 6.4.6).

When considering kinetic type models, two main groups can be considered:

i. The models based on data obtained from culture media which are not specific to a particular food product could be used as a first approximation of *Lm* behaviour. They are used to describe the possible impact of several factors such as the foods intrinsic properties (pH and a_w) as a model input. They are a good option to consider in the absence of challenge test data in the studied food product. However, a limitation is that some of these models can fail to accurately describe the microbial behaviour in foods (e.g. not considering the effects of the competitive indigenous micro-organisms). Note that it is possible to improve the robustness of these models developed in culture medium by validating them with challenge test data obtained in foods (ISO/DIS 23691).

ii. The models based on food are developed using challenge test data and then used to predict the microbial behaviour considering the physico-chemical characteristics of the food (e.g. pH, a_w, organic acids concentrations etc.) as input parameters, together with the storage temperature. Some simpler food models which consider the effect of only one input factor (e.g. temperature) have also been proposed to predict the behaviour of micro-organisms in particular foods without the need to specify the physico-chemical characteristics as they were implicitly included in the model parameters during its development. The limitation here is that changes in the food formulation causing significant modifications in the pH and / or other food intrinsic characteristics invalidates the use of the so-called simple food model. In addition, it is also possible to include other input factors (e.g. preservatives, modified atmosphere, competition from other micro-organisms, etc.) to give more robust predictions but this requires more input data.

In all cases, it is important to document the justification of the models used and the values of the input parameters. This is useful for example to identify which of two food products with different physico-chemical characteristics (e.g. pH, a_w) will permit the fastest growth of *Lm*, or how much time duration with abuse storage temperature will influence the growth behaviour of *Lm* in the food.

6.4.4. Defining the model inputs

It is essential to document the rationale for the models employed and the values of the input parameters used in generating the model's prediction. To ensure the software is used correctly, understanding the physicochemical properties of the food product is essential. This includes parameters such as pH, a_w, stationary phase and aqueous salt concentration (% salt in water), as well as the storage temperatures of the food product throughout its shelf-life duration over reasonably foreseeable conditions of distribution, storage and use.

FBOs should rely on robust data when selecting input values for the models. For instance, when inputting parameters such as the pH and a_w of the food product, the values utilised should be derived from multiple analytical tests to ensure both inter- and intra-batch variability are adequately captured. Since some specific physicochemical characteristics may vary slightly between different batches, the parameters selected should represent the worst-case scenario for the product under investigation (see section 6.2.3).

When determining the physicochemical properties of a multi-component composite product (e.g. a RTE sandwich consisting of meat, lettuce, bread, butter, and mayonnaise), it is crucial to measure the characteristics of each individual component separately but after the preparation of the composite food in order to allow the different ingredients to interact with each other ones. It is not advisable to homogenise the product under investigation by first subjecting it to blending, mixing, or dilution prior to analysing its physicochemical characteristics, such as pH and water activity (a_w). Understanding the characteristics of the constituent ingredients in multi-component composite food products is essential, as one component may support the growth of *Lm* more effectively than others. For example, RTE meat with a high pH and a_w can support the growth of *Lm* better than mayonnaise, which has a lower pH. If modelling is employed to predict the growth rate of various foodborne pathogens or indicators in a multi-component composite food product, the a_w and pH used for the model should correspond to the worst-case constituent ingredient.

6.4.5. Practical applications of predictive microbiology

Predictive microbiology may be useful for many applications, for example:

- to predict the growth probability of micro-organisms in foods using growth / no growth models,
- to predict bacterial growth in various conditions using kinetic models,
- to estimate the contamination level on a given day of the shelf-life,
- to evaluate the impact of the inter-batch variability using the stochastic approach,
- to optimise formulation (additives, pH, a_w, salt) to ensure safety by design,
- to evaluate the impact of disruption to the cold chain, and to test different storage scenarios,

- to set the adequate level of a Critical Control Point in a process (e.g. value of a Performance Objective (PO) at end of production to meet the final Food Safety Objective (FSO) of 100 cfu/g at end of shelf-life), and
- as a screening tool to identify the worst-case product within a group of similar products before performing a challenge test on the worst-case product only.

6.4.6. Example of commonly used predictive microbiology software

More than forty predictive microbiology software programmes have been identified to date, with multiple possible applications, including microbiological shelf-life predictions considering *Lm* (Possas, Valero, & Pérez-Rodríguez, 2022; Tenenhaus-Aziza & Ellouze, 2015). It is recommended to choose tools for which the models used have been the subject of scientific publications and validation in food. Several software tools with validated models are available to predict *Lm* growth. A **non-exhaustive** list is provided in Table 2 for information.

Table 2. Non-exhaustive list of available software tools to predict Lm growth

Software	Access	Cost	Associated publication	Modeling approach	Factors included	Matrix
FSSP	http://fssp.food.dtu.dk/	Free	(Dalgaard, and Mejlholm, 2019)	D	Temperature (°C), NaCl in water phase (%), pH, smoke components, phenol (ppm), CO2 (%) in headspace at equilibrium, Nitrite (mg/kg), organic acids (ppm) in water phase (acetic acid, benzoic acid, citric acid, diacetate, lactic acid, sorbic acid) with or without Lactic Acid Bacteria	Chilled seafood and meat products
Growth Predictor	https://www.foodsctech.com/growth-predictor	Free	https://zenodo.org/records/14281569	D+S	Temperature, pH, a_w, nitrite, CO_2, phenolic compounds, organic acids (e.g. lactic, acetic) and any inhibitory compound with its Minimum Inhibitory Concentration (MIC)	Chilled meat products. Models can be calibrated to different foods by adjusting the μ_{ref} value
IPMP Dynamic Prediction	http://www.ars.usda.gov/Main/Docs.htm?docid=25312	Free	(Huang, 2014)	D	Temperature, then implicitly deduced form the food model	Cooked pork, beef hot dogs, hard boiled eggs, salmon roe, fresh-cut cantaloupe
Sym'Previus	https://symprevius.eu	Commercial	(Couvert et al., 2017)	D+S	Temperature, pH, a_w, nitrite, CO_2, lactic, and any inhibitory compound with its Minimum Inhibitory Concentration (MIC)	Cold smoked salmon. Models can be calibrated to any foods by adjusting the μ_{opt} value
MicroHibro	https://www.microhibro.com	Free (registration	Cubero-González, S., et al. (2019); Pérez-Rodríguez, F. et al (2025)	D+S	Temperature, pH, aw, nitrite, CO_2, preservatives, packaging atmosphere, aw, NaCl, organic acids (e.g.,	Multiple food categories, including meat, fish, dairy, RTE

		required)			lactic acid), time-temperature series	products. Models support growth, inactivation, survival, and probabilistic QMRA modules. Models can be introduced by advanced users and/or validated/calibrated
ComBase	https://combase.errc.ars.usda.gov/ Note: Models developed on *L. monocytogenes* and *L. innocua* data	Free	(Baranyi & Tamplin, 2004) Baranyi J. and Roberts T.A. (1994).	D	- Temperature, pH and a_w/NaCl, OR - Temperature, pH, a_w/NaCl, CO2 OR - Temperature, pH, a_w/NaCl, lactic acid (ppm) OR Temperature, pH, a_w/NaCl, nitrite (ppm)	Culture medium

NA: Not Available, D: Deterministic, S: Stochastic; D+S: some modules use Deterministic models and some others use Stochastic models.

6.4.7. Advantages and limitations

Predictive microbiology models are useful to develop safe by design food products that do not support *Lm* growth. They are also useful to quickly predict the pathogen's behaviour in foods supporting growth at a limited cost (once the model is established and validated, it can be used several times without additional costs). Using these models, it is possible to simulate different scenarios to reflect the impact of changes in the temperature and composition of the cold chain during storage, the inter-batch variability for the studied food, and also the strain variability without conducting additional experiments. When validated with challenge test data, predictive microbiology models can be used to predict *Lm* behaviour considering an increased number of reasonably foreseeable sources of variability within a short time and without additional costs, compared to the results that could be obtained from a challenge test study alone.

However, the use of predictive microbiology models should be reserved to trained users. Indeed, the selection of the adequate model and the right input factors to be considered is sometimes challenging.

For example, if both broth and food models are available the food models should be preferred. However, this choice would not be recommended if the food used in the model development (e.g. milk) is significantly different from the food for which the predictions are required (e.g. meat).

In addition, the lag time prediction can be less robust than the maximum growth rates prediction. In the absence of a good understanding of the underlining hypothesis, it is best to use a worst-case approach and not include the lag time when performing predictions. The setting of the initial and final contamination levels when performing the simulations can also be challenging and have an important impact on the simulation results.

Also, organic acids are inhibitors that can be used as input factors to limit *Lm* growth and propose safe by design formulations. However, only their undissociated form is active and used to obtain correct predictions of *Lm* growth. The analytical method used to obtain the input data is also critical (best to utilise the same analytical method used during the model development).

Finally, when a predictive microbiology software is used, it is important for the user to be familiar with the layout of the tool. Indeed, the same models could be incorporated into different tools with different interfaces, and it is important to understand (or to get the appropriate training on) how to input the different values of the factors into the model, and how to read and interpret the output of the model simulations (e.g. deterministic vs. stochastic simulations).

Despite these limitations, predictive models remain valuable tools for estimating the growth of *Lm* in foods. With the new training curricula, the new generation of food safety professionals from CAs and from industry are well at ease to use these models and software as they have a good understanding of their limitations. If in doubt, FBOs should consult third party predictive microbiology experts or consider using other available studies (e.g. challenge tests).

6.5. Challenge tests

A challenge test is a laboratory-based study used to evaluate the microbiological safety of a product. It aims to validate the shelf-life under given storage conditions by providing information on the behaviour of *Lm* (growth, survival, or decrease) when artificially inoculated.

There are two types of challenge test:

- Challenge test assessing the growth potential (Δ) of the inoculated micro-organism;
- Challenge test assessing the maximum specific growth rate (μ_{max}).

A challenge test may be performed for a single product or for a product that represents a group of products. This should be clearly indicated in the test report.

6.5.1. Prerequisites and technical information on performing a challenge test

A challenge test is usually carried out by a laboratory on behalf of an FBO. It is important that the FBO provides the laboratory with the necessary information about the product and production process before starting a challenge test. This allows the laboratory to make informed decisions in the process of carrying out the challenge test, which includes:

- Identification of factors that have an impact on the growth of *Lm*,
- Characterisation of the product and assessment of the sources of variability in the product and production process (see section 6.2),
- Demonstrating that products analysed during the challenge tests are representative of the production.

The EURL *Lm* TGD (EURL *Lm*, 2021), in chapter 6.1, provides the important prerequisites that need to be considered before starting and carrying out a challenge test:

- Description of product (commercial name of the product, weight, etc.), new formulation, new product or a product with a production history;
- Processing conditions (at least the relevant ones in the production process: for instance, thermal treatment, drying, smoking, ripening, slicing, mincing, freezing, thawing, salt curing, packaging, etc.);
- Composition of the product (labelled on the product);
- Product characteristics including the variability between and within batches of the product. It is also important to note, for certain categories of food, if the values of certain characteristics change during the shelf-life (e.g. pH values in fermented products, cheeses; or a_w values in dry ham, hard cheeses);
- Packaging condition of the end-product (including a photo of the product);
- Storage conditions during the shelf-life (taking into account reasonably foreseeable conditions during transportation, storage at manufacturer, at retail and at consumer level);
- Shelf-life, recommended (instructions on the packaging) and reasonably foreseeable conditions of use of the product.

Details related to the methodology of carrying out a challenge test to assess the growth of *Lm* are available in the EN ISO 20976-1 and further specified in the EURL *Lm* TGD (EURL *Lm*, 2021). It is important to note that:

- To account for variation of the production process and of the product, it is recommended to conduct a challenge test on three different batches coming from different production days.
- The minimum physico-chemical characteristics to know before starting a challenge test are pH and water activity. In addition, other factors such as organic acids, should be measured, as far as they are relevant to control the growth of *Lm* in the product. Background indigenous micro-organisms such as mesophilic or psychrotrophic aerobic counts, lactic acid bacteria, *Pseudomonas* spp., yeasts and moulds may also be considered.

6.5.2. Challenge test assessing the growth potential

A microbiological challenge test assessing growth potential (Δ) is a laboratory-based study that measures the growth of *Lm* in an artificially contaminated food stored under foreseeable conditions at production, storage and use. It has to reflect the foreseeable conditions that might be expected to occur throughout the distribution chain, including storage conditions between production and consumption.

The growth potential (Δ) is the difference between the highest observed *Lm* concentration in $\log_{10}$ during the test and the initial *Lm* concentration in $\log_{10}$ at the beginning of the test.

Growth potential (Δ) = (highest observed *Lm* concentration) - (initial *Lm* concentration). See EURL *Lm* TGD paragraph 6.2.1 (EURL *Lm*, 2021).

During the challenge test, *Lm* enumeration is carried out on the day of contamination and at the end of the shelf-life, with intermediate sampling points that are distributed across the shelf-life.

Microbiological challenge tests assessing the growth potential (Δ) can be used to:

- Classify a food:

when $\Delta > 0.5$ $\log_{10}$, the food is classified into "RTE foods able to support the growth of *Lm* other than those intended for infants and for special medical purposes" (category 1.2),
when $\Delta \leq 0.5$ $\log_{10}$, the food is classified into "RTE foods unable to support the growth of *Lm* other than those intended for infants and for special medical purposes" (category 1.3),

- Quantify the growth of *Lm* in a food of category 1.2 according to defined reasonably foreseeable conditions between production and consumption.
- Determine the maximum concentration of *Lm* that may be present at the end of production stage in order to comply with the limit of 100 cfu/g at the end of the shelf-life.
- To fix intermediate limits during the process that must be low enough to guarantee that the limit of 100 cfu/g is not exceeded at the end of shelf-life (Fig. 4).

The disadvantage of this type of challenge test is the lack of flexibility in the interpretation: the results are only valid for the product tested under the specified conditions, so that new experiments have to be performed each time there is a change (e.g. use of different time-temperature profiles, change of ingredients or recipe).

The storage conditions applied during challenge testing (incubation of the test units) should comply with the conditions at which the product is most likely to be subjected to, in normal use, until the end of the shelf-life. This should include the foreseeable temperature range along the cold chain: from production to retail, storage at retail and storage by the consumer.

It is the responsibility of the FBO to ensure that the storage conditions used are realistic, considering that storage temperatures labelled on the packaging may not always be maintained throughout the cold chain (from production to consumption). If an inappropriate storage temperature (lower temperature than usually encountered) is used during the challenge test, there may be an underestimation of *Lm* growth and an overestimation of the safe shelf-life length. The temperature(s) used to determine shelf-life of the product has to be properly justified and documented by the FBO.

If no data on the temperature and duration of the cold chain is available, the default temperature in Table 4 in the EURL *Lm* TGD (EURL *Lm*, 2021) should be used. When data is available, the use of this information is preferred. In this case, the 95th percentile of the data observation should be used. The FBO should inform the laboratory of the appropriate time-temperature profile to be used during a challenge test, taking into account the destined retail market and the corresponding default temperature.

6.5.3. Challenge test assessing the maximum specific growth rate

A microbiological challenge test assessing the maximum specific growth rate (μ_{max}) is a laboratory-based study that measures the rate of growth of *Lm* in an artificially contaminated food stored at a fixed temperature. For a robust μ_{max} determination it is important not to perform the study at very low temperatures and to target an appropriate temperature to observe the entire kinetic characterising *Lm* growth over time. With a robust μ_{max} estimate, it is possible to simulate the reasonably foreseeable storage conditions including for example a difference between the temperature during transport, distribution and storage.

Microbiological challenge tests assessing the maximum specific growth rate (μ_{max}) can be used to:

- Assess and quantify the growth of *Lm* under different storage temperatures to estimate the total growth potential of *Lm* for the duration of the product shelf-life.
- Determine the maximum concentration of *Lm* that may be present at the production stage to comply with the limit of 100 cfu/g at the end of the shelf-life.
- Determine the concentration of *Lm* at a given day of the shelf-life if the initial concentration is known.

The challenge test is conducted at one constant temperature. The time of the experiment should be long enough to observe the entire *Lm* growth curve and this time can be longer or shorter than the studied shelf-life. During the challenge test, *Lm* enumeration is carried out across sampling points distributed across all growth phases.

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 specific growth rate (μ_{max}) of the bacteria.

The advantage of this type of challenge test that assesses the maximum specific growth rate is the flexibility: when determined in a given condition of time and temperature, the growth rate can be estimated in other time/temperature conditions without the need to conduct another challenge test, given that the cardinal values of the studied strain are determined.

One disadvantage of this type of challenge test is that it is not designed to determine the lag time, which can lead to a different estimated concentration of *Lm* depending on whether it is considered or not. It would be possible with adequate studies to better characterise the lag time.

6.5.4 Interpretation of the results of a challenge test

The FBO is responsible for the interpretation of the results of the challenge test.

Footnote 5 of Chapter 1, Annex 1 of Commission Regulation (EC) No 2073/2005, specifies that FBOs may fix intermediate limits during the process that must be low enough to guarantee that the limit of 100 cfu/g is not exceeded at the end of shelf-life. When the growth potential of *Lm* is known, corresponding intermediate limits may be set according to the Fig. 4.

When a food is able to support the growth of *Lm* ($\Delta > 0.5$ $\log_{10}$), the Δ value may be used to estimate the growth: highest concentration of *Lm* during the food shelf-life = initial concentration of *Lm* + Δ. In practice, the highest concentration of *Lm* may be used to determine if the limit of 100 cfu/g is exceeded or not, along the entire shelf-life of the food.

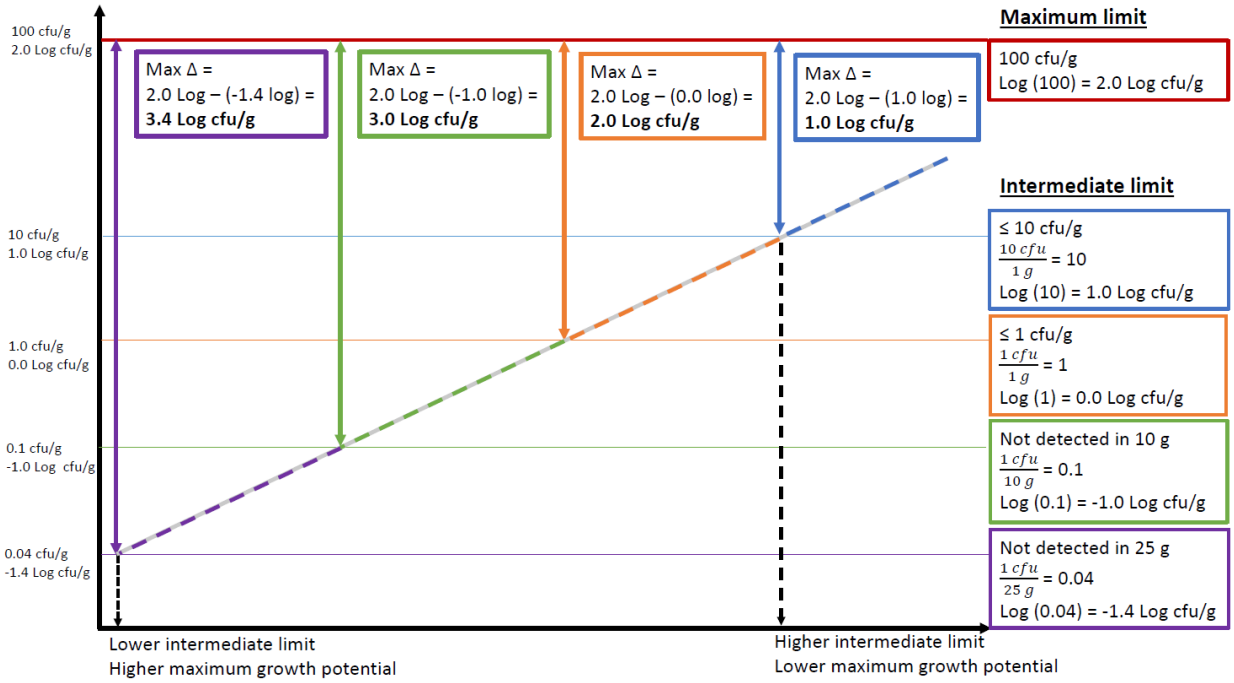

Figure 4. Relation growth potential to possible intermediate limit

More details related to the interpretation of the test results of a challenge test are available in the EURL *Lm* TGD (EURL *Lm*, 2021).

6.6. Durability studies

A durability study related to *Lm* is a laboratory study conducted to determine the concentration of *Lm* at the end of the shelf-life, in a naturally contaminated product stored under reasonably foreseeable conditions of temperature and shelf-life duration at all stages from production to consumption. The aim of durability studies is to verify that RTE foods naturally contaminated with *Lm* do not exceed the quantitative limit of 100 cfu/g at the end of the shelf-life (EURL *Lm*, 2021).

According to the EURL *Lm* TGD for conducting shelf-life studies on *Lm* in ready-to-eat foods (EURL *Lm* 2021), durability studies are most suited for verification purposes. This can be achieved by testing batches of products known to be contaminated with *Lm* at a low level (Case A). It may also be insightful when a batch is unexpectedly contaminated with *Lm* at levels that can be enumerated (Case B).

Durability studies alone should not be used to validate the microbiological shelf life of RTE foods concerning *Lm*. Instead, they should serve as a tool for shelf-life verification. While these studies use naturally contaminated batches, they can provide valuable complementary information to challenge tests, which remain the reference method for validation. The natural contamination in durability studies may more accurately reflect real-world conditions, as they account for factors

such as strain variability, potential cell injury or stress, pathogen distribution within the product, and initial *Lm* concentrations. However, these studies should be interpreted as supplementary to - and not as a substitute for - challenge tests conducted under worst-case conditions using scientifically validated methods.

6.6.1. Case A: RTE food able to support *Lm* growth and occasionally contaminated with a low level of *Lm*

Case A is where an FBO has already validated the shelf-life of RTE food supporting the growth of *Lm.* The FBO is well aware of the potential for *Lm* growth within the product based on the low level of *Lm* at the end of production (initial contamination), supported by data of the FBO (historical data). The durability study can be used in this case to verify whether the RTE food complies with the limit of 100 cfu/g *Lm* at the end of the shelf-life.
Guidance on how to carry out single random sampling for durability studies is provided in Annex 10.15 of the EURL *Lm* TGD (EURL *Lm*, 2021) and the protocol for the performance of the study is described in point 7.2 of the document. Quantitative microbiological analyses (i.e. the enumeration test for *Lm*) are performed on the samples at the end of the shelf-life on all the units stored according to the experimental storage conditions outlined in paragraph 6.2.2.6 of EURL *Lm* TGD (EURL *Lm*, 2021).

6.6.2. Case B: RTE food where a batch is unexpectedly contaminated with *Lm*

If a contamination is unexpectedly detected in a RTE food while it is still under the control of the FBO, the FBO could use this as an opportunity to perform a durability study in order to evaluate the growth of *Lm* in this naturally contaminated food product. This real-world scenario could bring additional insights in the growth of *Lm* in the food product and its shelf-life as previously validated as part of the FSMS.

To be able to interpret the results of the study, as many samples as practicable from the contaminated batch should be taken to increase the chance of selecting a contaminated unit (Avis 02-2016, SciCom AFSCA-FAVV; Avis 03-2022, SciCom AFSCA-FAVV). Samples should be taken as close to the production date as possible. Guidance on how to carry out the sampling is provided in Annex 10.15 of the EURL *Lm* TGD (EURL *Lm*, 2021) and the protocol for performing the durability study is described in point 7.2 of the EURL *Lm* TGD (EURL *Lm*, 2021).

Quantitative analyses are performed on some of the sample units that were produced as close as possible to the initial production time. The remaining sample units should be stored according to the recommended experimental storage conditions outlined in paragraph 6.2.2.6 of EURL *Lm* TGD (EURL *Lm*, 2021) and analysed at the end of the given shelf-life for the food product.

Quantitative analyses at other intermediate dates between the beginning and end of the labelled shelf-life are also recommended if feasible as it will provide additional useful information about the *Lm* growth in the RTE food product during its shelf-life. As the contamination levels might be low, it is useful to apply an enumeration method with a sufficiently low limit of quantification (LOQ) (e.g. < 10 cfu/g) in order to be able to interpret the results of the study.

6.6.3. Interpretation of the results of durability studies and recommended actions

For durability studies conducted on the same product type, produced under the same process and conditions, the expected estimated proportion of units potentially exceeding the limit of 100 cfu/g throughout the shelf-life can be calculated with a certain confidence level (e.g. 95%) by using the tool as provided in point 7.2.5. of the EURL *Lm* TGD (EURL *Lm*, 2021). The result is expressed as a confidence interval. The higher the number of sample units tested, the higher the chance of selecting a non-compliant sample and thus the narrower the confidence interval will be. If an FBO is able to gather a substantial set of results from durability studies, all complying with the limit of 100 cfu/g, these data will strengthen the confidence that the FSMS is functioning properly and that the shelf-life related to *Lm* was initially correctly set and validated. However, even if all results comply, there is still a proportion of units that could be non-compliant as shown by the calculator. Therefore, durability studies should not be used as a standalone tool for the validation of the shelf-life of a RTE food related to *Lm*. In combination with other studies, such as challenge tests or predictive microbiology, the studies can contribute to validate the shelf-life of a RTE food related to *Lm*.

In cases of non-compliant results of samples analysed during durability studies (i.e. the limit of 100 cfu/g is exceeded at the end of the shelf-life), this means that the hazard analysis within the HACCP study should be reviewed. When this occurs, it is crucial for public health that the consequences of the *Lm* limit exceeded 100 cfu/g in the RTE food product throughout its shelf-life are risk assessed and investigated thoroughly in a timely manner. This should trigger the tracking and detection of the source giving place to the non-compliance and its mitigation. The FBO is recommended to conduct a thorough root and branch investigation to identify the root cause of contamination. For example, this could be done by using structured problem-solving techniques such as root cause analysis, the '5 why's procedure', cause-and-effect diagrams etc. The Food Standards Agency in the United Kingdom has an eLearning on how to carry out root cause analysis targeted to food businesses (Food Standards Agency, 2024). A cause-effect diagram, such as a fishbone diagram, could be used to logically organise possible causes for a specific problem by graphically displaying them (Juran, 2018).

A review of the FSMS might be necessary, such as the selection of raw materials, a review of the *Lm* environmental monitoring program, a modification of the production process, an adaptation of the formulation of the product or the setting and validation of a shorter shelf-life. Non-compliant results might also be an indication of variability in the production process, meaning that the worst-case scenario was not properly assessed when setting and validating the initial shelf-life related to *Lm*.

6.7. How to combine the data generated by the studies to determine the shelf-life in relation to *Lm* and integrate them in FSMS

The studies (predictive microbiology, challenge tests, durability studies) described above in this section all contribute to a common goal: ensuring that foods on the market are safe and meet the microbiological criteria set in Commission Regulation (EC) No 2073/2005. The primary rationale for combining the data generated by these studies is to evaluate the information collectively to ensure that the concentration of *Lm* remains below 100 cfu/g throughout the entire shelf-life duration of the food product.

The studies comprise a collection of complementary resources which are useful in the three phases, setting, validation and verification of the shelf life, as illustrated in Figure 5.

For a new food product, certain studies are effective for screening to set the shelf-life. Two common approaches are benchmarking of the shelf life assigned to comparable products by other producers already on the market and literature studies performed for similar products.

After setting the shelf life, the next step is to validate the shelf-life. For this step, literature studies should be used in conjunction with other validation studies, as each food product has unique characteristics that may affect its shelf-life. Predictive models based on the physical conditions or experimental challenge studies should be preferred for validation of the shelf-. The results of these studies may indicate that the growth rate is higher or lower compared to the comparable products.

Predictive models can help identify the parameters that limit the growth of *Lm*. These parameters may include temperature, pH, a_w, and the presence of additives such as preservatives and indigenous micro-organisms. For example, if pH is determined to be the critical factor inhibiting growth, even a slight increase in pH due to minor deviations in the production process, could lead to increased growth of *Lm*. This could lead to the FBO setting a shelf-life duration that is too long and would allow the limit of 100 cfu/g of *Lm* to be exceeded throughout the shelf-life of the product. This is why it is important to take the worst-case scenario into account when using certain data to establish the shelf-life of the product (see section 6.2.3). In this example, to avoid such potential errors, measurements of pH could be included in the HACCP plan as a CCP. Consequently, shelf-life validation and the HACCP plan are interconnected tools that work in tandem.

Once the shelf-life has been validated, it is essential to verify the process by monitoring the presence of *Lm* in the environment and regularly testing food products to check if the pathogen is present in them at end of production and throughout their shelf-life. Such sampling cannot encompass the entire batch and therefore cannot serve as a substitute for the validation methods used to determine shelf-life. Consequently, the purpose of the sampling is to ensure that the conditions established for the product's shelf-life are being maintained. (European Commission, 2022; Regulation (EC) No 178/2002).

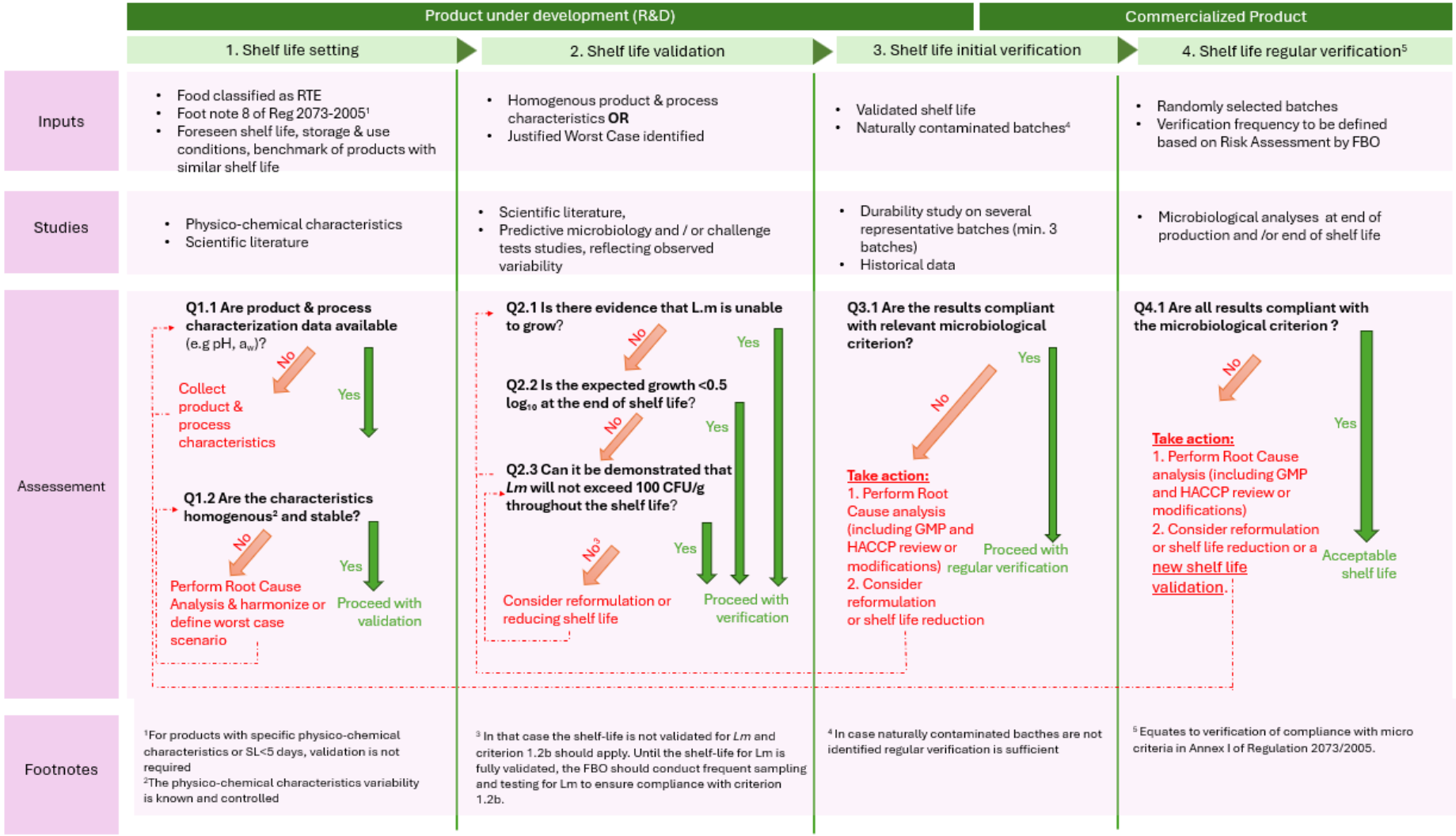

Figure 5. A flow diagram to demonstrate how the FBO can combine all the information gathered to validate and verify the shelf-life of RTE foods as regards Lm

6.8. Conclusions

All the documentation supporting the validation of *Lm* growth in the evaluated RTE food product(s) should be complied in a logical manner and maintained in a dedicated dossier, readily available upon request. This comprehensive record enables the FBO to demonstrate to the satisfaction of the CA that the shelf-life duration established in relation to the growth of *Lm* is accurate, evidence based and complies with the legal requirements. This dossier should include all supporting information (e.g. test certificates showing product's physico-chemical characteristics such as pH or a_w, copies of any relevant scientific literature or best practice guidance used as supporting evidence, data generated from predictive microbiological models, historical data, results of challenge studies etc.).

The shelf-life study should conclude with:

1. an assessment of *Lm* growth potential in the RTE product(s) under investigation,
2. a risk evaluation regarding compliance with the selected *Lm* criterion.

Any modifications to the product recipe, production process, storage conditions (time/temperature), or packaging may invalidate the shelf-life study results. In such cases, the study should be reconsidered or repeated to ensure continued compliance with food safety requirements.

7. Collaboration between FBOs for shelf-life determination

FBOs may choose to carry out shelf-life studies on their own, or they may collaborate with other FBOs in conducting shelf-life studies when they produce similar food products. Along with this collaboration, it is important that the FBO considers the environment of each individual food operation in which the RTE food is produced.

FBOs producing 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 operations requires the following aspects to be considered:

- The products should have the same physico-chemical characteristics (pH, a_w, salt content, concentration of preservatives, type of packaging, associated indigenous micro-organisms or any other characteristic important for the survival and growth of *Lm*) 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 *Lm*,
- The product recipe should be sufficiently similar and if not, the ingredients should be evaluated for their effects on the growth of *Lm*,
- 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 should consider 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 *Lm*, and
- Associated indigenous micro-organisms or starters should be identical, and if not, have the same effect on *Lm*.

The FBO should demonstrate to the CA that the products and the processing conditions are similar. 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 *Lm*. The FBO can also use relevant scientific literature and research data as supporting evidence for any conclusions made.

Any changes to the production process would require a re-evaluation of the validity of the collaborative study for that specific product. FBOs should submit the collaborative study to determine if they are conducted to the satisfaction of the CA. Some examples of previous collaborations by FBOs can be found in Appendix 2.

8. References

Actia Chlean Joint Technological Network (2021). Guide on best practices for sampling in the food industry. Available at: https://www.actia-asso.eu/en/surface-sampling/

Agencia española de seguridad alimentaria y nutrición (2019). Documento de orientación para la verificación de estudios de vida útil en relación con *Listeria monocytogenes* en alimentos listos para el consumo. Available at: https://www.aesan.gob.es/AECOSAN/docs/documentos/noticias/2019/verificacion_vida_util.pdf

Anses (2020). Data sheet on foodborne biological hazards: *Listeria monocytogenes*. Request No 2016-SA-0081. Updated: April 2020. Available at: BIORISK2016SA0081FiEN.pdf

Augustin JC, Zuliani V, Cornu M, Guillier L. (2005). Growth rate and growth probability of *Listeria monocytogenes* in dairy, meat and seafood products in suboptimal conditions. J Appl Microbiol. 2005;99(5):1019-42. doi: 10.1111/j.1365-2672.2005.02710.x. PMID: 16238733.

Baranyi J. and Roberts T.A. (1994). A dynamic approach to predicting bacterial growth in food. Int. J. Food Microbiol. 23, 277-294. doi: 10.1016/0168-1605(94)90157-0

Baranyi J, Tamplin ML. (2004). ComBase: a common database on microbial responses to food environments. J Food Prot. 2004 Sep;67(9):1967-71. doi: 10.4315/0362-028x-67.9.1967. PMID: 15453591.

Campden BRI (2022) G79 Practical control of *Listeria* in food production. Available at: https://www.campdenbri.co.uk/

Campden BRI (2020) G55/2 Cleaning and disinfection of food factories: a practical guide. Available at: https://www.campdenbri.co.uk/

Campden BRI (1999) G20 Effective microbiological sampling of food processing environments. Available at: https://www.campdenbri.co.uk/

Codex CXG 61-2007 (Rev. 2009) Guidelines on the application of general principles of food hygiene to the control of *Listeria monocytogenes* in food. Available at: https://www.fao.org/fao-who-codexalimentarius/codex-texts/guidelines/en/

Codex CXC 1-1969 (Rev. 2022) General principles of food hygiene. Available at: https://www.fao.org/fao-who-codexalimentarius/sh-proxy/en/?lnk=1&url=https%253A%252F%252Fworkspace.fao.org%252Fsites%252Fcodex%252FStandards%252FCXC%2B1-1969%252FCXC_001e.pdf

Couvert, O., Guégan, S., Hézard, B., Huchet, V., Lintz, A., Thuault, D., Stahl, V. (2017). Modeling carbon dioxide effect in a controlled atmosphere and its interactions with temperature and pH on the growth of *L. monocytogenes* and P. fluorescens. Food Microbiology, 68, 89-96. https://doi.org/10.1016/j.fm.2017.07.003

Cubero González, S., Possas, A., Carrasco, E., Valero, A., Bolívar, A., Posada-Izquierdo, G. D., García-Gimeno, R. M., Zurera, G., & Pérez-Rodríguez, F. (2019). *'MicroHibro': A software tool for predictive microbiology and microbial risk assessment in foods*. *International Journal of Food Microbiology*, **290**, 226–236. https://doi.org/10.1016/j.ijfoodmicro.2018.10.007

Dalgaard, P., Mejlholm, O. (2019). Modeling Growth of *Listeria* and Lactic Acid Bacteria in Food Environments. Chapter 20 in Bridier, A. (Ed.), Foodborne Bacterial Pathogens: Methods and Protocols, Methods in Molecular Biology, vol. 1918, https://doi.org/10.1007/978-1-4939-9000-9_20, © Springer Science + Business Media, LLC, part of Springer Nature 2019. Pp 247-264

Direction générale de l'alimentation (DGAL) (2009). Note de service relative à la classification du fromage Roquefort dans la catégorie 1.3 du critère *Listeria monocytogenes* défini dans le chapitre 1 de l'annexe I du règlement (CE) n 2073/2005 (DGAL/SDSSA/N2009-8004). Available at: https://info.agriculture.gouv.fr/boagri/instruction-N2009-8004

Direction générale de l'alimentation (DGAL) (2024). Instruction technique DGAL/SDSSA/2024-270, 29/04/2024. Available at: https://info.agriculture.gouv.fr/boagri/instruction-2024-270

European Commission. National Guides to Good Hygiene Practice for Food and Feed. Available at: https://webgate.ec.europa.eu/dyna2/hygienelegislation/.

European Commission, 2022, *C*ommission Notice on the implementation of food safety management systems covering Good Hygiene Practices and procedures based on the HACCP principles, including the facilitation/flexibility of the implementation in certain food businesses (2022/C 355/01; published on 16.9.2022)

European Food Safety Authority Panel on Biological Hazards (2020b) Koutsoumanis K, Allende A, Alvarez-Ordóñez A, Bolton D, Bover-Cid S, Chemaly M, Davies R, De Cesare A, Herman L, Nauta M, Peixe L, Ru G, Simmons M, Skandamis P, Suffredini E, Jacxsens L, Skjerdal T, Da Silva Felicio MT, Hempen M, Messens W and Lindqvist R. Guidance on date marking and related food information: part 1 (date marking). EFSA Journal 18(12):6306, 74 pp. Available at: https://doi.org/10.2903/j.efsa.2020.6306

European Food Safety Authority Panel on Biological Hazards (2021) Koutsoumanis K, Allende A, Alvarez-Ordóñez A, Bolton D, Bover-Cid S, Chemaly M, Davies R, De Cesare A, Herman L, Hilbert F, Nauta M, Peixe L, Ru G, Simmons M, Skandamis P, Suffredini E, Jacxsens L, Skjerdal T, DaSilva Felicio MT, Hempen M, Messens W and Lindqvist R. Guidance on date marking and

related food information: part 2 (food information). EFSA Journal 19(4):6510, 45 pp. Available at: https://doi.org/10.2903/j.efsa.2021.6510

European Food Safety Authority Panel on Biological Hazards (2018), Ricci A, Allende A,Bolton D, Chemaly M, Davies R, Fernandez Escamez PS, Girones R, Herman L, Koutsoumanis K,Nørrung B, Robertson L, Ru G, Sanaa M, Simmons M, Skandamis P, Snary E, Speybroeck N, Ter Kuile B,Threlfall J, Wahlstrom H, Takkinen J, Wagner M, Arcella D, Da Silva Felicio MT, Georgiadis M, Messens W and Lindqvist R. Scientific Opinion on the *Listeria monocytogenes* contamination of ready-to-eat foods and the risk for human health in the EU. EFSA Journal 2018;16(1):5134, 173 pp. Available at: https://doi.org/10.2903/j.efsa.2018.5134

European Centre for Disease Prevention and Control (ECDC) and European Food Safety Authority (EFSA) (2018) Joint ECDC-EFSA Rapid Outbreak Assessment. Multi-country outbreak of *Listeria monocytogenes* serogroup IVb, multi-locus sequence type 6, infections linked to frozen corn and possibly to other frozen vegetables – first update 3 July 2018. Available at: https://www.ecdc.europa.eu/sites/default/files/documents/2018_ECDC-EFSA_ROA_Update1_UI-444_Listeria_0.pdf

EURL *Lm* (European Union Reference Laboratory for *Listeria monocytogenes*) (2021) Technical guidance document on challenge tests and durability studies for assessing shelf-life of ready-to-eat foods related to *Listeria monocytogenes* Version 4 of 1 July 2021. doi: https://doi.org/10.5281/zenodo.7760700

EURL *Lm* (2023a) Technical Guidance Document on sampling the food processing area and equipment for the detection of *Listeria monocytogenes* V4 2023-10-03. doi: https://doi.org/10.5281/zenodo.8406616

EURL *Lm* (2023b) Tutorials for the implementation of sampling techniques. Available at: https://sitesv2.anses.fr/en/minisite/listeria-monocytogenes/tutorials-implementation-sampling-techniques

EURL *Lm* (2023c) Guidance document to evaluate the competence of laboratories implementing challenge tests and durability studies related to *Listeria monocytogenes* in ready-to-eat foods. doi: https://doi.org/10.5281/zenodo.7748234

Food and Agriculture Organization/World Health Organization (FAO/WHO) (2004). Risk assessment of *Listeria monocytogenes* in ready-to-eat foods. Technical report. Report no.: Microbiological Risk Assessment Series 5. Available at: https://www.who.int/publications/i/item/9241562625

Food and Agriculture Organization/World Health Organization (FAO/WHO) (2022). *Listeria monocytogenes* in ready-to-eat (RTE) foods: attribution, characterization and monitoring –

Meeting report. Microbiological Risk Assessment Series No. 38. Rome. doi: https://doi.org/10.4060/cc2400en

Food and Agriculture Organization/World Health Organization (FAO/WHO) (2016). Statistical Aspects of Microbiological Criteria Related to Foods. A Risk Managers Guide. Microbiological Risk Assessment Series, no 24. Rome. 120pp. Available at: https://apps.who.int/iris/handle/10665/249531

Food Safety Authority of Ireland (2005). The Control and Management of *Listeria monocytogenes* Contamination of Food. Available at: https://www.fsai.ie/publications/the-control-and-management-of-listeria-monocytogen

Food Safety Authority of Ireland (2006). 1st Trimester National Microbiological Survey 2006 (06NS1): Microbiological Safety/Quality of Raw Mushrooms. Available at: https://www.fsai.ie/getmedia/dcf6c81e-fa79-44f8-b106-a9baef51da52/raw_mushrooms.aspx?ext=.pdf

Food Safety Authority of Ireland (2011). *Listeria monocytogenes*. Microbial factsheet series. Issue 1. Available at: https://www.fsai.ie/publications/listeria-monocytogenes

Food Safety Authority of Ireland (2014) Guidance Note No. 27 Guidance Note on the enforcement of Commission Regulation (EC) No. 2073/2005 on microbiological criteria for foodstuffs. Available at: https://www.fsai.ie/

Food Safety Authority of Ireland (2021a) Identifying Appropriate Peer Reviewed Scientific Publications. Available at: https://www.fsai.ie/getmedia/729c2ab1-efa2-416e-a56c-5673a6395dab/guidance-on-identifying-appropriate-peer-reviewed-scientific-publications-1.pdf?ext=.pdf

Food Safety Authority of Ireland (2021b) Guidance Note 20 Industrial processing of heat-chill foods. Available at: https://www.fsai.ie/getmedia/68870b71-bd82-4303-90f2-c62b0e9b5afe/10144_fsai-guidance-note-20-accessible.pdf?ext=.pdf

Food Safety Authority of Ireland (2022) Guidance Note 18 Validation of product shelf-life. Available at: https://www.fsai.ie/getmedia/2d6fc2de-7e55-4ebf-808d-453f72c460ab/guidance-note-no-18-validation-of-product-shelf-life-(revision-5)-2.pdf?ext=.pdf

Food Standards Agency (2024). Elearning on root cause analysis. Available at: https://rcatraining.food.gov.uk/#home

FSSC 22000 (2023). Guidance document: environmental monitoring. Version 2. Available at: FSSC-22000-V6-Guidance-Document-Environmental-Monitoring-1.pdf

Huang L (2014). IPMP 2013--a comprehensive data analysis tool for predictive microbiology. Int J Food Microbiol. 2014 Feb 3;171:100-7. doi: 10.1016/j.ijfoodmicro.2013.11.019

International Commission on Microbiological Specifications for Foods (ICMSF) (2018). Microorganisms in Foods 7: Microbiological Testing in Food Safety Management. Springer. Available at: https://www.icmsf.org/

ISO/IEC 17025:2017 General requirements for the competence of testing and calibration laboratories. Available at: https://www.iso.org/standard/66912.html

ISO 18593:2018 Microbiology of the food chain - Horizontal methods for surface sampling. Available at: https://www.iso.org/standard/64950.html

ISO 20976-1:2019 Microbiology of the food chain — Requirements and guidelines for conducting challenge tests of food and feed products Part 1: Challenge tests to study growth potential, lag time and maximum growth rate. Available at: https://www.iso.org/standard/69673.html

ISO 20976-2:2022 Microbiology of the food chain - Requirements and guidelines for conducting challenge tests of food and feed products. Part 2: Challenge tests to study inactivation potential and kinetic parameters. Available at: https://www.iso.org/standard/79327.html

ISO/DIS 23691 Microbiology of the food chain — Determination and use of cardinal values. Available at: https://www.iso.org/standard/76665.html

Juran (2018) The ultimate guide to cause and effect diagrams. Available at: https://www.juran.com/blog/the-ultimate-guide-to-cause-and-effect-diagrams/

Leong D, Alvarez-Ordóñez A, Guillas F, Jordan K. Determination of *Listeria monocytogenes* Growth during Mushroom Production and Distribution. Foods. 2013 Nov 27;2(4):544-553. doi: 10.3390/foods2040544. PMID: 28239137; PMCID: PMC5302272.

ListWare - the Digital Shelf-Life Calculator. Available at: https://listware.animalia.no/

Mackey B.M. and Bratchell, N. (1989) The heat resistance of *Listeria monocytogenes*. L. Appl. Microbiol. 9, 89-94. doi: 10.1111/j.1472-765X.1989.tb00298.x

NIZO. Predicting *Listeria* growth in cheese. Available at: Phttps://www.nizo.com/cases/predicting-listeria-growth-in-cheese/

Opinion of the Scientific Committee on Food in respect of *Listeria monocytogenes*, expressed on 22 June 2000. Available at: https://food.ec.europa.eu/document/download/9d608c5c-0425-443f-8bc4-e63afb659cfb_en?filename=sci-com_scv_out25_en.pdf

Pérez-Rodríguez, F., Valero, A., & Bolívar, A., eds. (2025). *Basic Protocols in Predictive Microbiology Softwares*. Methods and Protocols in Food Science. Springer Nature. DOI: 10.1007/978-1-0716-4112-5

Possas A, Valero A, Pérez-Rodríguez F (2022). New software solutions for microbiological food safety assessment and management. Current Opinion in Food Science, Volume 44, 2022, 100814, ISSN 2214-7993, https://doi.org/10.1016/j.cofs.2022.100814

Spanu, C. and Jordan, K. (2020) *Listeria monocytogenes* environmental sampling programme in ready-to-eat processing facilities: A practical approach. Comprehensive Reviews in Food Science and Food Safety 19:2843-2861. doi: 10.1111/1541-4337.12619

SciCom, 2016. Avis 02-2016 du Comité scientifique de l'AFSCA sur tests de provocation et tests de vieillissement pour *Listeria monocytogenes* dans le fromage. Available at: https://scicom.favv-afsca.be/comitescientifique/avis/2016/_documents/Avis02-2016Listeriamonocytogenes_website_000.pdf

SciCom, 2022. Avis 03-2022 du Comité scientifique de l'AFSCA sur le potentiel de croissance de *Listeria monocytogenes* dans le beurre fermier à base de lait cru. Available at: https://scicom.favv-afsca.be/comitescientifique/avis/2022/_documents/Avis03-2022_SciCom2021-16_ListeriaBeurre.pdf

Skandamis, P. (2024). Growth Predictor: a predictive modelling and quantitative microbial risk assessment software. Zenodo. doi: 10.5281/zenodo.14281569

Skjerdal T, Gangsei LE, Alvseike O, Kausrud K, De Cesare A, Alexa EA, Alvarez-Ordóñez A, Moen LH, Osland AM, From C, Nordvik B, Lindbäck T, Kvello J, Folgerø B, Dommersnes S, Hauge SJ (2021). Development and validation of a regression model for *Listeria monocytogenes* growth in roast beefs. Food Microbiol. 2021 Sep;98:103770. doi: 10.1016/j.fm.2021.103770

Tenenhaus-Aziza F, Ellouze M. Software for predictive microbiology and risk assessment: a description and comparison of tools presented at the ICPMF8 Software Fair. Food Microbiol. 2015 Feb;45(Pt B):290-9. doi: 10.1016/j.fm.2014.06.026. Epub 2014 Jul 5. PMID: 25500394.

The Standing Committee on the Food Chain and Animal Health (2010) Guidance on the implementation of Articles 11, 12, 14, 17, 18, 19 and 20 of Regulation (EC) N° 178/2002 on General Food Law. Available at: https://food.ec.europa.eu/system/files/2016-10/gfl_req_guidance_rev_8_en.pdf

Tompkin, R.B. (2002) Control of *Listeria monocytogenes* in the Food-Processing Environment. Journal of Food Protection 65(4):709–725. doi: 10.4315/0362-028x-65.4.709

Wemmenhove, E. (2019). Risk assessment of *Listeria monocytogenes* in Gouda cheese. [internal PhD, WU, Wageningen University]. Wageningen University. doi: 10.18174/466848

9. Abbreviations

AI: artificial intelligence

CA: competent authority

CCP: critical control point

CoA: certificates of analysis

cfu: colony forming units

EC: European Commission

ECDC: European Centre for Disease Prevention and Control

EFSA: European Food Safety Authority

EU: European Union

EURL: European Union Reference Laboratory

FBO: food business operator

FIC: food information to consumers

FSO: Food Safety Objective

FSMS: food safety management system

g: gram

GHP: good hygiene practices

HACCP: hazard analysis and critical control points

HPP: high-pressure processing

ISO: International Organization for Standardization

Lm: *Listeria monocytogenes*

LOQ: low level of quantification

No: number

PO: Performance Objective

PRPs: Prerequisite Programmes

RTE: ready-to-eat

STEC: Shiga toxin-producing *E. coli*

TGD: Technical Guidance Document

10. Glossary

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.

Food Safety Objective:
a critical component of risk management in food safety typically expressed in terms of the number of pathogenic microorganisms (such as bacteria, viruses, or parasites) that are allowed per gram or millilitre of food. It serves as a target or benchmark that food producers, processors, and handlers must strive to achieve to ensure that the final product is safe for consumer.

Good Hygiene Practices (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.

Grey literature:
information produced on all levels of government, academia, business and industry in electronic and print formats not controlled by commercial publishing (Third International Conference on Grey Literature in 1997 (ICGL Luxembourg definition, 1997 - Expanded in New York, 2004))

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.

Lag phase: phase, directly after inoculation, during which the microbial population is adapting to the environment, before it enters the exponential growth phase.

Lag time (λ): kinetic parameter in time unit to characterise the duration of the lag phase.

Maximum specific growth rate: kinetic parameter to characterise the exponential growth phase, represented by the slope of the curve showing the evolution of the natural logarithm (μ_{max}) or decimal logarithm (V_{max}) of the population as a function of time, under constant growth conditions.

Performance Objective:
a quantitative statement of the maximum frequency and/or concentration of a hazard in a food at a specified step in the food chain, before the time of consumption, that is considered acceptable and will permit achieving the Food Safety Objective (FSO).

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).

Primary model:
mathematical model describing the changes of microbial concentration as a function of time under constant and known conditions of intrinsic and/or extrinsic factor(s)

Ready-to-eat (RTE) food:
Food intended by the producer for direct human consumption without the need for cooking or other processing effective to eliminate or reduce to an acceptable level micro-organism of concern, as defined in Article 2, point g) of the Commission Regulation (EC) No 2073/2005.

Retail:
The handling and/or processing of food and its storage at the point of sale or delivery to the final consumer. It includes distribution terminals, catering operations, factory canteens, institutional catering, restaurants and other similar food service operations, shops, supermarket distribution centres and wholesale outlets.

Safe by design:
The term “safe design” is used in different industries to refer to the proactive approach of designing products, systems, and environments that are safe to use. In the food sector, the microbiological application of the concept consists in formulating foods to prevent the growth of potentially contaminating micro-organisms.

Secondary model:
Mathematical model describing the effects of the intrinsic and / or extrinsic factor(s) (e.g. temperature, pH, a_w) on the parameters of the primary model (e.g. maximum specific growth rate)

Shelf-life:
Either period corresponding to the period preceding the minimum durability or the 'use by' date, as defined respectively in Articles 2 and 24 of Regulation (EU) No 1169/2011.

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:
Obtaining evidence that a control measure or combination of control measures, if properly implemented, is capable of controlling the hazard to a specified outcome (Codex CXC 1-1969, Rev. 2022).

Verification:
The application of methods, procedures, tests, and other evaluations, in addition to monitoring, to determine whether a control measure is or has been operating as intended (Codex CXC 1-1969, Rev. 2022).

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 Why's procedure:
Root cause analysis method that focuses on identifying the key problem to be solved through a series of 'Why?' related questions. These questions, if asked correctly, can be used to successfully trace back to the root of the issue. The principle indicates that 5 of these specific 'Why's?' are sufficient in determining the underlining causal problem. However, this is dependent on how specific the questions being asked are (Food Standards Agency (2024)).

11. Appendix 1: An example of a FBO documentation checklist to validate the shelf-life of RTE food with respect to *Lm*

As a FBO, you should include the following documentation in your shelf-life study to demonstrate[2] the growth of *Lm* in your food products should they become contaminated with the pathogen:

1. A detailed product specification should be documented for each product produced. This should detail all product related information. This will include (but is not limited to) the following:

   ☐ Name of food product
   ☐ List of ingredients and specifications for each ingredient
   ☐ List of legal and national guideline food safety criteria and process hygiene criteria relevant to the food product, including which microbiological criterion applies in relation to *Lm* under Commission Regulation No 2073/2005
   ☐ Packaging details and specifications for all packaging
   ☐ Shelf-life duration
   ☐ Labelling considerations (e.g. allergens, best before/use by date etc.)
   ☐ Storage, distribution and retail display conditions
   ☐ Consumer instructions for use on label if applicable

2. Understanding, measuring and describing the food products physico-chemical (or intrinsic and extrinsic) characteristics will help to identify (i) characteristics that will allow microbial survival and growth in/on the food and (ii) characteristics that will act, alone or in combination, as hurdles or barriers to microbial survival and/or growth in/on the food. This will include (but is not limited to) the following information (depending on which is applicable to your RTE food product):

   ☐ pH (demonstrate inherent variability linked to the product and possible worst-case scenario by measuring a number of batches (see section 6.2.3))
   ☐ Water activity (a_w) (demonstrate inherent variability linked to the product and possible worst-case scenario by measuring a number of batches (see section 6.2.3))
   ☐ Water content and salt content (%)
   ☐ Preservatives (type and added and/or final concentration in the end product)
   ☐ Other food additives (type and concentration)
   ☐ Natural indigenous micro-organisms or added micro-organisms (e.g. starter cultures)
   ☐ Mix and concentration of gases if used for modified atmosphere packaging
   ☐ Processing conditions (e.g. thermal treatment, chilling, smoking etc.)
   ☐ Historical data (this will help to show the possibilities for initial contamination of the RTE food product with *Lm* – (see section 6.2.2))

[2] This demonstration is not necessary for food covered by the pH and a_w provisions of footnote 8 of Regulation (EC) No 2073/2005.

☐ Storage conditions at each stage of the cold chain, including reasonably foreseeable conditions of distribution, storage and use (i.e. the foreseen shelf-life)

3. Once the RTE food product has been described in detail, use this information to compare your food product with existing published data (e.g. scientific journals, books, industry guides, etc.) on the survival and growth of *Lm* in food products with similar intrinsic and extrinsic characteristics . Review and document any information you find on foodborne outbreaks of listeriosis that occurred due to contamination of similar RTE food products in the past. It can also be useful to review if any recalls have been required for similar food products in the past due to possible contamination with *Lm*. This information is useful to understand the likelihood of *Lm* contamination in the RTE food product and how *Lm* might grow in the RTE food product should it become contaminated. This will include (but is not limited to) the following:

   ☐ A search of scientific journals, books, industry trade guides, research organisations, guidance from national competent authorities, web search engines etc. to gather scientific information and guidance relevant to the RTE food you are producing on the likelihood of contamination, and on how *Lm* might grow in your RTE food product should it become contaminated on the basis of its physico-chemical characteristics
   ☐ A search to gather information on any cases of listeriosis linked to the consumption of the RTE food product you are producing (e.g. in reports from ECDC and EFSA about foodborne outbreaks)
   ☐ Retain a copy of any relevant documentation or information you find to build your scientific evidence base regarding the possible growth and survival characteristics of *Lm* in your RTE food product

On the basis of the information gathered for points 1 to 3, it may be necessary for you to conduct additional studies to determine how *Lm* could grow in your food product if it was contaminated. In particular, it is important to determine whether *Lm* if present could exceed the microbiological criterion limit of 100 cfu/g throughout the shelf-life of your RTE food product. As necessary, the further studies you carry out may include:

4. If choosing to carry out mathematical modelling to predict the growth of *Lm* in your RTE food product, you are advised to consider the recommendations in the following list (which is not exhaustive):

   ☐ Determine if access to relevant knowledge and proficiency of predictive microbiology is available within your food business operation. If not, consider if the employment of third-party predictive microbiology experts would be possible. Do not proceed any further if no expertise is available
   ☐ Research the literature and / or different predictive mathematical modelling programmes available to assess if one would be suitable for your needs (see section 6.4)

☐ Based on your proficiency level in predictive microbiology, decide if you will code the adequate model in a programming software (e.g. R, Python, Matlab) or use a Predictive Microbiology tool where the adequate model is already available

☐ Decide if you need to use a model developed in broth or in a relevant food product and document the rationale for your decision

☐ Decide if you need to use a model that can take into account a wide number of physico-chemical characteristics simultaneously and document the rationale for your decision. The reasonably foreseeable conditions of storage temperature variations throughout distribution, storage and use of the RTE food is a mandatory factor that you must consider

☐ Identify the relevant input factors for the model and their associated values based on the data you have for your food product. The inputs permitted will depend on the model selected (e.g. food type, temperature, pH, water activity, salt (NaCl) concentration, indigenous micro-organisms, nitrite, organic acids, smoke [phenol]). To run the predictions, use foreseeable storage conditions for the chosen shelf-life and worst-case scenario in the absence of data

☐ Decide if you need to use a model that can take into account the variability of certain physico-chemical characteristics (e.g. pH, a_w) and document the rationale for your decision to use a deterministic model (considering no variability) or a stochastic model (considering variability)

☐ Use the information listed above to choose the most appropriate predictive mathematical modelling programme relevant for your food type and model inputs and document the rationale for your decision

☐ Determine the initial concentration of *Lm* likely in your product based on historical data (use worst-case scenario data) and document the rationale for your decision

☐ Choose not to include a lag time (i.e. stationary phase) when running the model in order to predict growth under the worst-case scenario if you have no scientific data to justify using a different value to represent the lag time

☐ Run the model

☐ List all your assumptions (e.g. no lag time as a worst-case scenario), input values (e.g. pH values based on data collected on several batches), and predictions (e.g. curves showing the changes in the *Lm* concentration over time) for inclusion in the predictive microbiology report

☐ Document your interpretation from the predictive microbiology model output

☐ Document your conclusions along with the rationale for them in your predictive microbiology report

5. If you decide to carry out a challenge test on your RTE food product, you are recommended to:

☐ Check that the challenge test has been carried out according to the EURL *Lm* TGD protocol (EURL *Lm,* 2021) by using the checklist in Annex 2 of the Guidance document on competence of laboratories implementing *Lm* shelf-life studies (EURL *Lm*, 2023c)

☐ Check that the report includes in an annex, an overview of the data coming from you as the FBO

6. If you decide to carry out a durability study on your RTE food product, you are recommended to:

   ☐ Check that the durability study has been carried out according to the EURL *Lm* TGD protocol (EURL *Lm*, 2021)
   ☐ Check that the durability study has taken into account reasonably foreseeable conditions of distribution, storage and use
   ☐ Check that the laboratory provided a report that outlines the purpose of the durability study, the conditions under which the durability study has been carried out, the results obtained and a conclusion
   ☐ Check that the report includes in an annex, an overview of the data coming from you as the FBO

7. The items listed in points 1 to 6 are the steps required to determine the potential growth of *Lm* in a RTE food product, and in particular to assess the possible risk of *Lm* growing to a level of greater than 100 cfu/g throughout the shelf-life of a RTE food product should that food product be contaminated. The items you have carried out as relevant to your RTE food product should be collated and filed together in a logical order to complete your shelf-life study dossier. It is recommended that you document a conclusion at the end of your shelf-life study which summarises all of the information you have provided with respect to demonstrating the growth of *Lm* in your RTE food product throughout its shelf-life. The following list of items (non-exhaustive) are suggested to be included in your concluding remarks:

   ☐ A statement on whether your RTE food product will or will not support the growth of *Lm* and the rationale for your conclusion
   ☐ A statement on the possible initial contamination of your RTE food product and the rationale for your conclusion
   ☐ A statement on whether the microbiological limit of 100 cfu/g *Lm* could potentially be exceeded during the shelf-life duration you propose for your product under reasonably foreseeable conditions of distribution, storage and use should your product become contaminated
   ☐ Document the rationale for any additional controls you may need to implement to minimise the growth of *Lm* in your product
   ☐ A statement on whether the shelf-life duration you propose for your product is valid and the rationale for your conclusion
   ☐ Include a list of any references you have used as supporting evidence at the end of the shelf-life study

12. Appendix 2: Examples of previous collaborations by FBOs

According to Art. 3.2 of Commission Regulation (EC) No 2073/2005, FBOs may collaborate with each other when conducting studies in accordance with Annex II to investigate the compliance of RTE foods that are able to support the growth of *Lm* with the microbiological criteria set down in food category 1.2 throughout the shelf-life. This appendix lists some relevant examples of FBO collaborations to demonstrate the growth behaviour of *Lm* in different RTE food types.

1. Assessment of *Lm* growth in Gouda cheese in the Netherlands

In the Netherlands, FBOs collaborated together to develop a calculation tool designed to conduct a risk assessment regarding the growth of *Lm* in Gouda cheese. This tool can effectively demonstrate that traditionally produced Gouda cheeses do not promote the growth of *Lm*. The calculation tool is based on a comprehensive study by Wemmenhove (2019). The work was supported by the Dutch Dairy Organization and the Dutch Dairy Board (Predicting *Listeria* growth in cheese https://www.nizo.com/cases/predicting-listeria-growth-in-cheese/).

2. Studies to classify Roquefort cheese as food category 1.3

In France, certain professional organizations have joined forces to classify certain types of products into food category 1.3 (i.e. RTE foods that are unable to support the growth of *Lm*). In 2008, the Confédération Générale de Roquefort submitted a protocol to classify Roquefort cheese in this category. The protocol was studied and validated by Anses, and Roquefort is now classified as "does not support the growth of *Lm*" (Direction générale de alimentation, 2009).

3. Challenge study on raw whole fresh mushrooms

Mushrooms (like many fruits and vegetables) are eaten in their raw and cooked form and therefore it is important that they are free from contamination (both microbiological and chemical). Data from a study (Food Safety Authority of Ireland, 2006) recorded *Lm* detection in 1.1% (8/727) of raw mushroom samples taken at retail units in Ireland. However, none of these samples had levels of *Lm* >100 cfu/g. A microbial working group comprising representatives of the Irish mushroom industry engaged with the regulatory bodies in Ireland and agreed that clarification as to the most appropriate food safety assessment criteria of *Lm* on refrigerated fresh whole closed cap prepackaged mushrooms was required. Thus, the Irish FBOs collaborated in conducting a challenge study to assess the growth potential of *Lm* on fresh whole mushrooms (*Agaricus bisporus*) (Leong *et al.*, 2013).

The results of the challenge test carried out in accordance with the parameters of the EURL *Lm* guidelines for challenge studies. It showed that *Lm* did not grow (i.e. the growth potential was < 0.5 $\log_{10}$) on refrigerated fresh whole closed cap prepackaged mushrooms throughout the shelf-life duration for this product. The competent authority in Ireland accepted the results of the challenge study. Based on this study refrigerated fresh whole closed cap prepackaged mushrooms are categorised as a product that is "*unable to support the growth of Listeria monocytogenes other than those intended for infants and for special medical purposes."* (i.e. they fall under food category 1.3 in Commission Regulation (EC) No 2073/2005).

4. Development of predictive modelling tool for RTE meat and composite products

In Norway, meat trade organisations and researchers collaborated to measure and document physico-chemical characteristics data (a_w, pH etc.) of common groups of RTE meat and composite products (i.e. products with more than one ingredient). They also collaborated to carry out challenge studies. This data was used to develop and validate a predictive modelling tool called ListWare (https://listware.animalia.no/, Skjerdal *et al*. (2021)).

The distinguishing feature of the ListWare tool, as opposed to standard predictive model tools, is that its user interface is based on the composition of foods, including ingredients, additives, and packaging conditions etc. The tool contains a database with the mapped variations of the products based on products on the market in Norway. The growth rates and estimated time until the 100 cfu/g limit is exceeded is given based on predictive models which were validated using the data gathered for these specific products. ListWare covers the mapping of variation, the growth rate estimation, and the shelf-life estimation.

5. Guides to good practice

In some countries, several professional organizations pre-define the shelf-life for certain products whose physico-chemical characteristics are known to be stable. The shelf-life for these products is defined in national guides to good practice. Medium to small-sized FBOs can choose to adopt the recommended shelf-life duration for the products defined in the guidance provided that their products have the same physico-chemical characteristics, and that the shelf-life is regularly verified. The European Commission maintain a register of national guides to good hygiene practice available at https://webgate.ec.europa.eu/dyna2/hygienelegislation/.

References:

Wemmenhove, E. (2019). Risk assessment of *Listeria monocytogenes* in Gouda cheese. [internal PhD, WU, Wageningen University]. Wageningen University. https://doi.org/10.18174/466848

NIZO. Predicting *Listeria* growth in cheese. Available at: https://www.nizo.com/cases/predicting-listeria-growth-in-cheese/

Direction générale de l'alimentation (DGAL) (2009). Note de service relative à la classification du fromage Roquefort dans la catégorie 1.3 du critère *Listeria monocytogenes* défini dans le chapitre 1 de l'annexe I du règlement (CE) n 2073/2005 (DGAL/SDSSA/N2009-8004). Available at: https://info.agriculture.gouv.fr/boagri/instruction-N2009-8004

Food Safety Authority of Ireland (2006). 1st Trimester National Microbiological Survey 2006 (06NS1): Microbiological Safety/Quality of Raw Mushrooms. Available at: https://www.fsai.ie/getmedia/dcf6c81e-fa79-44f8-b106-a9baef51da52/raw_mushrooms.aspx?ext=.pdf

Leong D, Alvarez-Ordóñez A, Guillas F, Jordan K. Determination of *Listeria monocytogenes* Growth during Mushroom Production and Distribution. Foods. 2013 Nov 27;2(4):544-553. doi: 10.3390/foods2040544. PMID: 28239137; PMCID: PMC5302272.

ListWare - the Digital Shelf-Life Calculator. Available at: https://listware.animalia.no/

Skjerdal T, Gangsei LE, Alvseike O, Kausrud K, De Cesare A, Alexa EA, Alvarez-Ordóñez A, Moen LH, Osland AM, From C, Nordvik B, Lindbäck T, Kvello J, Folgerø B, Dommersnes S, Hauge SJ (2021). Development and validation of a regression model for Listeria monocytogenes growth in roast beefs. Food Microbiol. 2021 Sep;98:103770. doi: 10.1016/j.fm.2021.103770

European Commission. National Guides to Good Hygiene Practice for Food and Feed. Available at: https://webgate.ec.europa.eu/dyna2/hygienelegislation/.