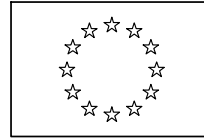




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EU COMMUNITY REFERENCE
LABORATORY FOR
LISTERIA MONOCYTOGENES

**Guide on measurement uncertainty for the enumeration of
*Listeria monocytogenes***

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Introduction & scope

A main purpose of the NRLs *Listeria monocytogenes* activities is to ensure the **reliability of the *L. monocytogenes* analyses** in the frame of official controls conducted in their respective countries. The measurement uncertainty (MU) associated to the analyses performed in each laboratory is recognized to be an important criterion to assess the reliability of these analyses. Thus each NRL *L. monocytogenes* should ensure that the laboratories approved in its country for *L. monocytogenes* official controls:

- (i) have estimated MU associated to their analyses for *L. monocytogenes* enumeration, according to the guidelines given in this document (see 2);
- (ii) and have obtained values which can be considered as acceptable (see 3).

This document intends to provide NRLs *L. monocytogenes* with some guidance and details regarding the estimation and interpretation of measurement uncertainty for the enumeration of *L. monocytogenes* in foods. Its content is based in particular on the training session that the CRL had organised for the NRLs on 9 April on this topic and on the outcome of the exchanges during this session.

The present guide is specifically targeted to the enumeration of *L. monocytogenes*, and the comparison of individual enumeration results to the quantitative limit $m=100$ cfu/g of certain *L. monocytogenes* criteria of the EC Regulation 2073/2005. However, it may also apply to the enumeration of any flora, and to the comparison of any enumeration result to a quantitative limit m/M of any microbiological criterion.

1. Definition of measurement uncertainty (MU)

Refer to ISO/TS 19036 amended¹, which includes the definition of the GUM (*Guide to the expression of uncertainty in measurement*).

Note that MU includes all sources of uncertainty linked to what happens in the laboratory to one given food unit or laboratory sample which has been sampled and which is received by the laboratory. The laboratory sample may consist in a packaged food, or an aliquot of bulk food. MU defined in such a way thus includes for example the effects of sub-sampling (e.g. taking a test portion of 10g or 25g from the laboratory sample) but excludes any uncertainty linked to sampling.

¹ ISO/Technical Specification 19036 (2006) *Microbiology of food and animal feeding stuffs -- Guidelines for the estimation of measurement uncertainty for quantitative determinations*, with Amendment 1 (2009) *Measurement uncertainty for low counts*

2. MU estimation

Use ISO/TS 19036 and its amendment for low counts which has just been published. Note that the EC *Guidance Document on official controls, under Regulation (EC) No 882/2004, concerning microbiological sampling and testing of foodstuffs*² also recommends in its clause 8.2.2 the use of ISO/TS 19036 for MU estimation in the context of official controls in food microbiology.

ISO/TS 19036 describes 3 options, in a decreasing order of preference, to estimate the reproducibility standard-deviation (s_R):

1. Option 1: intra-laboratory s_R ;
2. Option 2: inter-laboratory s_R derived from a method validation inter-laboratory study;
3. Option 3: inter-laboratory s_R derived from an inter-laboratory proficiency trial.

The uncertainty U (or MU) is derived from s_R as follows: $U = 2 \times s_R$

ISO/TS 19036 details the experimental design for option 1, and gives pros and cons of each option.

Please feel free to contact us if you have any difficulty to implement this ISO document, whatever option you choose.

3. Acceptable MU values

3.1 Introduction and role of NRLs

Once MU is estimated, we recommend to check that the obtained MU value is "acceptable".

As indicated before, the acceptability of the MU value appears to be an important criterion to assess the reliability of the analyses provided by a laboratory. This applies in particular to the laboratories involved in official controls. Thus as NRL you should check that the laboratories approved for *L. monocytogenes* official controls in your respective country have obtained such acceptable MU values.

² http://ec.europa.eu/food/food/controls/foodfeed/sampling_testing.pdf

3.2 Notion of acceptable MU

The notion of “acceptable” MU (or analytical tolerance) in food microbiology has been introduced in a recent AFSSA opinion³. MU can be considered as "acceptable" if it is not too large, relatively to values usually obtained in other laboratories. Table 1 (below), from the AFSSA opinion, gives guidance values of acceptable MU which are based on (i) results obtained in the frame of proficiency trials organised by RAEMA⁴, and (ii) results of ISO trials to estimate the MU component linked to the sub-sampling of the test portion and to the preparation of initial suspension⁵. The values given in the table are derived from thousands of data on several bacteria, including *L. monocytogenes*. From these data, it appeared that the guidance values of Table 1 depends mainly on:

- the matrix effect (homogeneous/heterogeneous);
- the number of colonies counted on plates (effect of the distribution of bacteria according to Poisson law);
- the presence or not of a confirmation stage in the enumeration method;
- intra/inter-laboratory reproducibility standard-deviation.

Based on the ISO trials quoted above, the following food matrix types can be considered as homogeneous:

- liquid products (e.g. milk, water, beverages) and powders (e.g. milk powder, egg powder);
- solid products which has been mixed, minced, stirred (e.g. minced meat, sausage meat, clotted cream, ice cream, soy cream).

Other products than these ones are considered as heterogeneous.

Table 1: Guidance values of acceptable MU for enumeration of bacteria (in log₁₀ cfu/g)

Total nb of colonies counted on plate(s) retained for enumeration	Homogeneous matrix		Heterogeneous matrix	
	Method without confirmation	Method with confirmation	Method without confirmation	Method with confirmation
≤5	0.7	0.7	0.7	0.8
6-10	0.5	0.6	0.6	0.7
11-15	0.4	0.5	0.5	0.6
>15-150 or >300, depending on case	0.3	0.5	0.5	0.6

³ <http://www.afssa.fr/Documents/MIC2007sa0174.pdf> in French, see p.9 and Table 1

⁴ Augustin, J.-C., Carlier, V. 2006. Lessons from the organization of a proficiency testing program in food microbiology by interlaboratory comparison: analytical methods in use, impact of methods on bacterial counts and measurement uncertainty of bacterial counts. *Food Microbiol.* 23, 1-38

⁵ Ah Soon, C. et Cornu, M. Report of 2003/2004 ISO trials on measurement uncertainty, June 2004, AFSSA-LERQAP, Maisons-Alfort, France

3.3 Acceptable MU for *L. monocytogenes* enumeration

Based on the approach described above in 3.2, we recommend to consider as acceptable the following MU values for *L. monocytogenes* enumeration:

- ca. 0.30 log₁₀ when sufficient colonies are counted on the plate(s) retained for enumeration (low numbers excluded) and when the product analysed is homogeneously contaminated (see 3.2);
- ca. 0.50 log₁₀ when sufficient colonies are counted on the plate(s) retained for enumeration (low numbers excluded) and when the product analysed is not homogeneously contaminated (see 3.2).

Thus if the estimated standard deviation s_R is above 0.15 log₁₀ with an homogeneously contaminated product, or above 0.25 log₁₀ with an heterogeneously contaminated product, then we recommend to check carefully the analytical method and, if necessary, to improve it. Improvement of the method may include:

- To improve the sub-sampling of the test portion and the preparation of the initial suspension, in order to be more representative of the whole laboratory sample (for example by increasing the weight of the test portion).
- To check the competence of the operators and the reproducibility of how they conduct the different steps of the analyse;
- To check the metrology of the apparatus (e.g. stability of temperature of incubators);
- To check the quality assurance of culture media;
- ...

In addition, as clearly emphasized in Amendment 1 to ISO/TS 19036, low colony counts increase drastically MU. Thus, when possible, low counts should be avoided, for example by increasing the range of tested dilutions or the number of plates for the first dilution.

Finally, if in the same organization, different laboratories have obtained different MU values for the same determination (e.g. *L. monocytogenes* enumeration), each laboratory has to use the MU value it has obtained. However, intensive exchanges between the laboratories are encouraged to compare their methodologies, both the analytical method itself and the MU estimation technique.

4. MU interpretation

MU interpretation, that is how to take into account MU when interpreting an enumeration result in terms of conformity to a limit of a microbiological criterion is dealt in the EC “Guidance Document on official controls, under Regulation (EC) No 882/2004, concerning microbiological sampling and testing of foodstuffs”:

Clause 8.2 states:

According to the strategy for setting microbiological criteria for foodstuffs in Community legislation, the general policy is that food business operators should always regard all test results above the limits as unacceptable regardless of the MU involved, whereas in the official controls the MU could be taken into account in order to be sure beyond reasonable doubt that the batch in question does not comply with the criterion.

Also clause 8.2.2 states:

In the context of official controls it is recommended the following principles are taken into account until more specified rules for the quantitative analysis have been established at Community level:

- *As regards food-borne pathogens, the highest acceptable result including MU should still be low enough to ensure a high level of human health protection. Particularly, in the context of enforcement actions the highest acceptable result must be considered carefully on a case-by-case basis. In Regulation (EC) No 2073/2005, only one quantitative limit is fixed for a pathogen as a food safety criterion, ie *Listeria monocytogenes* (100 cfu/g).*
- *Indicators are used to determine the acceptable functioning of a production process. Therefore, the rules for interpretation of results of these indicators related to process hygiene criteria in Regulation (EC) No 2073/2005 need not be as strict as in the context of food safety criteria.*

IMPORTANT NOTE: This aspect is not of the competence of the laboratories, thus in particular not the competence of the CRL and the NRLs, but of the risk managers (DG SANCO and national competent authorities).