



BTSF ACADEMY

Organisation and implementation of training activities to strengthen understanding, implementation and enforcement of EU law in the area of Sanitary and Phytosanitary Standards (SPS) in EU Member States and neighbouring non-EU countries

STM - Microbiological shelf-life studies of ready-to-eat foods related to *Listeria monocytogenes*

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Vilnius, Lithuania; Session 1: 14-17/04/2026; Session 2: 05-08/05/2026

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7. DURABILITY STUDY

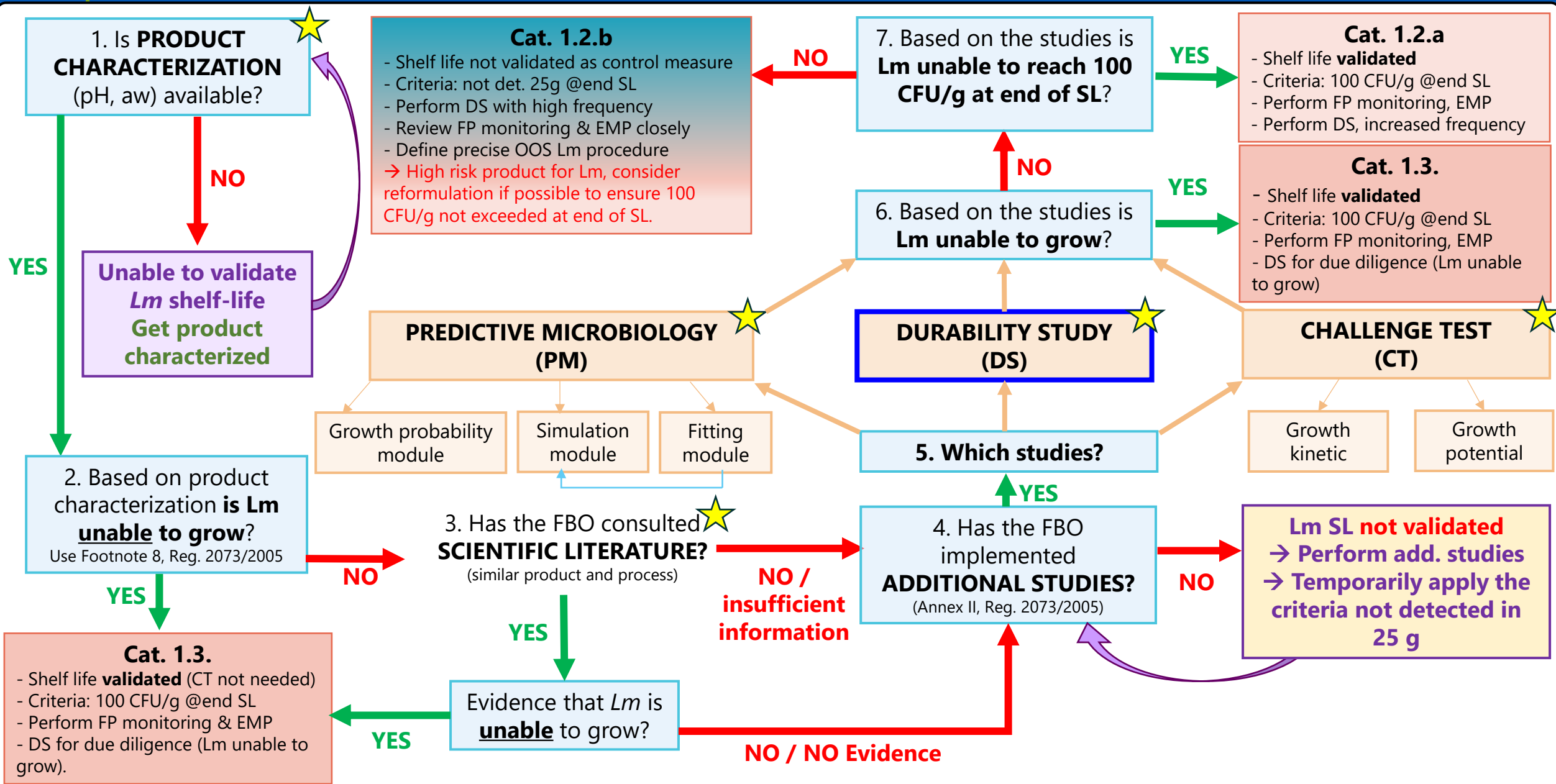
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Helene BERGIS

STM - Microbiological shelf-life studies of ready-to-eat foods related to *L.monocytogenes*

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Decision tree to identify if the shelf life is validated, under which category, which studies to perform and which limit to apply



BTSF Outline

Definition and Uses

Key steps

Example

Benefits and Limitations

DURABILITY STUDIES -> Regulation (EC) No 2073/2005 – Annex II

Studies to evaluate the growth or survival of the microorganisms of concern that may be present in the product during the shelf-life under reasonably foreseeable conditions of distribution, storage and use

Targeted at *Listeria monocytogenes* -> EURL *Lm* Technical Guidance Document:

A laboratory study conducted to determine:

- ✓ The concentration of *Lm* **at the end of shelf-life**
- ✓ In a **naturally contaminated food**
- ✓ **Stored under reasonably foreseeable conditions** from production to consumption

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Uses



Studies conducted by FBOs, once the shelf-life has been validated, to **verify** the determined shelf-life of RTE food



Tool used to **verify** that the quantitative *Lm* criterion (100 cfu/g) is not exceeding at the end of the shelf-life under foreseeable conditions of storage (*EURL Lm, Technical Guidance Document, 2021*)

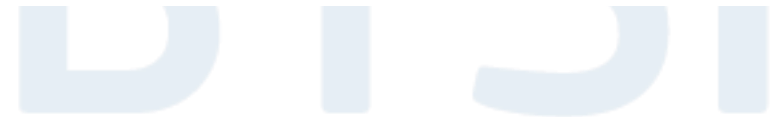


Studies conducted over time at regular intervals set by FBOS, on RTE foods already on the market

Inappropriate for the validation of RTE food shelf-life:

- Low prevalence and/or low level of *Lm* contamination
- Heterogenous distribution of *Lm* in solid food

In most cases, results will be obtained from samples not contaminated with *Lm* → **Unreliable results**



Steps of a durability study related to *Lm*:

1. Description of the food product
2. Sampling procedure
3. Storage of samples
4. Microbiological analyses
5. Results and Interpretation

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Gather **information** on:

- the composition of the food (list of ingredients)
- the main steps of the production process
- the physico-chemical and microbiological characteristics of the food
- the characterisation of the cold chain

RTE food able to support *Lm* growth where **low levels** of *Lm* contamination **frequently occur**

The sampling procedure has to be representative of the **production variability**.

All units of the production batch should have the same chance of being sampled



Simple random sampling of products from **the same batch**

Cold chain stage	Storage temperature			Storage duration		
					Shelf life (SL) ≤21 days	Shelf life (SL) >21 days
From manufacture until the arrival to the display cabinet	Temperature justified by detailed information ^(a)	Or if not known:	7 °C	Duration justified by detailed information	Or if not known:	One third of the total SL of the product
Retail: Display cabinet	Temperature justified by detailed information ^(b)		7 °C			One third of the total SL of the product
Consumer storage			10 °C			One third of the total SL of the product
						7 days
						1/2 (SL – 7 days)
						1/2 (SL – 7 days)

(a) The 95th percentile of the FBO's data observation

(b) The 95th percentile of the observations for the country where the stage of the cold chain is located

Source: Table 4 of the EURL *Lm* Technical Guidance Document (2021)

Analytical methods to be used can be:

- ✓ The reference method (Standard EN ISO 11290-2)
- ✓ Alternative methods validated in accordance to Standard EN ISO 16140-2
- ➡ Use a method with **a limit of enumeration lower than 100 CFU/g** (usually 10 CFU/g)

RTE food able to support *Lm* growth where **low levels** of *Lm* contamination **frequently occur**



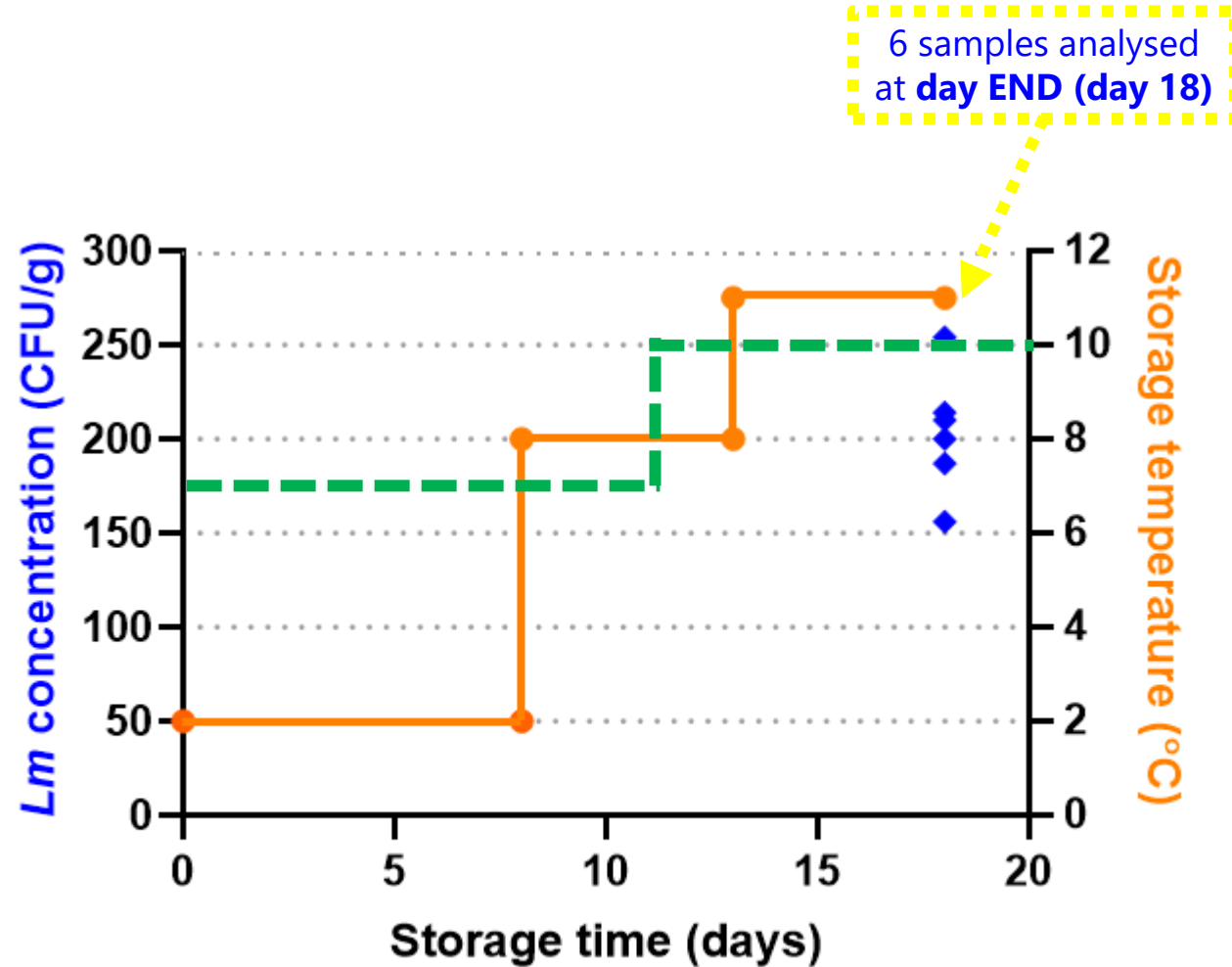
Analyses at **the end of the shelf-life**

Example of a durability study for a RTE food able to support *Lm* growth where **low levels** of *Lm* contamination **frequently occur**

In this example, storage conditions (temperature and duration) were justified by detailed information by the FBO.

Otherwise, for a shelf-life of **18 days**, the study conditions would be:
12 days at 7 °C + 6 days at 10 °C

(Ref: Table 3 of the EURL *Lm* Technical Guidance)



Steps to follow...

1. **Calculate the proportion of positive samples**, i.e. exceeding the limit of 100 CFU/g at the end of the shelf-life, **in the selected samples** (sub-population of the tested batch)
2. **Calculate a confidence interval associated** with that proportion
3. With the above... **Estimate the proportion of positive samples in the whole population** (the tested batch)

1. **Calculate the proportion of positive samples**, i.e. exceeding the limit of 100 CFU/g at the end of the shelf-life, **in the selected samples** (sub-population of the tested batch)

	Number of samples analysed at the end of the shelf-life	Number of samples over 100 CFU/g	Proportion of samples over 100 CFU/g
Batch A	5	0	0 %
Batch B	8	1	12.5 %

2. Calculate a confidence interval associated with that proportion

Batch A

Calculator:

Successes = Proportion =

Examined = Confidence =

Central Confidence Interval:

Lower limit = Upper limit =

Shortest Confidence Interval:

Lower limit = Upper limit =

Batch B

Calculator:

Successes = Proportion =

Examined = Confidence =

Central Confidence Interval:

Lower limit = Upper limit =

Shortest Confidence Interval:

Lower limit = Upper limit =

Number of samples
exceeding 100 CFU/g

Number of samples
analysed

Level of confidence
at 95 % (be sure at
95 % that the true
proportion of the
population is in the
range of the
confidence interval)

3. Estimate the proportion of positive samples in the whole population (the tested batch)

	Number of samples analysed at the end of the shelf-life	Number of samples over 100 CFU/g	Proportion of samples over 100 CFU/g	Estimated proportion of samples over 100 CFU/g (95 % confidence interval)
Batch A	5	0	0 %	0.4 % – 45.9 %
Batch B	8	1	12.5 %	2.8 % – 48.2 %

Central Confidence Interval:

Lower limit = 0.004211 Upper limit = 0.459258

Shortest Confidence Interval:

Lower limit = 0 Upper limit = 0.393038

Central Confidence Interval:

Lower limit = 0.028145 Upper limit = 0.482497

Shortest Confidence Interval:

Lower limit = 0.008629 Upper limit = 0.433445

Key steps - 5. Results

The more the samples are analysed, the narrower the confidence interval is

Number of samples analysed	Number of samples over 100 CFU/g	Proportion of samples over 100 CFU/g	Estimated proportion of samples over 100 CFU/g (95 % confidence interval)
5			0 % – 46 %
10			0 % – 28 %
15			0 % – 21 %
20			0 % – 16 %
25			0 % – 13 %
30	0	0 %	0 % – 11 %
35			0 % – 9.7 %
40			0 % – 8.6 %
...			...
95			0 % – 3.8 %
100			0 % – 3.6 %

The more the samples are analysed, the narrower the confidence interval is



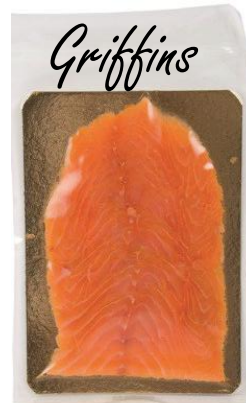
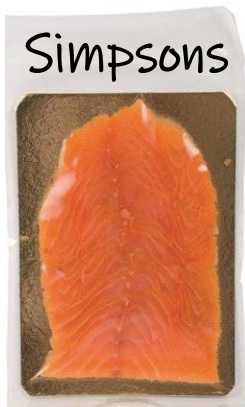
It is possible to **gather results of repeated durability studies** performed on the **same RTE food** obtained from the **same production process**



These repeated durability studies will contribute to **increase the level of confidence in the established shelf-life**

BTSF Example – Results Interpretation

Two FBOs of smoked salmon obtained the results below from durability studies relating to *Lm*



Number of
samples
analysed

12

Number of
samples over
100 CFU/g

0

Number of
samples
analysed

50

Number of
samples over
100 CFU/g


2

Can HOMER claim
to have **better**
results than PETER?



BTSF Example – Results Interpretation

Calculator:



# Successes = 0	Proportion = 0
# Examined = 12	
Confidence = 0.95	
<input type="button" value="Reset"/> <input type="button" value="Compute"/>	


Central Confidence Interval:

Lower limit = 0.001946 Upper limit = 0.247053

Shortest Confidence Interval:

Lower limit = 0 Upper limit = 0.205817

Calculator:



# Successes = 2	Proportion = 0.04
# Examined = 50	
Confidence = 0.95	
<input type="button" value="Reset"/> <input type="button" value="Compute"/>	

Central Confidence Interval:

Lower limit = 0.012299 Upper limit = 0.134586

Shortest Confidence Interval:

Lower limit = 0.006448 Upper limit = 0.12058



Peter's samples have exceeded the legal limit, but statistically, **the probability of exceeding the legal limit is higher for Homer** (25 % vs. 13 %)

Competent Authorities have to decide, based on a risk assessment, what the number of samples to be tested should be
→ an acceptable value for the upper limit

- ✓ A useful tool to **verify the established shelf-life**
- ✓ **Easy to carry out** in the laboratory, no specific equipment needed
- ✓ **No bias** concerning the **physiological state of the bacteria** in food because of the natural contamination
- ✓ Possibility to gather results from **repeated durability studies to increase the level of confidence in the established shelf-life**

- ❖ Durability studies **are not appropriate to validate the microbiological shelf-life** of RTE foods:
 - low-prevalence/low level of *Lm* contamination
 - heterogeneous distribution of *Lm* in solid food
- ❖ **Not possible to test all the foreseeable storage conditions**, therefore, it is not possible to extrapolate
- ❖ **Numerous samples are required for greater confidence in the statistical interpretation**

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Thank you!

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