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Report of the Scientific Committee of the Spanish Agency for Food Safety and Nutrition (AESAN) regarding the verification of *Listeria monocytogenes* shelf-life studies for ready-to-eat foods

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Abstract

The Spanish Agency for Food Safety and Nutrition (AESAN) and the competent authorities of various regional governments (autonomous communities) created a working group for the preparation of a "Guidance Document for the verification of *Listeria monocytogenes* shelf-lilfe studies for ready-to-eat foods. The Section of Food Safety and Nutrition of the Scientific Committee of the Spanish Agency for Food Safety and Nutrition was asked to revise the Guidance Document and propose practical examples for its better understanding.

The Guidance Document describes a procedure that serves as a tool for the competent inspection authorities in order to verify the suitability of the *Listeria monocytogenes* shelf-life studies prepared by food business operators dedicated to the manufacturing, packaging and repackaging of ready-to-eat foods.

The Document collects the existing information around the shelf-life studies and describes a procedure for the verification of said studies, including a checklist which enables the compliance with different requirementes of the presented studies to be checked.

The Excellence Network for quantitative biological risk assessment in Spain, BIOQURA, prepared a series of practical examples, of which a brief description is collected in the present report.

The Scientific Committee concludes that the Guidance Document is adequate, at present, for the purpose established. This Document should be regularly updated in light of the experience obtained from its application, progress in scientific knowledge, changes in the legislation and guidelines on this type of studies which may be developed at national or European Union level.

Key words

Shelf-life studies, Ready-to-eat foods, *Listeria monocytogenes.*

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1. Introduction

Regulation (EC) No. 2073/2005 (EU, 2005) establishes in its Article 3 that food business operators responsible for the manufacture of ready-to-eat foods are obliged to conduct shelf-life studies in order to investigate compliance with the microbiological criteria when these food may pose a *Listeria monocytogenes* risk for public health.

The Spanish Agency for Food Safety and Nutrition (AESAN) and various autonomous communities have created a working group to prepare a "Guidance document for the verification of *Listeria monocytogenes* shelf-life studies for ready-to-eat foods" (hereinafter the Guidance Document) which describes a procedure which serves as a tool for the competent inspection authorities to verify the suitability of shelf-life studies in relation to *Listeria monocytogenes* carried out by food business operators engaged in the manufacture, packaging and repackaging of ready-to-eat food.

The Section of Food Safety and Nutrition of the Scientific Committee of the AESAN have been asked to review the Document and to make contributions as they consider necessary and recommend practical examples for a better understanding and application by the competent inspection authorities.

2. Assessment of the Guidance Document proposal for the verification of *Listeria monocytogenes* shelf-life studies for ready-to-eat foods

The proposal of the Guidance Document brings together, on the one hand, the considerable amount of existing information available on shelf-life studies, both in terms of legislation and how to implement it, summarised in a relatively short text and, on the other hand, it describes a procedure for the verification of these studies. For this reason, it is considered relevant and may be of great use and interest for its end users.

The Document includes an introduction and sections describing the objective, the legal base and the reference documents. It includes a section of ready-to-eat food categories that are able or unable to promote the growth of *Listeria monocytogenes* in accordance with that established in Annex I of Regulation (EC) No. 2073/2005. In addition, there are sections allocated to shelf-life studies: types of studies (developed by a business or in collaboration with other businesses) and relevant information that must be provided. Lastly, the Guidance Document contains four Annexes with the specifications for the different types of shelf-life study, the hurdle technology, a decision tree for the classification of the food in the groups established in Regulation (EC) No. 2073/2005 and a checklist so that the official control inspectors can check compliance of the different requirements in the studies presented.

The Committee reviewed the Guidance Document and made several observations including how to more clearly separate the verification procedure from other more general information, avoid repetitions or relate the verification procedure more expressly to the checklist from one of the annexes. The checklists may become the tool that the competent authorities rely on to carry out their work. Nevertheless, given the diverse range of possible situations and the difficulty involved in the interpretation of whether or not a shelf-life study submitted by a business has been correctly prepared, these checklists may be of limited use in some of these possible situations. In this res-

pect, it may be useful, for those questions from the checklist for which it is considered necessary, to include a reference to the section of the Document in which this matter is discussed, or even a clarification of the matter.

Specific reference should be made to the guidance document to evaluate the competence of laboratories implementing challenge tests and durability studies related to *L. monocytogenes* in ready-to eat foods (ANSES, 2018) at different points in the Guidance Document. The inclusion is recommended, as a new Annex to the Guidance Document, of Annex 2 of the afore-mentioned document, which includes a checklist for assessing the technical competence of the laboratories performing challenge tests.

With respect to the request for practical examples which illustrate some of the possible situations which may occur, and to try to reduce the uncertainty faced by some competent inspection authorities, the Scientific Committee working group responsible for this report contacted the Network of Excellence for the biological quantitative risk assessment in Spain, BIOQURA, who were willing to prepare these practical examples.

3. Practical examples

It is assumed that the specific laboratory studies have been carried out by laboratories which comply with the "EURL Lm Guidance document: competence of laboratories implementing Lm shelf-life studies V2 - 7 May 2018" (ANSES, 2018).

The examples of the shelf-life studies have been prepared according to the questions used in the decision tree. A brief description is given below of the examples prepared:

- For the first question from the tree "Is food intended by the producer or manufacturer for direct human consumption without the need for cooking or other processing effective to eliminate or reduce *Listeria monocytogenes* to an acceptable level?" two examples of products have been prepared. These require heating prior to consumption and the question arises as to whether or not they are ready-to-eat: one is precooked and frozen lasagne which the consumer has to heat in a microwave following the instructions of the manufacturer. The other is three delights fried rice which must be heated in a frying pan. The question is whether this heating is sufficient to reduce *L. monocytogenes* to an acceptable level (>6 log cycles). After analysing the heat penetration results in the products, it is observed that heating the lasagne in the microwave does not guarantee this reduction. Therefore, from this point of view, it would be considered as a ready-to-eat food and should comply with the microbiological criteria applicable to this food group. In the case of three delights rice, the heating would guarantee the reduction of *L. monocytogenes*, and it would therefore not be considered as a ready-to-eat food and the microbiological criteria of *L. monocytogenes* would not apply.
- For the second question, "Does the RTE (ready-to-eat) food belong to the food categories in which *L. monocytogenes* is most probably absent or its growth is limited?", an example has been prepared consisting of a vacuum-cooked (*sous-vide*) meat preparation to which a heat treatment is applied which manages to inactivate more than 5 log cycles of *L. monocytogenes* compared to the previously packaged food and, a priori, without the possibility of recontami-

nation. In this case, although it is a ready-to-eat food product, under normal circumstances regular tests for this criterion would not be required.

- In relation to the fourth question, "comparing the product characteristics and scientific literature, is there evidence that *L. monocytogenes* does not grow in this product?", two examples have been prepared. A double example which aims to show the variability inherent to the product. The example involves the study of the specifications of the physical and chemical characteristics of two potato salad based products and, specifically, the pH, which is lower but close to 4.4, the growth limit of *L. monocytogenes*. One of the products has a very narrow pH range, all below the limit of 4.4 and, therefore, it would be considered that *L. monocytogenes* does not grow in this food product and the limit of 100 CFU/g would be applied throughout the shelf-life. The other would have a much greater variability, with part of the interval above a pH of 4.4, which would present doubts with respect to the possible growth of *L. monocytogenes* and it would be necessary to conduct complementary shelf-life studies. The other example includes a food product, feta cheese, which is not included a priori among those which do not permit the growth of *L. monocytogenes*, but in which the scientific literature shows it is unable to grow.
- In the fifth question, "has appropriate predictive microbiology (modelling) been performed?", a double example has been prepared to demonstrate one adequate use and one unsuitable use of predictive microbiology. In both cases, the predictive model used is appropriate for performing the study, but in one of the cases (fresh cheese), the company uses a storage temperature which is too low, and therefore the study is considered inadequate. The other case, salmon *sashimi* under modified atmosphere packaging, represents an adequate use of predictive microbiology, which demonstrates that it does not exceed 100 CFU/g of *L. monocytogenes* at the end of the shelf-life of the food product.
- For the sixth question, "Is there appropriate historical data for the growth of *L. monocytogenes* in the product and/or have the durability studies been performed as described in the European Union Reference Laboratory technical guidance document on the *L. monocytogenes* shelf-life studies for ready-to-eat food?", an example has been prepared on a durability study performed on a tabbouleh-type salad, which demonstrates the minimum number of units which the business should include in this study to guarantee that the 100 CFU/g limit is not exceeded during the product shelf-life.
- Lastly, in the seventh question, "has a challenge test been performed as described in the European Union Reference Laboratory technical guidance document on the *L. monocytogenes* shelf-life studies for ready-to-eat foods?", two examples have been used once again, one with coleslaw and another with pâté. Both are challenge tests to assess the growth potential. In the first, it is shown that the growth of *L. monocytogenes* is not supported while in the second it is supported.

In all the examples, the adequate conditions necessary for the performance of the study are discussed and, if the study is not sufficient, instructions are given for reaching a satisfactory result.

4. Assessment of the Guidance Document for the verification of *Listeria monocytogenes* shelf-life studies for ready-to-eat foods

The AESAN drafted a new Guidance Document for the verification of *Listeria monocytogenes* shelf-life studies for ready-to-eat foods which includes the suggestions of the Scientific Committee with respect to the structure of the Document, the checklists and the references to the guidance document to evaluate the competence of laboratories implementing shelf-life studies in relation to *Listeria monocytogenes*.

The final conclusion of the Scientific Committee is that the Guidance Document for the verification of *Listeria monocytogenes* shelf-life studies for ready-to-eat foods is adequate, at present, for the intended purpose.

The Guidance Document should be regularly updated in light of the experience obtained from its application, progress in scientific knowledge, changes in the legislation and the directives on this type of study which may be developed at national or European Union level.

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References

EU (2005). Commission Regulation (EC) No. 2073/2005 of 15 November 2005 on microbiological criteria for foodstuffs. OJ L 338 of 22 December 2005, pp: 1-26.

ANSES (2018). Agence nationale de sécurité sanitaire de l'alimentation, de l'environnement et du travail. European Union Reference Laboratory for *Listeria monocytogenes* EURL Lm Guidance Document to evaluate the competence of laboratories implementing challenge tests and durability studies related to *Listeria monocytogenes* in ready-to-eat foods. Version 2 – 7 May 2018.