EURL *Lm*

European Union Reference Laboratory for Listeria monocytogenes
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EURL *Lm* TECHNICAL GUIDANCE DOCUMENT on sampling the food processing area and equipment for the detection of *Listeria monocytogenes*

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GLOSSARY

CEN: European Committee for Standardization (https://www.cencenelec.eu/), is an association that brings together the National Standardization Bodies of 34 European countries.

EURL *Lm*: European Union Reference Laboratory for *Listeria monocytogenes*

FBOs: Food Business Operators

HACCP: Hazard Analysis and Critical Control Point

ISO: International Organisation for Standardarization (www.iso.org). ISO is an independent, non-governmental international organisation with a membership of 165 national standards bodies

MSs: Member States

NRL: National Reference Laboratory

RTE foods: Ready-to-eat foods

VBNC: viable but non culturable

WG: working group

FOREWORD

In 2010, according to a review of the literature undertaken by the European Union Reference Laboratory for *Listeria monocytogenes* (EURL *Lm*), EURL *Lm* and the network of National Reference Laboratories (NRLs) for *Listeria monocytogenes* agreed that the International and European Standard EN ISO 18593:2004, describing surface sampling technique (contact plates, stick swabs, sponges and cloths) for the detection or enumeration of bacteria in food processing area and equipment, did not give sufficient guidance specific to *L. monocytogenes* detection (see also Introduction). It was therefore agreed that the EURL *Lm* would produce a technical guidance document on this topic with the coordination of Brigitte Carpentier and Léna Barre (ANSES project leaders, France) and in collaboration with a working group (WG). In 2012, the EURL Lm guideline (version 3) on sampling of the food processing area and equipment for the detection of *Listeria monocytogenes* was published. After 2015, the ISO TC34/SC9/WG17 worked on the revision of the International and European Standard EN ISO 18593, based on the 2012 EURL *Lm* guideline. This led to the revision of the International and European Standard EN ISO 18593 which was published in 2018. Following the revision of International and European Standard EN ISO 18593 in 2018 and the revision of International and European Standard EN ISO 11290 - 1 and -2 in 2017, it was agreed that the EURL *Lm* would write a revision of this guideline in collaboration with a WG comprised 23 members from 12 EU Member States (MSs), belonging to NRLs and other organisations (see front page).

The fourth version of this Technical Guidance Document on sampling the food processing area and equipment for the detection of *Listeria monocytogenes* was approved by the EU Standing Committee on Plants, Animals, Food and Feed at its meeting of 3 October 2023.

1. INTRODUCTION

1.1 CONTEXT AND REGULATORY FRAMEWORK

It is now well established that ready-to-eat foods (RTE) can be contaminated during processing by subtypes of *Listeria monocytogenes* which persist in the food processing plants (Carpentier Cerf 2011). The revision of the International and European Standard EN ISO 18593:2018 does not give sufficient guidance or advice specific to *L. monocytogenes* detection from surfaces in the food chain environmental samples. Contact plates are not appropriate for the detection of *L*. *monocytogenes.* Wipe sampling techniques (stick swab and sponge/ cloth method) are the only appropriate techniques to use for *L. monocytogenes* detection. However, the stick swabs are not effective for sampling *L*. *monocytogenes* from surfaces other than hard-to-reach small areas.

Brauge *et al.* (2020a) carried out a European survey to collect information on how food business operators (FBOs) sample surfaces for detection or enumeration of *Listeria monocytogenes* in food processing plants. One hundred and thirty-seven questionnaires from 14 EU Member States were returned. The outcome of the survey showed that FBOs prefer friction sampling techniques (gauze pads, dry swab and sponges) over contact sampling techniques and several FBOs used combined surface sampling technique.

In the framework of Regulation (EC) No 2073/2005 (plus amendments), defining microbiological criteria for foods (Anonymous 2005), food chain environmental sampling (including processing areas and equipment) for *L. monocytogenes* detection on a routine basis according to a sampling programme is mandatory. Such sampling programmes aim at detecting and eliminating a persistent strain or, if elimination is impossible, to implement corrective actions to avoid food contamination by the pathogenic bacteria. An ineffective sampling programme or inappropriate sampling techniques may result in the false-negative detection of *L. monocytogenes* despite being present on surfaces. This will prevent implementing corrective actions and give a false sense of security. Furthermore, it was chosen not to address how to enumerate *L. monocytogenes* on surfaces for the following reasons. First, swabbing is not able to collect all the bacterial cells, the ratio of detached cells is variable and not foreseeable. Secondly, *L. monocytogenes* cells are not homogeneously distributed on the surfaces and the comparisons of results obtained sampling large and small areas would thus be invalid.

1.2 PERSISTENCE OF LISTERIA MONOCYTOGENES IN FOOD PLANTS

During the process of bacteria attachment and colonisation of surfaces their phenotypes change. They show, an increased exopolysaccharide production and a decreased susceptibility to disinfectant causing the formation of biofilm. Biofilms are an integral part of environment and allow bacteria to be less susceptible to environmental stress (disinfection, desiccation, starvation…). *L. monocytogenes* are able to form a biofilm and can be persistent for several months or years on surfaces in food processing environments. For example in Italy, *L. monocytogenes* were detected with a prevalence of 16% in environmental samples (n = 95 of samples collected) over a period of six-years (2003-2008) (Di Ciccio *et al.* 2012). In a Portuguese study carried out in cheese processing plants between 2003 and 2007, the prevalence of the micro-organism ranged from 6.7% in food contact surfaces (n = 224 of samples collected) and 11.5% in non-food product surfaces (n = 192 of samples collected) (Almeida *et al.* 2013). In Thailand, over a period of 1.5 years, the prevalence from a seafood processing plant was of 1.5% in samples from food contact surfaces (n = 195 of samples collected) and 4.5% fin samples from non-food contact surfaces (n = 177 of samples collected) (Vongkamjan *et al.* 2017). Many other evidence for persistence of *Listeria* in food processing environments have also been provided by a research team in Ireland in facilities from various food sectors (Leong *et al.* 2014), in dairy farms (Latorre *et al.* 2011) but also in retail deli environments (Wang *et al.* 2015). Brauge *et al.* (2020b) carried out an 8 month sampling campaign in four smoked salmon processing plants (plants A, B, C and D) and showed the increase of *L. monocytogenes* VBNC population mainly on the slicer blades after cleaning and disinfection operation in plant B.

Several researchers have reported that biofilms promote the viable but non culturable (VBNC) state which would also contribute to the high resistance of biofilms to stressful conditions (Flemming *et al.* 2016, Stewart Franklin 2008). In fact, the VBNC state refers to living bacteria that have very low metabolic activity and do not multiply. Consequently, VBNC cells do not grow on standard microbiological media but they have the ability to recover and become culturable under favourable conditions (*i.e.* resuscitation). Different environmental stresses could be responsible for the induction of the VBNC state during food processing, such as starvation, oxidative stress, temperature changes and disinfection. Overney *et al.* (2017) showed an induction of the VBNC state in *L. monocytogenes* biofilms formed by LO28 strain (serotype 1/2c) on stainless steel following a treatment with chlorinated alkaline cleaner used

at a concentration of 3% (vol/vol) or a quaternary ammonium-based disinfectant with a recommended concentration of 2% (vol/vol). Brauge *et al.* (2018) showed that *L. monocytogenes* VBNC populations in 48h biofilms had strongly increased following treatment with a NaOH solution at 0.5%. Brauge *et al.* (2020b) observed the decrease of *L. monocytogenes* viable culturable population and the appearance of *L. monocytogenes* VBNC population in 48h biofilms in response to treatment with quaternary ammonium- or hydrogen peroxide-based disinfectants.

Aalto-Araneda *et al.* (2019) studied 21 fish-processing plants and operational procedures relating to *L. monocytogenes* control. They showed the presence of viable culturable *L. monocytogenes* population only in sliced and mainly in gravad products of 7 plants because the slicing fish step is a critical point of contamination of the product. They identified different critical points in different fish-processing plants: the number of processing machines, deficiencies in the processing environment and machinery sanitation, and staff movement from areas of low toward high hygiene.

2. SCOPE

This guideline specifies where, how and when sampling should be performed to detect *L. monocytogenes* on surfaces of ready-to-eat food processing areas and equipment. This guideline is not designed to assess cleaning and disinfection efficiency.

This guideline is to be read in conjunction to the International and European Standard EN ISO 18593: 2018. The choice of sampling location(s), area and sampling times should be risk-based and on a case-by-case basis.

3. CHOICE OF SAMPLING LOCATIONS

For the choice of sampling locations, refer clause 7.2 of the International and European Standard EN ISO 18593:2018.

This guideline does not provide advice on sampling frequency, number of sampling points, validity of compositing or pooling of samples (International and European Standard EN ISO 6887-1:2017) or necessity to rotate sampling points.

4. TIME AT WHICH SAMPLING SHOULD BE PERFORMED

For the time of sampling and frequency, refer clause 7.4 of the International and European Standard EN ISO 18593:2018.

To increase the probability of detection of *L. monocytogenes*, sampling should be performed, if possible, during processing (depending on the process or food sector), after at least two hours of production or at the end of production runs i.e. before cleaning and disinfection. In some occasions sampling just before start-up of production (normally sometime after cleaning and disinfection) can be useful whether or not to detect persistent strains. In such cases, it is advisable to first disassemble the production equipment and then operate it without actual production, allowing to detect potential residues, even from challenging-to-access areas. Sampling after cleaning and disinfection operations may also offer informative results but should not be in itself considered as a guarantee with regard to the effectiveness of the cleaning and disinfection protocol (FDA 2017).

The state of the bacteria (viable culturable or VBNC) is different at the start or during or at the end of the production or after cleaning and disinfection operations. As the frequency of

sampling will differ depending on the food commodity being processed, the following examples or references to good hygienic practices are provided.

In processing lines where food products are manufactured from raw products which are not submitted to a treatment that reduces the level of microorganisms (raw cheeses for example), *L. monocytogenes* in a surface sample taken during the processing run may originate from these raw products as well as from the places where *L. monocytogenes* cells can persist in the food processing environment.

Sampling after cleaning and disinfection or at the beginning of production can be performed in addition to sampling performed during processing. Yet, this can lead to a false sense of security. Conversely, the detection of *L. monocytogenes* on food-contact surfaces after cleaning and disinfection indicates a serious failure in cleaning and disinfection procedures.

5. AREA TO BE SAMPLED

For the area to be sampled, refer the clause 7.3 of the International and European Standard EN ISO 18593:2018.

It is recommended in the International and European Standard EN ISO 18593:2018 that the total sampled area during a sampling campaign should be as large as possible to increase the probability to detect *L. monocytogenes*. In this regard, it is advised to sample between 1000 cm^2 and 3000 cm^2 (i.e. 0.1 m^2 to 0.3 m^2) when possible.

6. DILUENTS TO MOISTEN THE WIPE SAMPLING DEVICES

6.1 SIMPLE DILUENTS

For the simple diluents, refer the clause 5.1 of the International and European Standard EN ISO 18593: 2018 and the International and European Standard EN ISO 6887 part 1 and 5.

6.2 NEUTRALIZING DILUENTS

For the neutralizing diluents, refer the clause 5.3 and the Annex A of the International and European Standard EN ISO 18593:2018.

7. SAMPLING TECHNIQUES

A digital format of the guidelines including illustrative videos of the application of each sampling technique, is available on the EURL *Lm* website (https://sitesv2.anses.fr/en/minisite/listeria-monocytogenes/tutorials-implementation-sampling-techniques) as well as practical sheets for sampling techniques with several points: description of the sampling technique, which sampling device to choose, use protocol, affecting material nature, affecting sampling surface properties, affecting operator, limit of sampling technique (https://www.actia-asso.eu/en/surface-sampling/). FBOs are invited to download this material and use it in food processing places.

For sampling techniques, refer to clause 7.5.3 and 7.5.4 of the International and European Standard EN ISO 18593:2018 (with the exception of the use of contact plates which is not appropriate for the detection of *L. monocytogenes*).

Sampling surfaces by rinsing them is not recommended as rinsing does not have the same efficiency as wiping to detach microorganisms from the surfaces.

8. STORAGE AND TRANSPORT

For the storage and transport of samples, refer the clause 8.2 of the International and European Standard EN ISO 18593:2018.

9. METHOD OF ANALYSIS

After sampling, sample analysis will be performed according to International and European Standard EN ISO 11290-1: 2017 or a validated alternative method according to article 5 of Regulation (EC) No 2073/2005 as amended. In the event that *L. monocytogenes* positive samples are repeatedly found over successive sampling times at a same food processing site where corrective actions have already been implemented, subtyping *L. monocytogenes* isolates by a molecular typing method will be necessary. The typing of the isolates (like cgMLST) should establish whether the isolates belong to one single and thus persistent clone. It is strongly recommended that FBOs and laboratories store isolates to determine whether a strain is persistent or not and to allow the tracking of the contamination source. When food products entering processing premises are either raw or have been treated to reduce their microbial load (*e.g.*, pasteurization, microfiltration, etc.), the FBOs should, as part of their Hazard Analysis and Critical and Control Points (HACCP) plan, establish an acceptable number of positive samples. This acceptable number of positive samples may be set differently depending on whether the sample originates from a surface in direct contact with food products, or the sample originates from a surface without contact with food products. Surfaces in direct contact with food present a higher risk of food product contamination and should therefore be associated with a stricter threshold number of positive samples compared to surfaces without contact with food products.

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For the normative references, refer clause 2 of the International and European Standard EN ISO 18593 (2018)

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