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Review

The challenge of challenge testing to monitor *Listeria monocytogenes* growth on ready-to-eat foods in Europe by following the European Commission (2014) Technical Guidance document

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ABSTRACT

European Regulation (EC) No. 2073/2005 lays down the microbiological criteria for certain microorganisms in foods and the implementing rules to be complied with by food business operators (FBOs) in Europe when implementing general and specific hygiene measures. In relation to *Listeria monocytogenes*, this regulation covers primarily ready-to-eat (RTE) food products, and requires different microbiological criteria depending on the ability of the food product to support growth of *L. monocytogenes*. In addition, this regulation establishes that food safety is the responsibility of the FBO. The FBO can conduct studies to evaluate the growth of *L. monocytogenes* that may be present in the product during the shelf-life under reasonably foreseeable storage conditions of distribution, storage and use in order to investigate compliance with the criteria throughout the shelf-life of the product. The European Union Community Reference Laboratory for *L. monocytogenes* published a revised technical guidance document in June 2014 for conducting shelf-life studies on *L. monocytogenes* in RTE foods. This review article describes the recently published European guidance document, with special focus on the design of challenge studies to determine the growth potential of *L. monocytogenes* on foods. Information is given particularly on what a challenge test is and when one is advisable. The factors to be considered and the laboratory methodology to be applied when performing a challenge test to determine the growth potential of *L. monocytogenes* in a defined food matrix are also described. Results of recent research articles applying challenge tests to determine the growth of *L. monocytogenes* in a range of foodstuffs are summarized and discussed. Finally, recommendations for obtaining data that can contribute to any further revision of the guidance document and for addressing the main challenges of challenge testing for FBOs are presented.

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1. Introduction

Listeria monocytogenes is a Gram-positive, facultatively anaerobic, non-sporeforming rod involved in cases of food-borne listeriosis infection. *L. monocytogenes* is widely distributed in the environment and has been isolated from a variety of sources, including soil, vegetation, silage, faecal material, sewage and water. It is frequently present in raw foods of both plant and animal origin, and it can be found in cooked foods due to post-processing contamination. Thus, it has been isolated from foods such as raw and unpasteurized milk, cheese, ice cream, raw vegetables, fermented meats and cooked sausages, raw and cooked poultry, raw meats, and raw and smoked seafood. In addition, its ubiquitous presence also leads to the potential for contamination of the food processing environment, where occurrence and persistence of *L. monocytogenes* is frequent (Fox, Hunt, O'Brien, & Jordan, 2011; Nakari et al., 2014; Vongkamjan, Roof, Stasiewicz, & Wiedmann, 2013).

Although listeriosis is a relatively rare disease, it can be life threatening, and is of particular concern for pregnant women, the elderly and immunocompromised individuals, with fatality rates of 20 to 30% being common among hospitalised patients (Vazquez-Boland et al., 2001). Listeriosis occurs in different forms: neuromeningeal, maternal-neonatal and febrile gastroenteritis, and in serious cases it can lead to brain infection and even death. According to the latest EU summary report on zoonoses, zoonotic agents and food-borne outbreaks (European Food Safety Authority [EFSA], 2014), 1642 confirmed human cases of listeriosis were reported in the European Union in 2012, representing a 10.5% increase compared with 2011. The EU notification rate was 0.41 cases per 100,000 population, with the highest member state specific notification rates observed in Finland, Spain and Denmark. On average, 91.6% of the cases were hospitalised. This is the highest proportion of hospitalised cases of all zoonoses under EU surveillance. A total of 198 deaths due to listeriosis were reported by 18 member states in 2012, which was the highest number of fatal cases reported since 2006.

European Regulation (EC) No. 2073/2005 (European Commission (EC), 2005) lays down the microbiological criteria for certain microorganisms in foods and the implementing rules to be complied with by food business operators (FBOs) when implementing general and specific hygiene measures. In relation to *L. monocytogenes*, this regulation covers primarily RTE food products, and requires the following: (i) in RTE products intended for infants and for special medical purposes *L. monocytogenes* must not be present in 10×25 g; and (ii) in RTE products other than those for infants and special medical purposes different microbiological criteria apply depending on the ability of the food product to support growth of *L. monocytogenes*. Thus, for RTE foods unable to support the growth of *L. monocytogenes*, the levels should be <100 CFU/g throughout the shelf-life of the product ($n = 5$; $c = 0$). On the other hand, in RTE foods that are able to support the growth of the bacterium, *L. monocytogenes* must not be present in 5×25 g samples at the time of leaving the production plant; however, if the producer can demonstrate, to the satisfaction of the competent authority, that the product will not exceed the limit of 100 CFU/g throughout its shelf-life, the level should be <100 CFU/g throughout the shelf life of the product ($n = 5$, $c = 0$). In addition, this regulation establishes that the safety of the food is the responsibility of the FBO who can conduct studies to evaluate the growth of *L. monocytogenes* that may be present in the product during the shelf-life under reasonably foreseeable storage conditions of distribution, storage and use in order to investigate compliance with the criteria throughout the shelf-life of the product. This triggers the question on how the FBO decides if the product is able or unable to support the growth of *L. monocytogenes*, and how compliance with the 100 CFU/g limit throughout the shelf-life can be demonstrated. In this regard, the Directorate-General of Health and Consumers (DG SANCO) of the European Commission published a document directed at Food Business Operators who produce ready-to-eat (RTE) foods aimed to help them to demonstrate to the satisfaction of the competent authority that their products comply with the Community Regulation, to understand the range of different approaches available to help establish a safe product shelf-life in relation to *L. monocytogenes*, and to classify their products into RTE foods in which growth of *L. monocytogenes* can occur or in RTE foods in which growth of *L. monocytogenes* will not occur during their shelf-life (DG SANCO, 2008).

Determining the ability of foods to support the growth of *L. monocytogenes* is not simple since many RTE foods are traditionally produced in local regions using variable formulations which may have an impact on the fate of *L. monocytogenes*. The Food Standards Agency of New Zealand has recently published guidelines for undertaking challenge studies (Food Standards Agency New Zealand [FSANZ], 2014), although this document is not specifically related to *L. monocytogenes*. On the other hand, Canada also has guidelines which specifically relate to *L. monocytogenes* (Health Canada, 2012). In Europe, in order to facilitate the task of performing challenge studies, the European Union Community Reference Laboratory for *L. monocytogenes* prepared a technical guidance document in 2008 in collaboration with seven laboratories, including six National Reference Laboratories for *L. monocytogenes* (European Commission [EC], 2008). This guidance document was aimed at describing the microbiological procedures for determining growth of *L. monocytogenes* using challenge tests in the frame of the application of the Regulation (EC) No. 2073/2005. The content of this technical guidance document has been reviewed by Beaufort (2011). However, feedback from food processors and independent laboratories indicated a need for the revision of the guidance document and to develop a more user-friendly set of guidelines to facilitate such analyses. In September 2012 the revision of the "EURL *Lm* Technical Guidance document for conducting shelf-life studies on *L. monocytogenes* in ready-to-eat foods" commenced. The European Union Community Reference Laboratory for *L. monocytogenes* established a working group of representatives of 10 national reference laboratories, 1 associate national reference laboratory and 1 laboratory on behalf of a national reference laboratory, and the updated version of the technical guidance document has been recently published (European Commission (EC), 2014).

This review article describes the above mentioned, recently published European guidance document, with special focus on the design of challenge studies to determine the growth potential of *L. monocytogenes* on foods. Particularly, information is given on what a challenge test is, when one is advisable, the factors to be considered and the laboratory methodology to be applied when performing a challenge test to determine the growth ability of *L. monocytogenes* in a defined food matrix. Moreover, results of recent research articles applying challenge tests to determine the growth of *L. monocytogenes* in a range of foodstuffs are summarized and discussed. Finally, recommendations for obtaining data that can contribute to any further revision of the guidance document are presented.

2. Application of challenge tests on *L. monocytogenes* in the food industry following the EU Technical Guidance document

The growth ability of *L. monocytogenes* in food products may be estimated based on specifications of physico-chemical characteristics of the product, consultation of the available scientific literature, or predictive mathematical modelling. However, in most cases growth assessment will involve laboratory-based studies, so-called challenge tests. A challenge test can be defined as a laboratory-based study that measures the growth of *L. monocytogenes* in artificially contaminated food stored under foreseeable abuse conditions of transportation, storage at retail and at consumer level. As a primary objective, challenge tests aim to determine whether or not a particular food product has the ability to support growth of *L. monocytogenes*. An indication of the growth potential is obtained from the difference between the $\log_{10}$ CFU/g at the end of the shelf-life and the $\log_{10}$ CFU/g at the beginning of the test. When this difference is greater than 0.5 $\log_{10}$ CFU/g the food is classified into RTE foods able to support the growth of *L. monocytogenes*. Alternatively,