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Guidelines on sampling the food processing area and equipment for the detection of *Listeria monocytogenes*

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FOREWORD

According to a review of the literature undertaken by the European Union Reference Laboratory for *Listeria monocytogenes* (EURL *Lm*), the EURL Lm and the network of National Reference Laboratories (NRLs) agreed that the International Standard ISO 18593, describing surface sampling methods for the detection or enumeration of bacteria in food processing area and equipment, does not give sufficient guidance specific to *L. monocytogenes* detection (see also introduction). It was thus agreed that the EURL *L. monocytogenes* would write a guidance document on this topic, in collaboration with a working group (WG). This WG comprised 20 members from 7 EU Member States (MSs), belonging to 3 NRLs and other organizations (see front page).

Apart from the literature review and the contributions from the WG members, these guidelines were based on a large survey conducted in 2010 on surface sampling practices (137 respondents from 15 EU MSs, belonging to FBOs, service providers in charge of hygiene control, official control services,…), which showed a great variety of practices and some wrong ones, emphasizing the need of a European document.

Several drafts have been prepared, each submitted to a consultation of, in a first step, all NRLs which consulted at national level operators practicing surface sampling, and then the WG members.

The current version 3 results from an agreement of all NRLs at their annual workshop on 28-30 March 2012 and takes into comments received from 3 EU MSs (UK, NL, DE), in the frame of a MS consultation made by DG SANCO. This version has been agreed by the EU MSs' Standing Committee on the Food Chain and Animal Health at its meeting of 18 July 2012 and a further written 2-week consultation.

INTRODUCTION

It is now well established that ready-to-eat foods can be contaminated during processing by subtypes of *Listeria monocytogenes* which persist in the processing plants [1]. Sampling processing areas and equipment for *L. monocytogenes* on a routine basis according to a sampling scheme is thus necessary and mandatory, in the frame of the EC Regulation 2073/2005 defining microbiological criteria for foods [2]. Such sampling schemes 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. Several guidance documents are published in this regard [3-5]. An ineffective sampling programme or ineffective sampling techniques may result in the non-detection of *L. monocytogenes* when it is present. This will prevent implementing corrective actions and give a false sense of security.

The International Standard ISO 18593 [6], which describes surface sampling methods to detect or enumerate viable micro-organisms, does not give sufficient guidance or advice specific to *L. monocytogenes* detection. Wipe sampling methods (swab and sponge/ cloth method) are the only appropriate methods to use for *L. monocytogenes*. The ISO standard does not describe when sampling should be performed or what areas should be sampled. The present guidelines aim to compensate for this gap when implementing Article 5.2 of EC Regulation 2073/2005 [2]. Furthermore, it was chosen not to address how to enumerate *L. monocytogenes* on surfaces for the following reasons. First swabbing does not detach all bacterial cells and the proportion of detached cells is unknown and variable. Secondly *L. monocytogenes* cells are not evenly distributed on a surface and comparisons of results from large and small areas would thus be invalid.

After sampling, sample analysis will be performed according to EN ISO 11290-1 or validated alternative methods according to article 5 of Regulation (EC) No 2073/2005[1]. In the event that "excessive" *L. monocytogenes* positive (see section 5) samples are found over successive sampling campaigns at a same food processing site where preventing actions have already been implemented, subtyping *L. monocytogenes* isolates by a molecular method (such as Pulsed-field gel electrophoresis or Multi-locus variable number tandem repeat analysis or Fluorescent amplified fragment length polymorphism) will be necessary to establish whether the isolates belong to one single and thus persistent clone.

[1] According to the requirements of EC Regulation 2073/2005, the present document is only devoted to the detection of *L. monocytogenes*. Meanwhile, it may be interesting to know that, as suggested by the Codex Alimentarius [3], "effective monitoring programs may also involve testing for *Listeria spp*.; their presence is a good indicator of conditions supporting the potential presence of *Listeria monocytogenes*".

2 SCOPE

These guidelines specify where, how and when wipe sampling should be performed to detect *L. monocytogenes* on surfaces of ready-to-eat food processing areas and equipment.

NOTE 1: Surfaces of the processing area and equipment are not the only places to monitor, the sampling scheme should also include processing aids (such as compressed air, ice, brine solution, water, drain water), whose sampling techniques are not covered in this document.

NOTE 2: No advice on sampling frequency, number of sampling points, validity of compositing (pooling) the samples or necessity to rotate sampling points will be given here as they have to be chosen on a case-by-case basis, with a risk-based approach.

NOTE 3: These guidelines are not designed to assess cleaning and disinfection efficiency.

3 NORMATIVE REFERENCES

The following referenced documents should be used for the application of this document.

ISO 6887-1 Microbiology of food and animal feeding stuffs — Preparation of test samples, initial suspension and decimal dilutions for microbiological examination — Part 1: General rules for the preparation of the initial suspension and decimal dilutions

ISO 7218 Microbiology of food and animal feeding stuffs — General requirements and guidance for microbiological examinations

ISO 11290-1 Microbiology of food and animal feeding stuffs — Horizontal method for the detection and enumeration of *Listeria monocytogenes* — Part 1: Detection method

ISO/TS 11133 Microbiology of food and animal feeding stuffs — Guidelines on preparation and production of culture media

4 CHOICE OF SAMPLING LOCATIONS

L. monocytogenes can be found on visually clean surfaces but it is most frequently found at wet and soiled places where the bacterium is able to grow and persist [1, 7]. Hard to reach places such as holes or crevices in fibrous, porous, rusting and hollow materials, poorly cleanable equipment are potential harbourage sites that should be sampled. It can be difficult to sample unreachable areas where food debris can collect. These areas should be sampled after dismantling the equipment with the maintenance team. It is not recommended to take a sample by rinsing such areas as rinsing does not have the same efficiency as wiping for detaching the microorganisms from the surfaces.

Sampling should be done frequently in areas where the food product is exposed to contamination, but it may be interesting to also sample, less frequently, in areas where it is not (storage areas).

The choice of sampling location has to be chosen according to historical data linked to each factory and after step-by-step examination of the process. A non-exhaustive list of places to choose sampling locations is given below [4, 5, 8]:

- Non-food contact surfaces: Drains, floors, pools of water on the floor, cleaning tools, wash areas, in-floor weighing equipment, hoses, hollow rollers for conveyances, conveyors, equipment framework, internal panel of equipment, condensate drip pans, forklifts, hand trucks, trolleys, trolley wheels, trash cans, freezers, ice makers, cooling fins in condensers, aprons, walls, ceilings, cold spots where water condenses as wet insulation in walls or around pipes and cooling units, rubber seals around doors, especially in coolers, contents of vacuum cleaners, door handles and taps.
- Food contact surfaces: Conveyor belts, slicers, cutting boards, dicers, hoppers, shredders, blenders, peelers, collators, filling and packaging equipment, containers, other utensils, non-disposable gloves.

5 TIME AT WHICH SAMPLING SHOULD BE PERFORMED

The detection of *L. monocytogenes* can be difficult if samples are taken immediately or soon after cleaning and disinfection. Cells, because of the injury caused by the chemical agents used for cleaning and disinfection, can be still alive but non-culturable, therefore not easily detectable [8]. Furthermore cells remaining in harbourage sites despite cleaning and disinfection can also be undetected, while they are more accessible to sampling once dislodged during processing because equipment vibrates and/or because foods and liquids come in contact with harbourage sites [9].

Therefore, to increase the probability of detecting a persistent strain, sampling should be performed during processing, after at least two hours of production or at the end of production runs i.e. before cleaning and disinfection [4, 8-11]. 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. In plants, where pasteurized products or raw materials not frequently contaminated are processed (pasteurized cheeses for example), *L. monocytogenes* in a surface sample should be investigated as a persistent *Listeria*.

When foodstuffs entering the processing premises are raw or have been treated to decrease their microbial load (by pasteurization, microfiltration etc.)[2], the food business operators (FBOs) should, as part of their HACCP plan, establish acceptable number of positive samples which can be set differently for the detection of *L. monocytogenes* on food contact surfaces and non-food contact surfaces. Corrective actions should be implemented, according to the FBO HACCP plan, when the established number of positive samples is exceeded[3]. Of course, when raw food is processed, sampling after cleaning and disinfection or at the beginning of

[2] Foods and ingredients should be analyzed according to the HACCP plan.

[3] Acceptable number of positive sample could be zero when foodstuffs entering the processing premises have been treated to decrease their microbial load.

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.

When samplings are not performed daily, they should not be performed always on the same day(s) of the week. It may be appropriate to take samples following servicing of equipment, repairs to equipment, construction and increased production as these can increase the risk of *L. monocytogenes* contamination.

6 DILUENTS TO MOISTEN THE WIPE SAMPLING DEVICES

6.1 SIMPLE DILUENTS

In easy-to-reach areas which are sampled during or at the end of processing, simple diluents containing no neutralizer should be used to moisten stick swabs and the other wipe sampling equipment.

Recommended diluents are: peptone solution at 1 g/l, peptone saline or quarter-strength Ringer's solution, distributed in tubes or bottles, and sterilize for 15 min at 121 °C.

Phosphate buffered diluents are not recommended to be used for cells stressed by hostile conditions of food processing premises (salt, acid, cleaning and disinfecting products etc.) since they may have a deleterious impact on their culturability [12].

Similarly, it is recommended not to use a neutralizing diluent when no residual disinfectant is expected. A neutralizer used to quench residual disinfectant can have a slight deleterious impact on bacterial cells and it is likely that such an impact would be greater when cells are stressed. Indeed, it was shown that neutralizers reduce the rate of *Salmonella* isolation from field samples [13].

Fraser broth or half Fraser broth should not be used in place of a diluent since they could favour growth of *L. monocytogenes* in the processing site.

6.2 NEUTRALIZING DILUENTS

In any areas where residues of disinfectants are expected, or when samples are taken immediately after disinfection, neutralizing diluents should be used to moisten stick swabs or other wipe sampling equipment.

When chlorine or chlorine releasing compounds are used, sodium thiosulfate should be chosen as a neutralizer. For other active substances a number of neutralizers are available (see EN 1276 [14], EN 1650 [15], EN 13697 [16] and EN 13704 [17]) yet none of them is appropriate for all disinfectants ("universal") [18]. A neutralizing diluent which may be used in most situations is described in Table 1. It should be distributed in tubes or bottles, and sterilize for 15 min at 121 °C.

Table 1: Neutralizing diluent which can be used in most situations (adapted from ISO 18593 [6])

Component	Conc.
Sorbitan monooleate (Polysorbate 80)	30 g/l
Lecithin	3 g/l
Sodium thiosulfate	5 g/l
L-Histidine	1 g/l
Saponin	30 g/l
Peptone	1 g/l
Sodium chloride	8.5 g/l

7 CULTURE MEDIA

See EN ISO 11290-1 Standard for culture media.

8 APPARATUS AND GLASSWARE

For general requirements, see EN ISO 7218.

For requirements specific to *L. monocytogenes,* see EN ISO 11290-1 and amendment(s).

Usual microbiological laboratory apparatus and, in particular, the following.

8.1. Wipe sampling devices

Stick swab*, sterilized stick tipped with cotton or synthetic material individually contained in a sterilized tube. The material used should be documented free of inhibiting substances.*

NOTE For some types of surface, the cotton residues can contaminate the internal parts of these surfaces after sampling.

Sponge, woven or unwoven cloth or gauze pads, *sterilized and individually packed in a sterilized plastic bag. The material used should be documented free of inhibiting substances.*

8.2. Disposable sterilized gloves (optional)

8.3. Cool box, with ice packs, insulated, capable of maintaining the samples between 1 and 8°C during transportation to the laboratory (see EN ISO 7218)

8.4. Sterilized absorbing paper, capable of absorbing stagnating water (i. e. pools of water on the floors)

8.5. Mixer, for mixing liquids in tubes

8.6. Peristaltic homogenizer, to prepare initial suspensions by peristaltic movement

9 AREA TO BE SAMPLED

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, 10, 11] i.e. when the areas are open and flat (conveyors, shelves, etc.).

Stick swab should be used to sample hard-to-reach small areas (e.g. inside hollow rollers, motor housing).

Sponge, woven or unwoven cloth or gauze pad should be used to sample large areas. In contrast with stick swabs they can be rubbed more vigorously over surfaces and are highly absorptive. It is not advised to use templates or graduate rulers, they can transfer contamination and/or their disinfection can interfere with the test, however the sampled area should be known approximately. For this purpose, the operator may remember that the length of the forearm, from the tip of the middle finger up to the elbow (a cubit), is around 45 cm. Similarly, when fingers are spread, the distance between the tip of the thumb and that of the little finger (a span) is approximately 20 cm.

The size of the sampled area should be consistent so that trends can be monitored over time.

10 PREPARATION OF SAMPLING DEVICES

No equipment which has been inside a microbiology laboratory should be taken into a food production area due to the risks of introducing contamination.

Equipment for surface sampling and protective clothing should be stored and handled separately from laboratory operations, especially the laboratory premises dealing with pathogen analysis.

Operators should ensure that no item used for sampling is left in the production area. For this purpose, counting these items before and after sampling is recommended.

10.1 STICK SWAB

Stick swab can be used dry or moistened. In the event that the area to sample is wet, a dry swab should be used, and in the event that the area to sample is dry, a moistened swab should be used. In the event that sampling has to be done in a wet or dry place where residues of disinfectants are expected (which is frequent in hard to reach areas) the swab should be moistened with a neutralizing diluent (6.2).

Moistened swabs can be prepared aseptically in the laboratory before the sampling campaign. The end of the swab should touch slightly the surface of the diluent (6.1 or 6.2) so that the swab does not drip [8]. Then, return the swab to the tube which is then tightly closed to maintain both sterility and humidity.

10.2 SPONGE, WOVEN OR UNWOVEN CLOTH OR GAUZE PAD

Prior to commencing the taking of samples, moistened wiping devices can be prepared in the laboratory before the sampling campaign with an appropriate and recorded volume of sterilized diluent (6.1 or 6.2) so as it does not drip. After moistening the wiping device, close the plastic bag in a manner that will ensure sterility and keep humidity.

If moistening the sampling devices is performed in the processing area, the diluent should not be stored in glass bottle.

11 SAMPLING

11.1 STICK SWAB METHOD

Remove a swab from its tube and rub as vigorously as possible without disintegrating it and rotate the swab on the inside of the piece of equipment or over any other hard-to-reach area to be sampled.

Put the swab in the original tube; close it so that the swab is protected from contamination and its end remains still moist at the analysis.

After sampling, the sampled area should be wiped down with an alcohol wipe.

11.2 SPONGE/CLOTH /GAUZE PAD METHOD

In the event that the area to be sampled is too wet (i.e. pools of water on the floor), the liquid in excess should firstly be removed by gently applying sterilized absorbing paper.

Open the plastic bag containing the wiping device. Remove aseptically the wiping device with a sterile gloved hand. Alternatively the wiping device can be gripped through the plastic bag with pulling the reversed bag over the hand as shown on Figure 1.

Wipe vigorously with a zigzag motion the whole chosen surface in two perpendicular directions, changing the face of the wiping device. Return the wiping device to the plastic bag and close it so that it is protected from contamination and it still remains moist at the analysis.

Figure 1: Wiping device gripped through a plastic bag

After sampling, the sampled area should be wiped down with an alcohol wipe.

12 TRANSPORT OF SAMPLES STORAGE AND STARTING OF THE ANALYSIS

Transport the samples in a cool box (8.3) between 1 and 8°C.

If necessary, store the samples at the laboratory at 3°C±2°C. Examine the samples as soon as possible, preferably not later than 24 h after receipt at the laboratory and in any case not later than 36 h after sampling, according to EN ISO 7218 Standard (clause 8.3, 3rd paragraph before end and the next paragraph on perishable products). The length of time before analysis should be recorded and written in the analysis report.

13 SAMPLE ANALYSIS

13.1 STICK SWAB METHOD

Add a sufficient volume and at least 9 ml of half Fraser broth (see EN ISO 11290-1) in the tube containing a swab so that the tip is completely immersed in the broth.

Thoroughly mix the contents of tubes containing swabs using a mixer (8.5) for 30 s.

Then perform the detection of *L. monocytogenes* according to the EN ISO 11290-1 Standard method or a validated alternative method.

13.2 SPONGE/CLOTH /GAUZE PAD METHOD

Add 9 times the weight of the moistened wiping device, as prepared in 10.2, of half Fraser broth (see EN ISO 11290-1) in the plastic bag containing the wiping device which must be completely soaked in the broth.

Treat the contents of the bags in a peristaltic homogenizer (8.6) for 1 min.

Then perform the detection of *L. monocytogenes* according to the EN ISO 11290-1 Standard method or validated alternative method.

14 EXPRESSION OF RESULTS

Results should be reported as: presence or absence of *L. monocytogenes* at the sampling location. Indicate, if known, the size of the sampled area.

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