PO Box 10800 Market Harborough LE16 0HU
www.chilledfood.org

Action following the detection and/or enumeration of *Listeria monocytogenes* (*Lm*) or *Listeria* species (*L. spp*) in food including at levels below the limit of quantification (<LOQ)

This document provides guidance on action to be taken following the detection and/or enumeration of Lm or *L. spp* in food, and information on the relevance of Uncertainty of Measurement to results.

When a laboratory is asked to test a foodstuff, it could be for *L. spp* or *Lm,* the latter being the only *Listeria* species for which legal limits are set. Whether detection or enumeration is required determines the choice of method, i.e., ISO 11290-1 or 11290-2, respectively.

Enumeration of the number of a particular organism or group of organisms in a sample requires the sample to be diluted by one tenth using a buffered diluent and sampling 1 gramme by directly plating onto a specifically designed 'selective' agar to allow any organisms present to grow under ideal conditions (nutrients and temperature) to be seen as a colony. The result will be reported as a number of colony forming units in 1 g (cfu/g). The lowest level of *Listeria* reported by traditional enumeration methodologies is typically <10cfu/g or <20cfu/g, which are limits of quantification (LOQ). This means *Listeria* may be present at lower levels i.e., 1-9cfu/g or 1-19cfu/g respectively.

For organisms that are rarely found or found in very low levels a **detection** method can be used in addition to quantification e.g., *Listeria spp*, which is much more sensitive but will not give a quantitative result. For detection, a larger 25g sample is used and the very low number of cells potentially present 'enriched' by allowing them to grow under ideal conditions to levels which enable them to be detected on the specifically designed 'selective' agar plate. This result will be reported as 'detected' or 'not detected' in 25g.

More sensitive **detection** methodologies will typically detect *Listeria* (species or *monocytogenes*) down to 1 cfu in the sample. Due to the additional enrichment stages for *Listeria* detection, results will be available **after** the enumeration results (if carried out at the same time).

Both tests (enumeration and detection) should be carried out on the same product on the same day, which could be:

- end of life for products where *Listeria* may grow, to support the assigned shelf life, or
- start of life if *Listeria* cannot survive in the product, or to demonstrate GHP during manufacture

Actions required on detection

When considering actions required following the isolation of *Lm* in a food sample it must be remembered that:

1. Food safety cannot be guaranteed by testing

   Each test is based on a sample taken from a batch and pathogen detection cannot indicate contamination throughout the batch nor can a result of not detected indicate that there is no contamination in the whole batch. This is why HACCP has to be used to control food safety and testing

can only be used to verify HACCP and its CCPs. Therefore, detecting *Lm* in a food sample indicates a possible failure in HACCP or its supporting prerequisites which requires immediate investigation and action.

2. Not all *Listeria spp* are pathogenic – only Lm is

   Lm is the only human pathogen and food safety *L. spp* hazard. *L. spp* (other than *Lm*) do not pose any food safety concerns and **must only** be used as indicator organisms as they can potentially indicate future sources of *Lm* contamination and can be used to proactively control *Lm*.

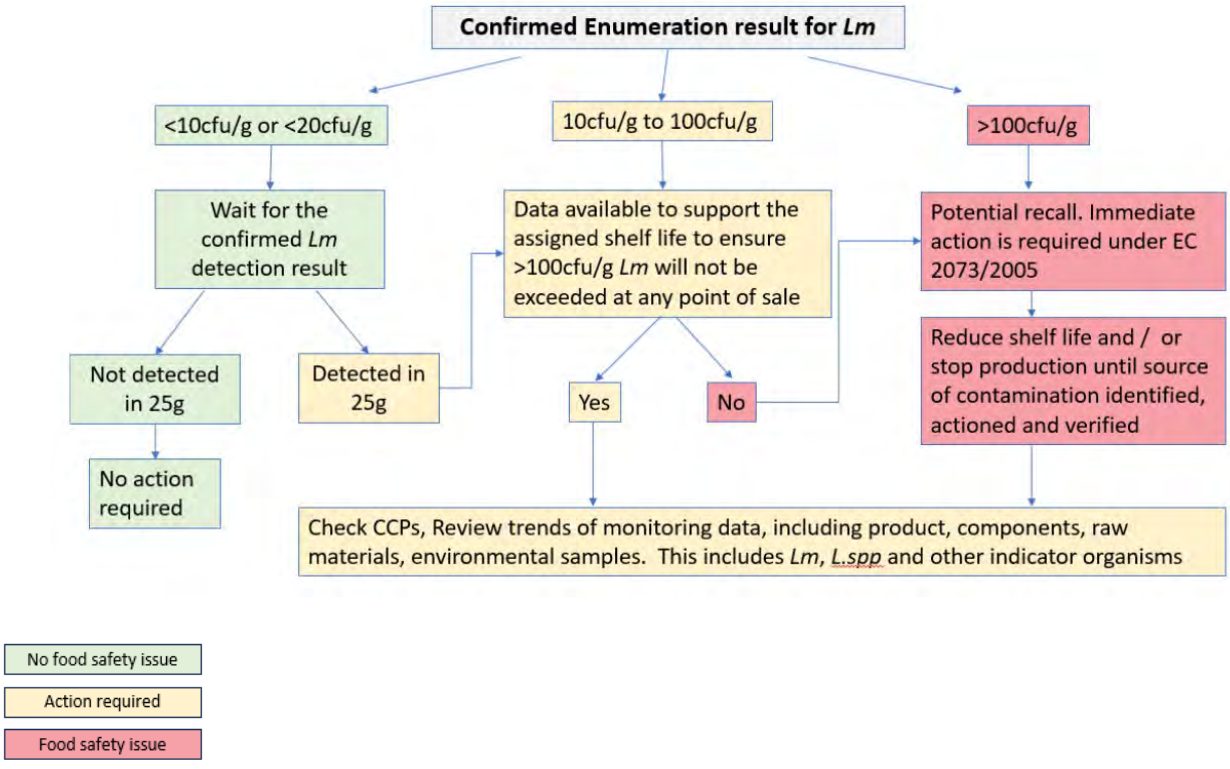
3. No method can be 100% accurate

   Some *L. spp* can be misidentified as *Lm* using routine confirmatory methods, this is explained further below.

4. Out of specification results

   Any out of specification result must not be actioned by retesting even for other microorganisms. **Microbiological testing can only be used as verification of the efficacy of a corrective action taken following an out of specification result.**

Figure 1: **Decision tree showing the appropriate action to be taken for *Lm* results to identify food safety issues and comply with legislation**



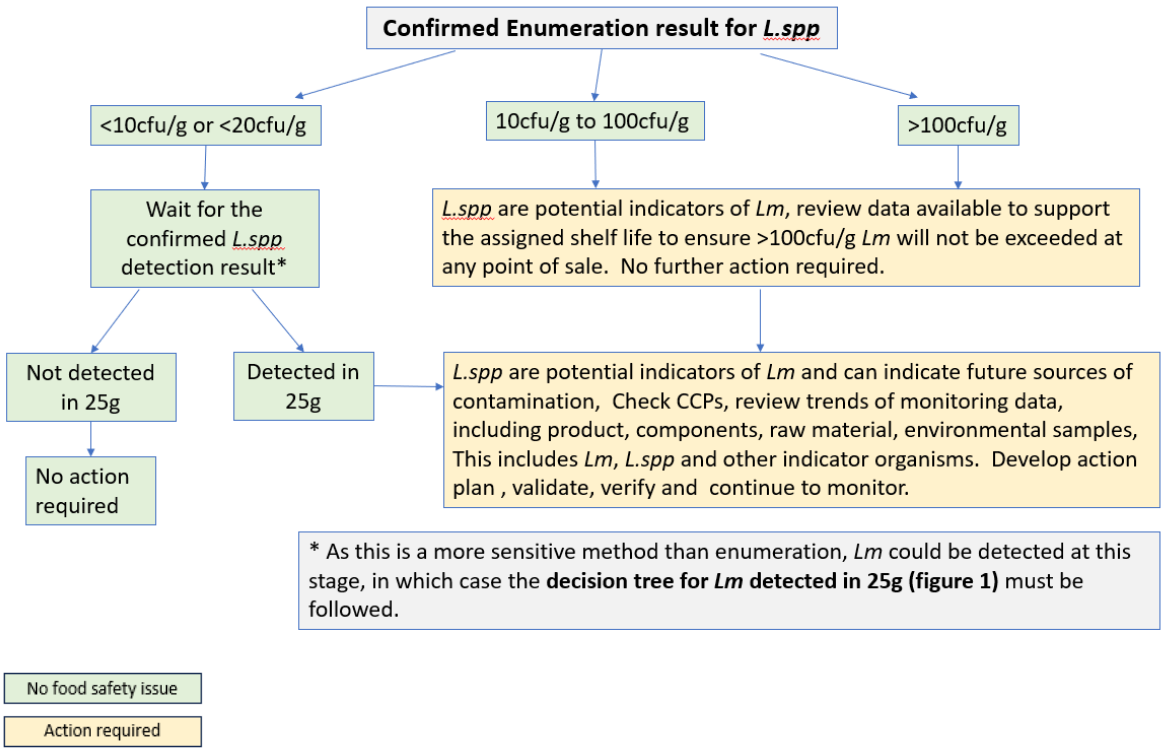
Relevance of detecting *Listeria spp*

With the development of whole genome sequencing, 28 species of *Listeria* have to date been discovered. These have been categorised into 2 groups *Listeria* sensu stricto and *Listeria* sensu lato[1]. Biochemical galleries will currently distinguish between *monocytogenes, innocua, seeligeri, ivanovii, welshimerii* and *grayii*, but not other species.

Listeria sensu lato species are **not** closely related to *Lm*, they are not pathogenic, are non-motile and some cannot grow at chilled temperatures, therefore these species cannot be used as indicator organisms. However, some are incorrectly identified as other *L. spp* (usually *L. ivanovii*) but not *Lm* using routine methodology.

Listeria sensu stricto are those species closely related to *Lm* and can be used as index indicator organisms. There are only 9 Listeria sensu stricto species, all of which are detected and confirmed as *L. spp* using most existing classical and rapid methods. However, *L. marthii*, *L. swaminathanii* and *L. cossortiae* are usually identified incorrectly as *Lm* but are NOT pathogenic.

Figure 2: **Decision tree showing the appropriate action to be taken for *L. spp* results to identify food safety issues and comply with legislation**



[1] See Wiedmann (2023). Is it a *Listeria* sensu stricto or sensu lato species? Why understanding the difference is important. https://www.chilledfood.org/wp-content/uploads/2023/08/IAFP-Listeria-sensu-lato-or-stricto-5_8_23_Webinar.pdf (accessed 6 September 2023)

Addendum: Note on Uncertainty of Measurement (UoM)

UoM is a tolerance applied to enumeration methods only and calculates the effect that the variables within the analysis have on the final result, e.g., tolerances on measurements used, such as weights and temperatures, differences in media batches and methods, staff technique differences.

As detection methods have enrichment stages, enumeration cannot be established, but the sensitivity of the method can be measured i.e., the lowest number of cells the method can detect in 25g.

Uncertainty will not be the same on all methods or for all laboratories and may change over time but should in a well-managed laboratory be relatively stable without any major fluctuation over time.

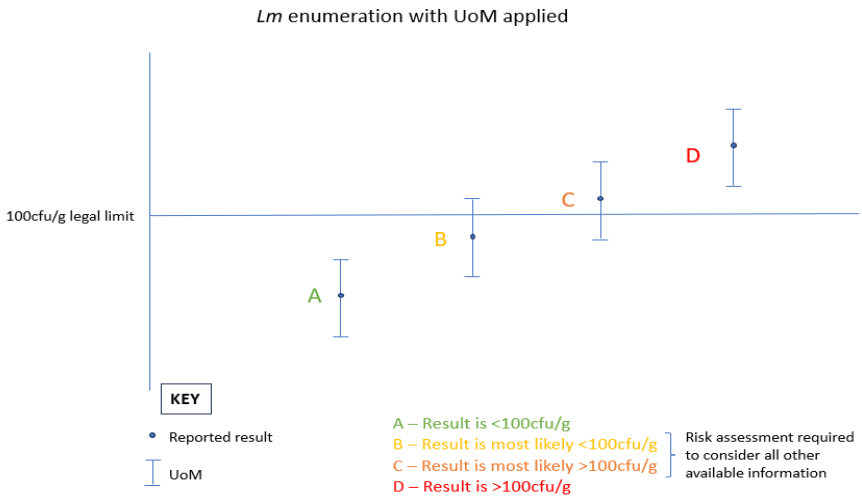
UoM will be available from a laboratory for a specific test but is not required to be reported on test certificates as the reported result will be the median. The potential lower result cannot be used for an out of specification reported result, nor can the upper result be used for reported results within specification.

BRCGS Food Safety Issue 9[2] requires (Clause 5.6.2): "Where applicable, the measurement uncertainty associated with laboratory test results shall be considered."

All reported results must be interpreted and risk assessments carried out by an appropriately trained person, considering the context of the result e.g., epidemiological data, baseline trend data, hygiene indicators, product type and use, and the likelihood of making people ill. **The detection or enumeration of a pathogen alone may not represent a risk to public health.**

In the event of a potential food safety issue, the UoM should be considered in the risk assessment. Ensure that the UoM is relevant to the time the result was generated as UoM may change over time due to method and staff changes. The UoM can indicate the accuracy of the reported result.

Figure 3: **Diagram to show the application and relevance of UoM**



[2] https://www.brcgs.com/our-standards/food-safety/issue-9-revision/