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**Accompanying note: revision of the  
EURL Lm Technical Guidance Document  
for conducting shelf-life studies on *Listeria  
monocytogenes* in ready-to-eat foods  
(draft Version 3-20/02/2014)**

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The “EURL Lm Technical Guidance Document for conducting shelf-life studies on *Listeria monocytogenes* in ready-to-eat foods” has been revised since September 2012 by EURL Lm and a working group of representatives of 10 NRLs, 1 associate NRL and 1 laboratory on behalf of a NRL.

The enclosed draft third version shows important modifications in the structure and the content of the document, compared to the 2<sup>nd</sup> version (November 2008). This revised version introduces a first step of review of data provided by the food business operator (FBO) on the selected product, before starting a challenge test, relying on the expertise of the laboratory. This new version is more detailed in its whole content (addition of an introduction and of several explanatory annexes).

The main modifications between the 2<sup>nd</sup> and the 3<sup>rd</sup> version are listed below:

1. In the 2<sup>nd</sup> version, an **introduction** gathered Foreword and Scope (details about each shelf-life study and selection of appropriate microbiological procedures). In the 3<sup>rd</sup> version, the introduction gives some general aspects about *Listeria monocytogenes*, ready-to-eat foods, legislative background and another EU Guidance Document dedicated to FBOs. The scope is separated from the introduction.
2. The **scope** of the 3<sup>rd</sup> version differentiates validation of shelf-life (with challenge tests) from verification of shelf-life (with durability studies).
3. Whereas the 2<sup>nd</sup> version introduced the challenge tests assessing the growth potential, the 3<sup>rd</sup> version develops a new part: **Review of data** which is based on Annex II of the Regulation (EC) No 2073/2005 and the EU Guidance Document dedicated to FBOs.



#### 4. About the **challenge test assessing the growth potential**:

- Related to batches, the 2<sup>nd</sup> version recommended at least 3 batches. The 3<sup>rd</sup> version gives some tools to determine the number and the choice of batches (predictive microbiology, calculator developed and provided by EURL *Lm*, batches that are the most favourable for *L. monocytogenes* growth).
- Related to strain selection, the 2<sup>nd</sup> version recommended to use a mixture of at least 3 strains, one was a reference strain and the others came from the same or similar food matrix. The 3<sup>rd</sup> version gives some information about the number and the choice of strains (mixture of at least 2 strains whose one has to have known growth characteristics), strains characteristics and storage.
- Related to the preparation of inoculum, the 2<sup>nd</sup> version recommended to perform prior trials, to stop the 2<sup>nd</sup> subcultures at the late exponential phase or early stationary phase, to mix subcultures at the same concentration and to check the inoculum concentration on TSA. The 3<sup>rd</sup> version recommends to stop the 2<sup>nd</sup> subcultures at stationary phase, to mix subcultures in equal quantity and to check the inoculum concentration on selective agar.
- Related to the preparation and inoculation of test units:
  - The 2<sup>nd</sup> version recommended to prepare at least 3 test units at “Day 0” and at least 3 others at “Day End” for the measurement of physico-chemical characteristics and the same scheme for the test units dedicated to the enumeration of the associated microflora was adopted. The 3<sup>rd</sup> version recommends to prepare at least 1 test unit at “Day 0” and at least another one at “Day End” for both the measurement of physico-chemical characteristics, gas atmosphere and the enumeration of the associated microflora.
  - For the non-inoculated test units dedicated to the detection of *L. monocytogenes*, the 2<sup>nd</sup> version indicated that the challenge test could be continued even if *L. monocytogenes* was present at “Day 0”. The 3<sup>rd</sup> version recommends to stop the test.
  - The contamination level was fixed at 50-100 cfu/g in the 2<sup>nd</sup> version while in the 3<sup>rd</sup> version it is fixed at around 100 cfu/g.
  - A new item is added in the 3<sup>rd</sup> version: a challenge strain control.
- Related to storage conditions:
  - In the 3<sup>rd</sup> version there is an additional decision level (FBO’s data) and the 75<sup>th</sup> percentile in the 2<sup>nd</sup> version becomes 85<sup>th</sup> percentile.
  - About storage duration, the 3<sup>rd</sup> version recommends to apply 1/3 of the total shelf-life of the product for each stage of the cold chain, whatever the shelf-life duration.
- Related to the measurement of physico-chemical characteristics, the 2<sup>nd</sup> version recommended to measure pH and  $a_w$  while the 3<sup>rd</sup> version recommends to measure in addition other factors such as preservatives.
- A new paragraph about gas atmosphere is added in the 3<sup>rd</sup> version.



- Related to microbiological analyses, the 3<sup>rd</sup> version requires accreditation for the detection and enumeration of *L. monocytogenes* and it requires regular participation to proficiency testing trials for other microbiological parameters.
  - Related to the calculation of growth potential, the maximum standard deviation at “Day 0” in the 2<sup>nd</sup> version was set at 0.3 log<sub>10</sub> cfu/g whereas it is set at 0.5 log<sub>10</sub> cfu/g in the 3<sup>rd</sup> version.
  - Related to the test report, additional information is listed in the 3<sup>rd</sup> version .
5. About the **challenge test assessing the maximum growth rate**:
- Related to strain selection, the 3<sup>rd</sup> version specifies that one of 2 selected strains has to have known growth characteristics.
  - Related to the preparation of inoculum, the 2<sup>nd</sup> version recommended to prepare 2 subcultures at 37°C until the beginning of the stationary phase. The 3<sup>rd</sup> version recommends to apply the same instructions as for challenge test assessing the growth potential, except that the 2<sup>nd</sup> subcultures are not mixed.
  - Related to the preparation and inoculation of test units:
    - The 2<sup>nd</sup> version recommended to detect *L. monocytogenes* at “Day 0” and enumerate it at “Day End”. The 3<sup>rd</sup> version specifies to detect *L. monocytogenes* at “Day 0” and at “Day End”.
    - In the 2<sup>nd</sup> version, at least 3 test units at “Day 0” and at least 3 others at “Day End” were prepared for the measurement of physico-chemical characteristics and 2 or (10 to 15) test units were dedicated to the enumeration of the associated microflora. The 3<sup>rd</sup> version recommends to prepare at least 1 test unit at “Day 0” and at least another one at “Day End” for both the measurement of physico-chemical characteristics, gas atmosphere and the enumeration of the associated microflora.
  - For the measurement of physico-chemical characteristics, gas atmosphere and microbiological analyses, the same modifications as for challenge test assessing the growth potential are made.
  - Related to the calculation of the maximum growth rate, the software Microfit used in the 2<sup>nd</sup> version is replaced by the software DMFit in the 3<sup>rd</sup> version. By taking into account the confidence interval in the 3<sup>rd</sup> version, the highest limit of this confidence interval is retained for further calculations.
  - Related to the test report, the 3<sup>rd</sup> version lists additional information.
6. About the verification of the shelf-life (**durability studies**), in the 3<sup>rd</sup> version, some details about implementation of EC Regulation 2073/2005 are introduced and the test report is deleted.
7. Nine **annexes** are added in the 3<sup>rd</sup> version, providing a decision tree, flow diagram of the studies and several examples.



This draft version 3 represents an agreement of WG members and of all NRLs (the draft has been circulated to all NRLs by circular letter dated 04/11/2013). Only on one aspect, a complete consensus of WG members could not be reached: the calculation method for the growth potential. The majority of the WG members agreed to calculate the growth potential by the difference between the median  $\log_{10}$  value at the final analysis day and the median  $\log_{10}$  value at the initial analysis day, for each batch. The BE and NL NRLs didn't agree with it and preferred to calculate the difference between the maximal  $\log_{10}$  value at the final analysis day and the minimal  $\log_{10}$  value at the initial analysis day, for each batch. Thus the 2<sup>nd</sup> calculation method is introduced in the 3<sup>rd</sup> version in a note, as an alternative calculation method.