Qualitätssicherung. **Vom Landwirt bis zur Ladentheke.**

Supporting document listeria prevention for **slaughtering, deboning and processing**

QS. Ihr Prüfsystem für Lebensmittel.

IFS International Featured Standards

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Quality assurance and safe food are the common goal of QS and IFS. To achieve this goal, the highest hygiene requirements must be met in the food chain. For both standards, comprehensive guidelines and supporting documents have been developed together with experts. In addition to the present supporting document, you will find the IFS guideline for an foreign effective body management under the following link. **IFS Foreign Body Management**

IFS Foreign Body Management

1 Scope

This supporting document assists companies in the meat industry in evaluating the *listeria* risk of their own plants. It points out which influencing factors can be considered when evaluating the risk so that the assessment can be executed as accurately as possible.

In addition, this supporting document helps in classifying products in accordance with Regulation (EC) No 2073/2005 by using a decision tree. Recommendations for sampling, analysis and result evaluation are given and best-practice advice is shown for different companies, which can be considered in the evaluation of their own plants.

2 Introduction

At the end of 2017, the European Food Safety Authority (EFSA) published an extensive study on *Listeria monocytogenes* (*L.m.*) named "*Listeria monocytogenes* contamination of ready-to-eat foods and the risk for human health in the EU". According to the publication, the incidence of listeriosis diseases increased steadily between 2008 and 2013 but flattened out after this period. The declining figures indicate a fundamental improvement in the situation. In particular, the increased sensitivity of food manufacturers is one of the key factors. The study proves that more than 90% of listeriosis cases were caused by “ready-to-eat” (RTE) products that contained more than 2,000 colony-forming units per gram (CFU/g).

Fish and fishery products are the most common cause of listeriosis infection according to the EFSA study, followed by meat and meat products. Although there appears to be a high variability in the virulence potential of the different *L.m.* strains, the study currently suggests that all *L.m.* strains can lead to listeriosis in humans.

Due to the high adaptability of *listeria*, one must pay special attention towards the persistence of *L.m.* because of its capability to form biofilms and can therefore be 100 to 1000 times more resistant to cleaning and disinfection than planktonic cells. *L.m.* has a selective advantage over many other bacteria, as it can grow at cold-storage temperatures, tolerates high salt concentrations and can even survive freezing.

In practice, it is therefore important to consider all possible sources of input along the value chain.

efsa
European Food Safety Authority

“The EFSA study proves that more than 90% of listeriosis cases were caused by “ready-to-eat” (RTE) products”

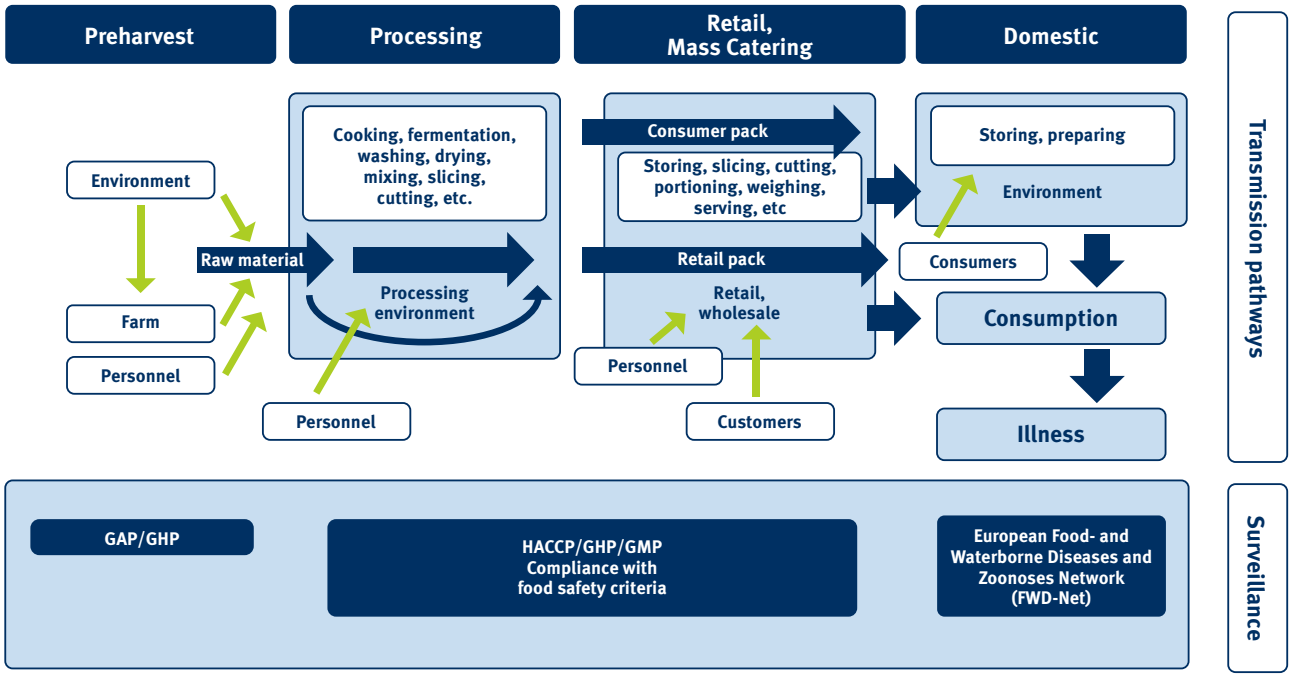

Figure 1: Schematic overview of transmission pathways and the control system of *L.m.* in RTE products

The EFSA's comprehensive study on the presence of *L.m.* in food production environments resulted in an average of 12.6% positive findings. Samples used for the study were taken at twelve different plants that handle raw animal materials. It is therefore of the utmost importance to detect the entry of *listeria* into the value chain as early as possible by taking appropriate measures to reduce or, wherever possible, eliminate the bacteria and prevent it from establishing itself in the production environment.

According to the study, positive analytical results can be divided into three groups:

- Repeated positive results in different departments and areas
- Repeated positive results in the same areas
- Occasional positive results at certain points

3 Legal background

Examination of surfaces

Extracts from the Commission Regulation (EC) No 2073/2005 on microbiological criteria for foodstuffs, Article 5(2) explain that:

"Samples shall be taken from processing areas and equipment used in food production, when such sampling is necessary for ensuring that the criteria are met. In that sampling, the ISO 18593:2018 shall be used as a reference method.

Food business operators manufacturing RTE products, which may pose a *Listeria monocytogenes* risk for public health, shall sample the processing areas and equipment for *Listeria monocytogenes* as part of their sampling scheme."

Sampling of surfaces

Guidelines on sampling and further obligations of the producer are described in Regulation (EC) No 852/2004 and in the "Guidelines on sampling the food processing area and equipment for the detection of *Listeria monocytogenes* Version 3 – 20/08/2012" of the European Union Reference Laboratory for *L.m.* There is no legal requirement on the frequency of sampling, which must be determined by the food business operator in a risk-oriented manner.

The reference method for correct sampling can be found in ISO 18593:2018. Further recommendations have been published by the European Reference Laboratory for *L.m.*

Samples may be taken during production, after at least two hours of production or at the end of production (before cleaning and disinfection). If sampling is not done daily, weekday sampling should be varied. Sampling after repair work, after increased production and in areas which are not directly involved in the production (e. g. cold storage) should also be scheduled.

Microorganisms are often found in damp, dirty areas of the plant but they can also multiply on seemingly clean surfaces. Places which are difficult to access for cleaning and disinfection (holes, cracks, hard-to-clean equipment) are potential enrichment areas for *L.m.* that should be sampled. According to ISO 18593:2018 these are subdivided as follows:

- Surfaces without food contact (e. g. drains, floors, door handles, gaskets, trash cans, etc.)
- Surfaces with food contact (e. g. conveyor belts, cutting boards and machines, funnels, hands, gloves, etc.)

The sampling area must be defined, always selecting a representative section of the surface which is scheduled for sampling. For the general detection of microorganisms, the sampled area should be as large as possible in order to increase the probability of detecting low bacterial counts. If possible, an area between 1,000 and 3,000 cm² should be examined. Whereas, for the quantitative determination of microorganisms, a representative area of maximum 100 cm² should be chosen.

In accordance with the relevant ISO standard, depending on the texture of the surface and the purpose of the examination, contact plates, sterile swabs, sterile wipes or sterile sponges may be used for sampling. The use of contact plates is only possible on smooth, even surfaces. Sterile swabs should be used for hard-to-reach uneven surfaces (up to 100 cm²). Large surfaces (over 100 cm²) shall be sampled with sterile wipes or sponges. The area and sampling location should be accurately described to facilitate subsequent evaluation. To ensure correct adherence to the selected areas, templates can be used.

After sampling, appropriate measures must be taken to ensure that no residues of the sampling material remain at the sampled area (cleaning) and that the area is disinfected again. According to ISO 18593:2018, sterile cloths soaked in alcohol are suitable for this purpose.

It is important to note that contact plates are **not** suitable for qualitative detection.

Product examination

According to Regulation (EC) No 2073/2005, the sampling and testing frequency for RTE products must be determined by the food manufacturer based on risk evaluation as part of his internal HACCP concept. The competent authority may verify compliance and initiate further samplings and investigations.

Annex 1, Chapter 1 of Regulation (EC) No 2073/2005 describes the food safety criteria of various products regarding the detection of *L.m.*

Food is divided into three categories for *listeria* sampling

- **Category I**
  RTE products intended for infants or special medical purposes:

  The sample must consist of ten sample units of 25 g each. A detection of *L.m.* in a sample unit leads to an unsatisfactory result.

- **Category II**
  RTE products able to **support** the growth of *L. monocytogenes* other than those intended for infants or for special medical purposes (related to a_w-value, pH-value, salt content of the product, etc.):

  If it is not possible for the food manufacturer to satisfactorily demonstrate to the competent authorities (e. g. scientific elaborations regarding the growth dynamic of *L.m.* in the product) that at the end of the shelf life 100 CFU/g is not exceeded, a random sample must be taken consisting of five sample units of 25 g each, before the food leaves the direct control of the food business operator who produced it. A detection of *L.m.* in only one sample unit leads to an unsatisfactory result.

  If the food business operator can prove that 100 CFU/g is not exceeded even at the end of the shelf life, then a value above 100 CFU/g must not be detected in **any** of the five sample units at the end of the shelf life. When in doubt, it may be necessary to establish intermediate limit values to be used for evaluation during the procedure.

- **Category III**
  RTE products other than those intended for infants or for special medical purposes, that **do not support** the growth of *L.m.* (see Figure 2, products referred to in point 4):

  Under normal circumstances it does not make sense to test certain foods for *L.m.* because a contamination is highly unlikely. This includes, for example, foodstuffs which undergo heat treatment in the final package which kills *L.m.*, and very dry foodstuffs (see Regulation (EC) No 2073/2005 Chapter 1). If an analysis is executed, five sample units of 25 g each must be analysed. A bacterial count of 100 CFU/g may not be exceeded in any sample unit.

All product analysis must consider the variability of the microorganisms as well as the processing and storage conditions. These factors must also be considered when selecting sampling points and frequencies.

Article 5 of Regulation (EC) No 2073/2005 provides that the food business operator may use alternative methods of sampling and analysis if it can be proven that they produce equivalent results. According to ISO 6887-1:2017-07, for the pure detection of *L.m.* it is possible to merge the individual samples (pooling) to form a collective sample (pool). Samples may only be pooled for qualitative analysis and it must be ensured that they originate from the same batch.

4 Influencing factors and self-assessment

The first step in assessing the *listeria* risk on company level is the analysis of the relevant influencing variables and the evaluation of the manufactured products. The assessment always takes place within the framework of the existing HACCP concept. This supporting document does not replace the operational HACCP concept, but gives suggestions and hints for effective control and management of the *listeria* risk. This helps to improve the systematics of the HACCP concept regarding *listeria*.

4.1 Product groups

In order to execute an evaluation of the products and product groups, a comprehensive description of the products and their use should first be provided. This should include at least the following criteria:

- Description of the products (examples)
  - Carcasses for deboning
  - Meat cuts for further processing
  - Standardised products for specific product groups, e. g. differentiated according to raw sausages, cooked sausages or raw materials for minced meat production
  - Type of storage (chilled or frozen)
  - Type of packaging
- Description of use (examples)
  - The product concerned is an RTE product.
  - The product concerned is intended as a raw material for an RTE product.
  - The product concerned is intended for a relevant consumer group (e. g. the elderly, those with weakened immune systems, young children, etc.).

In the case of RTE products, a decision must be made based on scientific findings (literature, challenge test, mathematical model calculations or stability studies; Article 3(2) in conjunction with Annex II of Regulation (EC) No 2073/2005) into which category of the regulation the food is to be classified in.

It is also advisable to classify foods that are not ready for consumption (and therefore have no legal obligation to be analysed for *L.m.*, e. g. fresh meat that is not ready for consumption) in terms of their potential risk of contamination with *L.m.* In line with the process concept, it is also advisable to evaluate the hazard potential of these products.

According to the description of the products and their use, a distinction can be made between the risks posed by the product.

- RTE products which, according to Regulation (EC) No 2073/2005, are to be classified in category 1.2 or 1.3 of Annex I, Chapter 1, form the critical group. Whether the food classifies as category 1.2 or 1.3 must be decided by the food business operator himself. The criteria from Regulation (EC) No 2073/2005 and the guidance document SANCO/1628/2008 can provide useful information (see also Figure 2).
- All products which are not RTE products, but which may be used as a raw material to produce an RTE product, represent an indirect hazard.

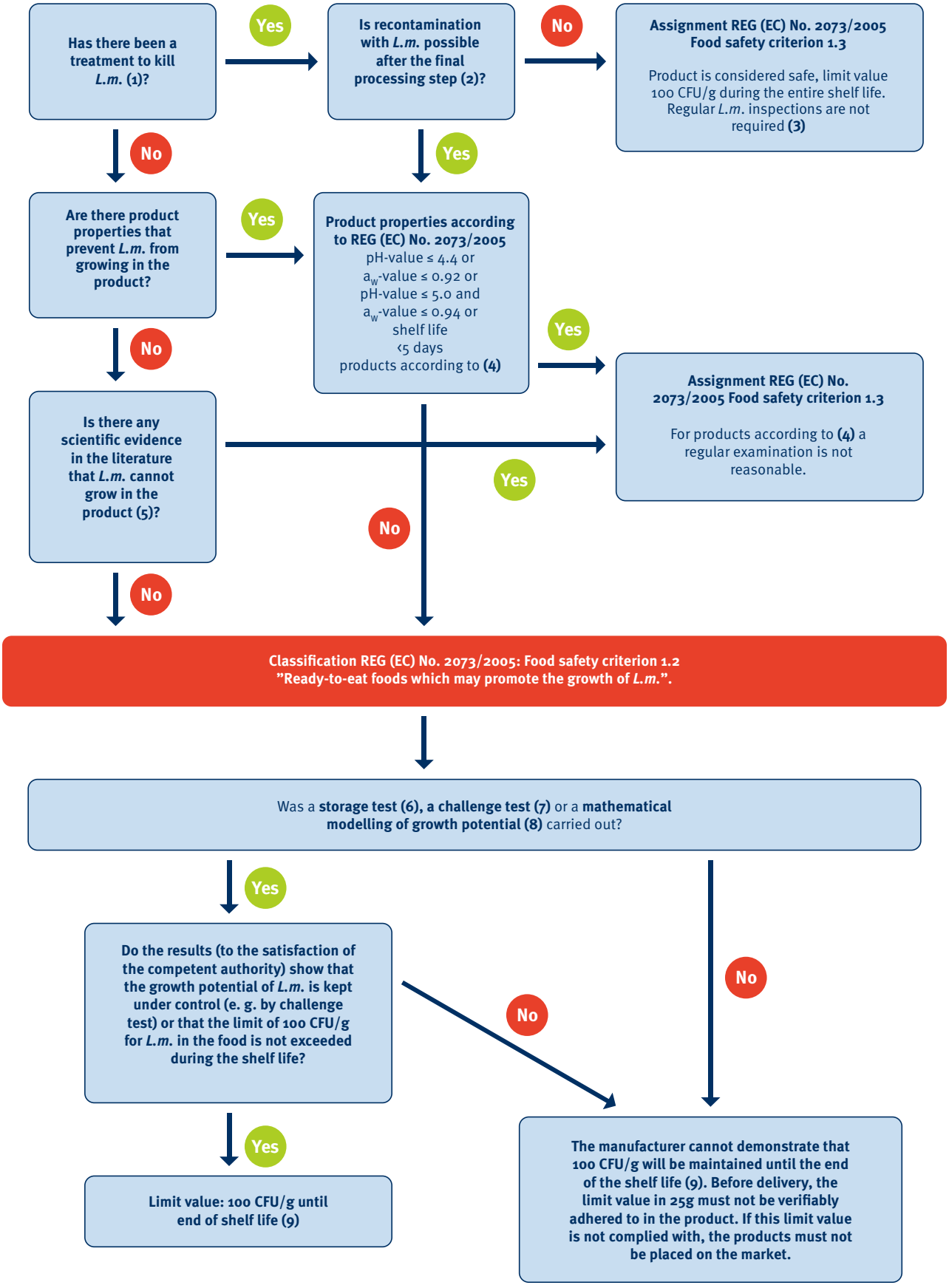

Figure 2: Decision tree for the classification of products according to Regulation (EC) No 2073/2005. Information on (1) to (9) can be found in table 2.

Table 1: Information regarding the decision tree for the classification of products according to Regulation (EC) No 2073/2005

TERM	DEFINITION/EXPLANATION
Storage test	The storage test allows the determination of the shelf life of food regarding *L.m.* by storing uninoculated samples. Storage tests reproduce natural conditions better than challenge tests but are usually limited by a low initial *L.m.* concentration.
Challenge test	The challenge test is used to determine the growth of *L.m.* in artificially inoculated samples to the end of shelf life with the aim of demonstrating that the growth potential of *L.m.* is kept under control (≤0.5 log CFU/g). Such a test, combined with a low concentration of *L.m.*, can ensure that the limit of 100 CFU/g is not exceeded.
Mathematical model	Predictive microbiology (modelling) aims to predict the behaviour of microorganisms in food during production or storage. The models have been developed to predict microbial behaviour when the physicochemical properties of the food (e. g. pH-value, a_w-value, etc.) and storage temperature are known.

Table 2: Information regarding the decision tree for the classification of products according to Regulation (EC) No 2073/2005

NOTES	PARAMETERS	EXPLANATIONS
1 Treatment / Eradication	Heating (at least pasteurisation)	Adequate pasteurisation depending on the matrix must be guaranteed. This should be coordinated between the food business operator and the laboratory.
2 Recontamination		Recontamination is possible, for example, if a product has not been heated in the final packaging.
3 Product is considered safe	e. g. heating or at least pasteuristaion in final package.	The *listeria* is killed and recontamination is impossible.
4 Information in the footnotes of Regulation (EC) No 2073/2005	Regulation (EC) No 2073/2005 lists several product categories for which a regular inspection is not useful (e. g. biscuits, beer, wine, sugar, chocolate products, live mussles, salt, etc.).	Depending on scientific evidence, other products may also belong to criterion 1.3 (limit value 100 CFU/g). For frozen products, it must be ensured before freezing that the conditions of Regulation (EC) No 2073/2005 are fulfilled. *Listeria* do not multiply during storage of deep-frozen RTE products, which corresponds to the classification of these products in category 1.3.

NOTES	PARAMETERS	EXPLANATIONS
5 Scientific evidence	The conditions of the examined food production must be found in the scientific literature.	Literature must refer to models that are close to real food production. Also, the storage conditions (in particular: realistic temperature profiles) must correspond to reality.
6 Storage tests	Storage tests according to the document "Technical guidance document for conducting shelf-life studies on *Listeria monocytogenes* in RTE foods".	Storage tests demonstrate that the limit value of 100 CFU/g is not exceeded until the end of the shelf life. The statistical principles described in the document must be considered.
7 Challenge tests	Challenge tests according to the document "Technical guidance document for conducting shelf-life studies on *Listeria monocytogenes* in RTE foods".	Challenge tests ensure that *L.m.*'s growth potential is kept under control (≤ 0.5 log CFU/g).
8 Mathematical modelling of growth potential		The available growth models refer to the physiochemical profile of a food. If the modelling is not suitable to sufficiently reflect reality, additional storage tests or challenge tests should be executed.
9 Shelf life		Shelf life either implies the period up to the use-by date or the date of minimum durability.

Best-practice notes

- Evaluation of all relevant products within the framework of the HACCP concept
- Categorisation of products according to Regulation (EC) No 2073/2005
- Consideration of critical consumer groups
- Consideration of raw materials for RTE products within the framework of the HACCP concept and mention of them in the specification

4.2 Challenges in the product groups

In addition to the categorisation of products and product groups, the actual occurrence of *L.m.* is an important factor. This requires a comprehensive trend analysis of the available sample results. Both product analysis and environmental analysis should be considered. In general, there are no guidelines for the examination frequency.

The examination frequency and the number of samples should be based on the operational conditions and be included in the framework of the HACCP concept. Reliable results can be seen when a regular and systematic examination has been conducted. Typically, the evaluation of the results is based on the evolution over time, and the long-term evolution should be considered over months, quarters or years. Short evaluation periods can only be useful if the analyses are just a few days apart.

Examples of evaluation

Table 3: Evaluation of *L.m.* evidence in RTE products and raw materials for RTE products that have not been subjected to a manufacturing process that inhibits the growth of *L.m.* or kills it over a long period of time (e. g. one year)

FREQUENCY OF POSITIVE RESULTS	RISK POTENTIAL
No positive results	Very low
Sporadic positive results	Medium
Regular positive results	High

Table 4: Evaluation of *L.m.* evidence on raw materials for products that have been subjected to a manufacturing process that inhibits the growth of *L.m.* or kills it (e. g. heating, drying etc.) over a long period of time (e. g. one year)

FREQUENCY OF POSITIVE RESULTS	POTENTIAL RISK
No positive results	Very low
Sporadic positive results	Very low
Regular positive results; trend shows steady improvement	Low

The deterioration of the results or the sudden occurrence of cumulative positive results should always lead to a more intensive investigation of the cause.

Best-practice notes

- Systematic evaluation of the *L.m.* analyses
- Conclusion of the results regarding processes and products
- Evaluation at regular intervals in a trend analysis

4.3 Spatial plan / material flow

The complete representation of all product and material movements can serve as the basis for the assessment of the company regarding the material flow. For this purpose, the company should present the following material flows:

- Raw materials
- Auxiliary materials
- Semi-finished products
- Finished products
- Packaging materials and reusables

The type of plan should be chosen in a way which ensures an easy identification of all crossing points. Existing hygienic areas in the production should be separated by colour codes. A distinction is usually made between low-risk, high-care and high-risk areas.

In practice, it has been established that the equipment used (pallet trucks, pallets, containers, etc.) is only utilised within one hygiene area in order to minimise cross-contamination between hygiene areas.

It is important to ensure that there is a clear separation of product groups. Different product groups in one area entail major risks and should be avoided wherever possible. This includes, for example, the combined processing of raw meat and pasteurised goods (e. g. cooked sausage, cold cuts) on the same premises. If the products are processed at different times, the pasteurised RTE products should be produced first.

In addition to the material flow, the design and structural condition of the buildings and equipment are important. The persistent characteristic of *L.m.* must be considered, as *L.m.* is likely to form biofilms after the settlement of recesses in combination with moisture. It is therefore of utmost importance that surfaces in the operating environment and surfaces in contact with foodstuffs are easy to clean, dry and as undamaged as possible.

The following points are relevant for the evaluation of buildings and equipment:

- Walls
  - Solid, easy to clean, no cavities or holes
  - Hollow walls / lightweight construction panels / constructive installations and extensions with recesses
  - Floor transition (moulding, transition from wall to floor, cavities, gaskets, etc.)
- Ceilings
  - Suspension or solid ceiling, surface structure, holes, etc.
  - Ventilation and condensation water
  - Accessibility and cleanability
  - Evaporator installation, position, arrestors and insulated drip tray
- Floors
  - Material easy to clean and undamaged on the surface
  - Drains and their positioning
  - Slope to the drains
  - Type of drain (gutter, fixed drain, gully with insert, etc.)
- Equipment
  - No cavities, easy to clean, no standing moisture
  - Fabric conveyor belts free of damage
  - Hygienic design
- Zone Principles
  - Suitable hygiene areas with physical barriers (e. g. hygiene sluices) are installed. Alternative methods (e. g. foam carpets with disinfectant) are installed at relevant locations.

Best-practice notes

- Analysis of all material flows
- Focus on crossing points and the effective delimitation of specific hygiene sectors
- Periodic site inspections to identify and repair defects (e. g. cavities, accumulations of moisture due to design, surfaces difficult to clean or damaged, etc.)
- Preferential installation of drains with removable inserts instead of permanently installed drains and gutters.

4.4 Personnel flow and staff hygiene

The movement of both material and personnel should also be transparent. In the visualization, the hygiene areas and the location of the hygiene sluices should be illustrated. Furthermore, access to break rooms and outdoor areas should be properly designated. In practice, it has proven useful that employees from different hygiene areas use differently coloured work clothing so that they can be easily distinguished from each other.

The rules concerning staff hygiene should include the use and application of suitable detergents and disinfectants, the use of protective clothing and the procedures regarding changing of clothes and hygiene sluices. A special focus should be on changing and cleaning shoes when switching between hygiene areas, alternatively, overshoes can be used. In practice, the risk of bacterial carry-over is significant at the entrances and exits to hygienic areas when moist cleaning systems are used. These areas should thus be given special attention when monitoring the environment.

Staff training

The training of staff on hygiene should cover the basic information about *L.m.*

This includes:

- Importance and occurrence of *L.m.*
- Typical pathways of contamination
- Structural and technical requirements
- Application of detergents and disinfectants
- Application of effective monitoring concepts

Best-practice notes

- Systematic analysis of the personnel movements.
- Analysis should focus heavily on crossing points and effective installation of hygiene sluices.
- Use of differently coloured clothing to differentiate between hygiene areas (e. g. differently coloured hairnets).
- The introduction of shoe changing in high-risk areas was proven to be more effective than the use of shoe cleaning equipment.
- Inclusion of low-risk areas in analysis and measures.

4.5 Cleaning and disinfection

The persistent characteristics of *listeria* must be considered in the planning of cleaning and disinfection measures. This applies particularly to recesses, cavities and damp places.

Cleaning frequencies

In practice, daily cleaning has been proven successful. Intermediate cleaning by using only water should be avoided. If it is indispensable to clean intermediately using water, e. g. during slaughtering, surge water should be used in such a way that there is as little splashing as possible and a distribution of germs via aerosols is prevented. The use of high- or low-pressure equipment should be avoided for intermediate cleaning.

Combined processes (scrubbing and suction machines) have been proven successful in cleaning cold stores and corridors. This way the contamination risk by floors can be reduced. This technique should be combined with a suitable disinfectant. Drains and gullies should always be cleaned with a special cleaning device (e. g. by usage of a brush with a diameter smaller than that of the drain to avoid splashing water) instead of high-pressure or low-pressure equipment. Stagnant water (especially in drains) should be avoided.

During the execution of cleaning and disinfection measures, condensation should be prevented, and special attention should be paid to existing high-pressure lines. In the past, positive results have often been achieved by using high-pressure equipment. To avoid water condensation, adequate ventilation of the cleaned areas and the air pressure level between the individual hygiene areas should be considered.

In general: the more often, the better!

Drying periods

Each cleaning and disinfection cycle should be followed by an appropriate drying time. Even during production times, all production rooms should be kept as dry as possible.

Detergents and disinfectants

When selecting detergents and disinfectants, the particularities of *listeria* should be considered. It is important that the specification of the disinfectant used include information on its efficacy against *Listeria spp.* and *L.m.* The effectiveness of most approved detergents and disinfectants has been tested on low protein and fat loads and planktonic cells. Therefore, the practical suitability of the efficacy studies should be questioned when selecting the agents and, in case of doubt, own investigations should be carried out. It is very important that there are manufacturer specifications for the application of cleaning and disinfecting agents (e. g. water temperature, application concentration and exposure time) and that these are observed in practice. Hot water or steam can also be used for disinfection of objects that are difficult to access.

Cleaning equipment and hoses

Cleaning equipment, tubes and similar equipment should be included in the cleaning schedule. Their permanently damp and partially warm storage carries the risk that contamination remains undetected and can spread over large areas. Over time, hoses become porous both on the outside and inside and thus offer ideal recesses for the growth of *listeria*. The same applies to spray guns and their handles, valves, connectors and the protective clothing of the cleaning staff. For these reasons, special attention should be paid to the storage conditions and the periodic inspection of the cleaning equipment. This equipment should be cleaned, disinfected and inspected for damage at regular intervals. In practice, it has proven useful to place hoses and cleaning nozzles/guns in appropriate disinfectant solutions once a week in order to ensure internal and external disinfection.

Cleaning and disinfection of commodities

All commodities with direct or indirect contact with the product can be a carrier of *listeria* and should be considered in the cleaning plan in addition to building and plant components. For example, big boxes made of plastic are often used to store or transport products both internally and to the customer. Frequently, this involves crossing hygiene areas and possible contamination between different companies. Large containers must not be damaged and should be free of open cavities. Containers with structural cavities should not be used at all.

In addition to cleaning and disinfection, the storage of consumer goods is also an important factor. Storage areas can be arranged in such a way that subsequent contamination is avoided and transportation to the place of use contains as little risk of contamination as possible. In practice, mistakes are often made by storing disinfected commodities (e. g. empties) in unsuitable places and crossing unnecessary low-care or unclean areas on their way to use.

Practice has shown that *L.m.* has established itself in the production environment and can be part of the microbial flora for months or years. Particularly susceptible areas are hollow rollers on conveyor belts, cracks in equipment (e. g. rollers), fits between furnishings (e. g. between floor-to-wall), rubber seals, switches, porous conveyor belts, slicers and reusable gloves.

Best-practice notes

- Establishment of a cleaning and disinfection plan
- Comprehensive list of handling and storage of all relevant commodities and cleaning equipment
- Cleaning of evaporators as well as mechanical cleaning and disinfection of the floors in cold-storage rooms and corridors
- Manual cleaning of gullies and drains at the end of the cleaning process
- Sufficient drying period after cleaning and disinfection

4.6 Control of cleaning and disinfection

Regular checks are recommended to ensure the effectivity of cleaning and disinfection. Typically, the cleaning control includes a visual inspection of equipment and rooms before each start of production. The process should be defined in standard operating procedures. Impeccable basic hygiene helps to reduce or control the risk of *L.m.* contamination. In the production of meat and meat products, the control should also include microbiological tests (e. g. contact plate or wipe samples), via testing for the aerobic mesophilic bacterial count (AMK) and enterobacteria (Ent.). This microbiological control can be carried out either immediately after cleaning or before the start of production (after disinfection).

After sampling, appropriate measures shall be taken to ensure that no residues of the sampling material remain at the sampled area (cleaning) and that the area is disinfected again. According to ISO 18593:2018, sterile cloth soaked in alcohol is suitable for this purpose.

A typical cleaning control plan can be based on the following principles:

Table 5: Example of a typical control plan

AREA	FREQUENCY	METHOD	LIMIT VALUE
Entire company	Before each start of production	Visual	No visible contamination
Surfaces with food contact immediately after cleaning and disinfection	Weekly, different days, approx. 10 to 20 samples	According to ISO 18593:2018	AMK < 100 CFU/100 cm² Ent. 0 CFU/100 cm² *L.spp.* 0 CFU/100 cm²
Surfaces with food contact immediately before production	Weekly, different days, approx. 10 to 20 samples	According to ISO 18593:2018	AMK < 10 CFU/cm² Ent. < 1 CFU/cm² *L.spp.* risk-oriented depending on the product

Hotspots

The sampling areas should be shown in the business plan in order to identify local accumulations (so-called hotspots) more easily. For this purpose, the results should be recorded over a longer period and evaluated regularly (graphically if necessary).

When sampling surfaces which are in contact with food, the largest possible areas should be selected for sampling. The use of sponges that can wipe off areas of multiple 1,000 cm² has proven useful. Alternatively, whole components can be sampled. A wipe sample should be taken in such a way that any existing biofilms are removed as well. Gullies and drains should also be sampled. The use of swabs should be limited to hard-to-reach areas and recesses. Dilution solutions must contain a de-inhibitor if residues of cleaning agents and disinfectants are suspected during sampling. It neutralizes the residues, reducing the probability of false negative results.

Best-practice notes

- Consideration of *listeria* during the control of cleaning and disinfection
- Marking of sampling areas in the operational plan and determination based on a risk assessment
- Execution of a weekly examination in high-risk areas with ten samples on different days of the week
- Creation of a sample plan with the possibility of statistical evaluation (e. g. trend analysis)

4.7 Monitoring of products, raw materials and settings

In addition to cleaning and disinfection control, it is essential to monitor products, raw materials and the production environment in order to prevent *listeria*. Besides the investigation of raw materials, control mechanisms should be arranged with the supplier since the results of the raw material investigations can only verify compliance with these mechanisms.

The company should develop a sampling plan that includes risk-based raw materials, semi-finished products and finished products. For some products, sampling frequencies are already required in Annex I of Regulation (EC) No 2073/2005, but not for *Listeria spp.* and *L.m.* For this purpose, the food business operators have to define the sampling frequencies by themselves, considering the identified risks and the structure of their companies. An inspection for *Listeria spp.* can be used as an indicator for the possible occurrence of *L.m.* and should be used when testing environmental samples and food contact surfaces. Approximately 40% of the positive *Listeria spp.* detection is related to *L.m.* Unless further testing for *L.m.* is performed, any positive *Listeria spp.* detection should be assumed to be *L.m.*

If the testing of environmental samples always leads to negative results, the sampling plan should be reviewed.

When testing products and surfaces with food contact, the following points should be considered:

- Material and personnel flow
- Raw materials and packaging (e. g. E2-boxes, H1-pallets, plastic big boxes, etc.)
- Production environment (ceilings, walls, floors, drains, etc.)
  - All cavities (e. g. behind or at mouldings or transitions from panel walls to the floor or rollers on roller conveyors, etc.)
  - Floors in front of and behind hygiene sluices and sole cleaning equipment in hygiene sluices
  - All gates and doors in contact with the floor (also on installations such as cooking cabinets, etc.)
  - Gaskets and insulating material as well as panel wall fillings
  - Doors, walls and ceilings
  - All forms of condensation water (also in connection with pressurised air systems such as vacuum or compressed air systems)
  - All accumulations of stagnant water especially with contact or connection to floor inlets
  - Wall and ceiling ducts for sewage pipes

- Food-contact surfaces (e. g. plastic conveyor belts, particularly those with fabric inserts, cutting boards, dosing and filling devices, slicers, etc.)
- Damage to respective surfaces (e. g. cracks, recesses, etc.)

Sampling areas shall be established according to the results of a risk-based assessment.

Table 6 presents examples of sampling raw materials and products with related sampling frequencies, procedures and limit values. The recommendations are not legally binding, except the limit values for RTE products of categories 1.2 and 1.3 of Regulation (EC) No 2073/2005.

The examples may be adapted by the food manufacturers taking into account, among other things:

- **Treatment procedures during production**
- **Growth and contamination potential of *L.m.* in the respective product**
- **Level of processing**
- **Shelf life**
- **Quantity of product manufactured**
- **Storage conditions of the product (chilled or unchilled)**
- **Expected consumers**

If the limit values specified here or the derived internal limit values are exceeded, measures must be taken to prove that the *L.m.* risk is under control.

The following factors should be considered when defining the measures which will be taken after positive *L.m.* detection on food contact surfaces:

- **Can the food promote the growth of *L.m.*?**
- **Is it a surface which gets into contact with food?**
- **How often has *L.m.* already been detected in this area?**

It is advisable to dismantle, clean and disinfect the equipment and systems for which positive *L.m.* evidence has been provided. In some cases, the use of heat (at least 71°C for 20 to 30 minutes) has also proven to be effective if the practical possibility exists. Smaller objects can also be placed in hot water with detergent.

If legal limit values in accordance with Regulation (EC) No 2073/2005 are exceeded, it must be checked whether the food can be subjected to further processing in order to achieve compliance with the criteria. Otherwise, it must be checked whether the food may not be placed on the market or whether a recall or withdrawal of goods must be initiated.

Listeria are exposed to several stress factors in food processing and production, such as nutrient deficiency, extreme temperatures, salt, low water activity and biocides. These stressors can favour the occurrence of cells that are not able to grow on classical cultivation media but are nevertheless able to live and possibly become infectious. This cell status has been described as viable but nonculturable.

Table 6: Sample plan with examples for testing for *L.m.*, specifying frequency, procedure and limit value

OBJECT	FREQUENCY	PROCEDURE	LIMIT VALUE
Raw materials for RTE products that are not subjected to any process that inhibits or kills the growth of *L.m.*	Risk-oriented (e. g. 1x monthly)	Consideration of this supporting document, GMP, GHP etc. by the supplier Supplier monitoring and evaluation	*L.m.* not detectable in representative sample quantity (e. g. 5 x 25 g or meat juice sample)
Raw materials for RTE products that are subjected to a process that inhibits or kills the growth of *L.m.*	Risk-oriented (e. g. 1x monthly)	Consideration of this supporting document, GMP, GHP etc. by the supplier Supplier monitoring and evaluation	Individual definition between producer and supplier
RTE products of category 1.2 (compliance with *L.m.* ≤ 100 CFU/g during shelf life could be demonstrated)	Risk-oriented (e. g. weekly on different days)	Quantitative evidence	*L.m.* ≤ 100 CFU/g during shelf life
RTE products of category 1.2 (compliance with *L.m.* ≤ 100 CFU/g during shelf life could not be demonstrated)	Risk-oriented (e. g. weekly on different days)	Enrichment in 25 g	*L.m.* not detectable in 25 g before the food has left the direct control of the food business operator who produced it
RTE products of category 1.3	Risk-oriented (e. g. weekly on different days)	Quantitative evidence	*L.m.* ≤ 100 CFU/g during shelf life

The Food Service and Inspection Service (FSIS) recommends the practice of the "Hold and Test" method when inspecting food contact surfaces during production. It is recommended to block products manufactured at the time of sampling until satisfactory results are obtained. In the case of positive results, the batch may be released after a statistically qualified *L.m.* examination. It is advisable to define a procedure agreed beforehand with the authority.

Generally speaking, positive results on surfaces in contact with foodstuffs are hardly avoidable. According to EFSA, environmental samples for *L.m.* inspection provide positive results in an average of 12% of cases. Investigations of surfaces in food contact from different federal states (in Germany) show positive results of between 4% and 6%. The aim of monitoring food-contact surfaces is to sample as intensively as possible in order to identify all sources of contamination. In case of positive results, the company should intensively sample the affected areas for a short period of time after measures have been taken to demonstrate that the *L.m.* risk has been permanently eliminated. Afterwards, the original sampling frequency can be applied again. The aim of sampling should be to obtain realistic results of the situation in the company, in order to be able to react as early as possible and in a selective manner to sources of input of *L.m.* and not to assume a false sense of security.

Common mistakes:

- Wrong selection of sampling areas

  Few or no samples on food contact surfaces

- Incorrect sampling technique

  Incorrect sampling equipment (e. g. contact plate instead of sponge), too dry, incorrect wiping technique, surfaces too small, no neutralizing agent (de-inhibitor), too many samples after cleaning and disinfection

- Wrong interpretation of individual results

  Monitoring of surfaces that get into contact with food can only be effectively evaluated by regarding the trend of at least the last four to eight samples. This trend should be evaluated on a minimum annual basis (preferably twice a year or every quarter).

- Too much focus on product analysis

  The product analysis can only be used to verify the entire hygiene measures and processes.

Best-practice notes

- Establishment of a *listeria* monitoring system based on corresponding risk assessments with the aim of obtaining results that can be statistically evaluated in a trend analysis
- Sampling points traceable in the company plan
- "Hotspot analysis" and trend analysis according to product groups and hygiene areas (ideally related to each production line)
- Cleaning and disinfection control parallel to monitoring of surfaces in contact with foodstuffs
- Training of the responsible employees in sampling technology

5 Best-practice notes

5.1 Slaughtering

In addition to the recommendations in Chapter 4, the following best-practice notes apply to the slaughtering of cattle and pigs:

Product groups

- Evaluation of all product groups in a risk analysis
- Special consideration of the mammary glands when slaughtering lactating sows due to increased *L.m.* risk

Room plan and material flow

- Effective separation between clean and unclean areas
- Effective separation of waiting, anaesthesia and slaughtering areas from other areas of the slaughtering process
- Regular inspections and repairs – especially of equipment for treating the surface of carcasses
- The clean areas of slaughtering in particular should be kept dry
- Enough ventilation to avoid condensation water
- Prevention of condensation and accumulation of moisture on floors in cold rooms for carcasses
- Enough distance between carcasses and floor

Personnel flow and hygiene

- Separate entrances and exits for the stable and unclean slaughter areas
- Accessibility of the clean area only through hygiene sluices
- Separation of employees from different hygiene areas, also in the break rooms
- Opportunities for apron and hand hygiene in the vicinity of every relevant workplace

Cleaning and disinfection

- Sufficient drying phase after cleaning and disinfection
- Effectiveness of the cleaning agents and disinfectants used against *listeria*
- Regular change (e. g. once a week) of disinfectants to prevent resistance. Follow the instructions for use and dosage exactly!
- Special consideration of cavities and seals on tools (e. g. saws, etc.) as well as equipment (e. g. product extraction, collecting channels, hygiene sluices, etc.) in the cleaning plan
- Intermediate cleaning according to specified frequency without the use of high- or low-pressure equipment
- Regular cleaning and disinfection of the floors in the cold stores
- Special attention to intermediate cleaning and disinfection of tools in contact with the product in order to avoid the risk of cross-contamination
- Consideration of ground level discharges, gutters, hygiene sluices as well as the whip system during cleaning control
- Hygienic design of the slaughtering and gutting equipment, as well as the machines and building structure in the production environment
- Consideration of cavities and recesses in wet cleaning due to the danger of biofilms
- Production environment allows easy cleaning, and food contact surfaces are smooth, non-absorbent, sealed and allow good water drainage

5.2 Deboning

In addition to the recommendations in Chapter 4, the following best-practice notes apply to the cutting of beef and pork:

Product groups

- Evaluation of the product groups as part of a risk analysis
- Special consideration of meat used as raw material for RTE products of categories 1.2 or 1.3 (see chapter 4.1).
- Special consideration of sow meat due to a tendency of higher *listeria* risk

Room plan and material flow

- Conception of the plant rooms with the aim of minimising the accumulation of moisture during ongoing operation
- Systematic detection and repair of damage to surfaces in contact with foodstuffs
- Special attention to cutting boards, cutting belts, skinning and membrane skinning machines
- Reduction of condensation water to a minimum (if necessary, use of ventilation systems)
- Floor drains with removable inserts instead of gutters

Personnel flow and hygiene

- Suitable entrances and exits for the various hygiene areas
- Access to clean areas only through hygiene sluices
- Separation of employees from different hygiene areas, also in the break rooms

Cleaning and disinfection

- Sufficient drying phase after cleaning and disinfection
- Effectiveness of the cleaning agents and disinfectants applied against *listeria*
- Regular change of disinfectants (e. g. once a week) to prevent resistance. Follow the instructions for use and dosage exactly!
- Special consideration in the cleaning plan of cavities and seals on tools (e. g. saws, etc.) and of equipment (e. g. product extraction, floor passages, collecting channels, hygiene sluices, etc.)
- Intermediate cleaning according to specified frequency without the use of high- or low-pressure equipment
- Regular cleaning and disinfection of floors in cold stores
- Decontamination of work equipment with dry processes (such as cold disinfection) instead of hot water
- Special attention to tools that get in contact with the product, such as cutting boards and cutting belts, as well as the structures and machines above them
- Consideration of ground level discharges, gutters and hygiene sluices during cleaning control
- Hygienic design of the deboning equipment, machines and building structure in the production environment
- Consideration of cavities and recesses when wet cleaning due to the risk of biofilm formation
- Production environment allows easy cleaning, and food contact surfaces are smooth, non-absorbent, sealed and allow good water drainage

Monitoring of products, raw materials and environment

- Evaluations of the microbiological monitoring of the cut meat with the possibility of drawing conclusions about product groups, the chronological development (trends) and the success of the executed hygienic measures.

5.3 Processing

In addition to the recommendations in Chapter 4, the following best-practice notes apply to the processing of meat, meat products, meat preparations and ready meals with meat content:

Product groups

- Evaluation of the products within the framework of a risk analysis and allocation based on comprehensible documentation (e. g. in the form of the decision tree) into the respective categories of the Regulation (EC) No 2073/2005
- Special consideration of raw materials used for RTE products of the categories 1.2 or 1.3 (see Chapter 4.1) as well as for minced meat and raw meat preparations

Room plan and material flow

- Design of the operating rooms with the aim of minimising the accumulation of moisture during ongoing operation
- Spatial separation – ideally separate rooms or buildings – of raw, fermented and heated meat products instead of merely chronological separation with a significantly higher risk
- Identification of crossing points in the company and control by suitable measures (e. g. decontamination foam carpets, pallet transfer stations, etc.)
- Systematic detection and repair of damage in rooms and on surfaces with food contact – especially in plants in high-care and high-risk areas (e. g. slicers, packaging plants, filling systems, freezers, etc.)
- Consideration of the interior of the systems
- Explicit monitoring of sensitive components such as smoke and cooking systems, freezers and the effectiveness of hygiene sluices
- Reduction of air humidity through ventilation systems
- Reduction of water condensation to a minimum
- Application of clean-room-technologies and microbiological control of the indoor air in high-risk areas
- Floor drains with removable inserts instead of gutters

Personnel flow and hygiene

- Suitable entrances and exits for different hygiene areas
- Accessibility of the clean area only through hygiene sluices
- Instead of shoe-cleaning systems in high-risk areas, implement changing of shoes with additional decontamination
- Change of protective clothing for employees in high-risk areas at risk-based intervals
- Separation of employees from different hygiene areas including break rooms

Cleaning and disinfection

- Sufficient drying phase after cleaning and disinfection
- Effectiveness of the cleaning agents and disinfectants used against *listeria*
- Regular change of disinfectants (e. g. once a week) to prevent resistance. Follow the instructions for use and dosage exactly!
- Special consideration of cavities and seals on tools (e. g. saws, etc.) and equipment (e. g. product guides, transport and feed equipment for packaging material, hygiene sluices, etc.) in the cleaning plan. This also includes plant components close to the floor (e. g. drains, doors of cooking cabinets, etc.)
- Consideration of hard-to-reach areas by using special systems for fogging disinfectants
- Suspended ceilings above critical areas (e. g. high-risk areas) must be kept clean and decontaminated
- Intermediate cleaning according to predetermined frequency and without the use of high- or low-pressure equipment
- Decontamination of tools with disposable wipes in combination with disinfectants
- Regular cleaning and disinfection of the floors in the cold stores for semi-finished goods, ideally with the help of suitable cleaning equipment

Monitoring of products, raw materials and environment

- Examination of the production environment and equipment is the core element of prevention
- Examination of semi-finished and finished products to verify the executed hygienic measures
- Regular evaluations in order to draw conclusions about product groups, production lines, the development over time (trends) and the success of the performed hygiene measures
- Hygienic design of the processing equipment (packaging machines, filling machines, etc.) and the production environment
- Consideration of cavities and recesses in wet cleaning due to the risk of biofilm formation
- Production environment allows easy cleaning, and food contact surfaces are smooth, non-absorbent, sealed and allow good water drainage

5.4 Sampling and methods

Sampling of surfaces

- Determination of the area to be examined as a representative section of the surface
- Selection of a large area for the qualitative detection of microorganisms in order to increase the probability of detection of even small bacterial counts (if possible, an area between 1,000 and 3,000 cm²)
- For quantitative detection of microorganisms, select a representative area of maximum 100 cm²
- Usage of sterile, buffered peptone water as diluent for swabs, cloths or sponges
- Adding neutralisation agents only if residues of disinfectants are suspected, otherwise the test results will be influenced
- Usage of sampling material which is basically sterile and inhibitor-free

  Material to be used:

  - **Swabs** should be used for hard-to-reach, uneven surfaces (maximum 100 cm²). They consist of a fragile handle with a head (e. g. cotton). A defined, marked area is swabbed, after which the swab is transferred into a tube with dilution or neutralisation medium. The choice of wet or dry swabs depends on whether the surface is wet. For dry surfaces, wet swabs should be used and vice versa.
  - **Sterile wipes or sponges** should be used for particularly large surfaces (larger than 100 cm²). Wipes and sponges can also remove biofilms. After sampling, the sampling material is placed in a sterile plastic bag containing dilution liquid.
- Exact description of sampled area and sampling location
- Always sample the same area and location to allow a trend analysis
- Work with templates to ensure correct adherence to the selected surfaces
- Cleaning and disinfection of the sampled area to avoid contamination by culture medium residues
- Cooled transportation to the test laboratory within four hours (1 °C to 4°C)
- Analysis of the samples in the laboratory within 24 hours

Sampling of foods

- Representative sample selection and composition
- Consideration of the process steps within production when selecting samples, e. g. after mincing, after slicing, at the exit of the freezer, when inserting into the package, etc.
- Coordination of the sample quantity – depending on the examination spectrum – with the laboratory and consideration of any follow-up analysis. In most cases, approx. 100 g per sample material is advisable.
- Cool storage of sample material until transport
- No freezing of samples, because depending on the process, false results may occur
- Always keep sample material cool during transport
- When examining samples, it must be clear whether an individual examination (e. g. 5 x negative/positive result in 25 g) or a pool examination (e. g. 1 negative/positive result in 125 g) is required
- When pooling, it must be considered that it will take more time to identify the food with a positive result compared to the single analysis of the samples

Sampling of carcasses and raw materials

- Inspection of carcasses during the control of raw materials. The correct sampling is described in ISO 17604:2015.
- Always choose the same sampling methods and sampling areas in order to be able to execute trend analyses.
- The determination of the sampling areas is process-oriented and reflects the critical points in the process.
- Examples of sampling:
  - **Surface samples** (removal of tissue with a defined surface)
    A template or a sterile cork drill with a defined size is used for the extraction. Results are given as CFU/cm² (e. g. per 10 cm², 50 cm², 100 cm² etc.). Here, too, several samples can be pooled into one.
  - **Swabs** (e. g. for cattle)
    Sampled areas must either be as large as possible or focused on known contamination spots (ISO 17604:2015). The result is given as CFU/cm². The choice of sampling material (swab, cloth, etc.) is made according to the conditions of the carcass.

Microbiological detection of *L.m.*

- An examination for *listeria* may only be executed in appropriately equipped laboratories. The corresponding competence is proven by an accreditation according to ISO 17025:2018.
- The detection of *L.m.* is usually conducted according to the specifications of ISO 11290:2017-1 (qualitative detection) or ISO 11290:2017-2 (quantitative detection). Presumptive *L.m.* or *L.spp.* are subsequently confirmed by suitable morphological, genotypic or biochemical analysis.
- Alternative detection methods are permitted if they provide at least equivalent results to the reference method (e. g. PCR analysis) and are validated and certified by third parties in accordance with Article 5 of Regulation (EC) No 2073/2005 in accordance with ISO 16140-2:2016-11 or other internationally recognised similar protocols.

6 Definitions

6.1 Abbreviations

AMK:	Aerobic mesophilic bacterial count
a_W-value:	Ratio of the water vapour pressure above the foodstuff to the water vapour pressure of pure water.
EFSA:	European Food Safety Authority
Ent.:	Enterobacteria
FSIS:	Food Safety and Inspection Service
GAP:	Good agricultural practice
GHP:	Good hygienic practice
GMP:	Good manufacturing practice
HACCP:	Hazard analysis and critical control points
CFU:	Colony-forming units
log:	Logarithmic unit of measurement for the description of germ reduction
L.m.:	*Listeria monocytogenes*
L. spp.:	*Listeria species pluralis*
pH-value:	Negative decadic logarithm of the hydrogen ion concentration
RTE products:	Ready-To-Eat products

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