



BTSF ACADEMY

Organisation and implementation of training activities to strengthen understanding, implementation and enforcement of EU law in the area of Sanitary and Phytosanitary (SPS) standards 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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Challenge tests for shelf-life determination

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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



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Learning Objectives

1. Explain the design of a growth potential and growth rate study
2. Identify the steps in designing those studies
3. Know the advantages and disadvantages of both types of studies
4. Evaluation of a growth potential and growth rate study report

BTSF Challenge tests for shelf-life determination

- Challenge test: Laboratory study to assess the growth, survival or inactivation of microorganism(s) artificially inoculated in a food (ISO 20976-1)
- Two types of challenge tests (CT) can be used to study the growth of *Listeria monocytogenes* (*Lm*) in an artificially inoculated RTE food:
 - 1: CT assessing the growth potential (Δ)
 - 2: CT assessing the maximum growth rate (μ_{\max})

BTSF Challenge test assessing the growth potential

- The growth potential is defined as:

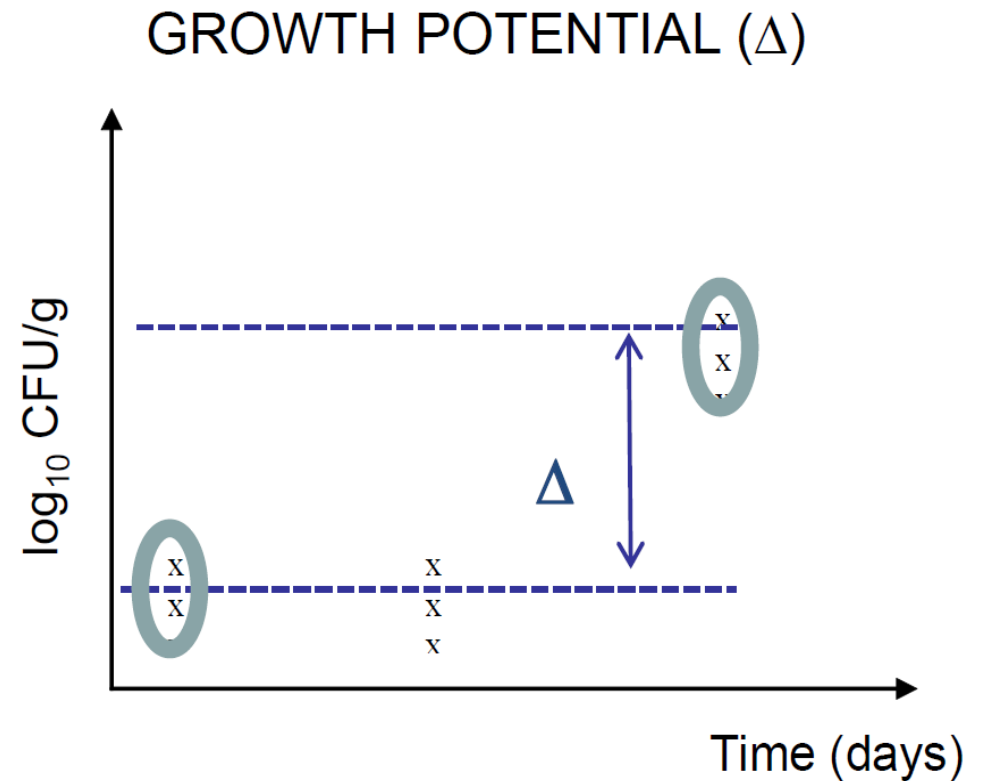
Growth potential (Δ) = (highest observed *Lm* concentration) – (initial *Lm* concentration)

- Initial *Lm* concentration is the concentration (cfu/g) added to the food at time of inoculation

$$\Delta = \log_{\max} - \log_i$$

\log_{\max} = highest value observed

\log_i = level at $t=0$



BTSF Challenge test assessing the growth potential

- The determined growth potential (Δ) is used to classify the food according to EC Reg. 2073/2005
- Is linked to whether the food can or cannot support the growth of *Lm*.
- Category 1.2 foods are **able** to support the growth of *Lm*
- Category 1.3 foods are **not able** to support the growth of *Lm*

1.2	Ready-to-eat foods able to support the growth of <i>L. monocytogenes</i> , other than those intended for infants and for special medical purposes	<i>Listeria monocytogenes</i>	5	0	100 cfu/g ⁽⁵⁾	EN/ISO 11290-2 ⁽⁶⁾	Products placed on the market during their shelf-life
			5	0	Absence in 25 g ⁽⁷⁾	EN/ISO 11290-1	Before the food has left the immediate control of the food business operator, who has produced it
1.3	Ready-to-eat foods unable to support the growth of <i>L. monocytogenes</i> , other than those intended for infants and for special medical purposes ⁽⁴⁾ ⁽⁸⁾	<i>Listeria monocytogenes</i>	5	0	100 cfu/g	EN/ISO 11290-2 ⁽⁶⁾	Products placed on the market during their shelf-life

BTSF Challenge test assessing the growth potential

- If $\Delta > 0.5 \log_{10} \text{ cfu/g}$:
 - ➔ the RTE food is **able** to support growth of *Lm*.
 - ➔ RTE food classified in category 1.2
- If $\Delta \leq 0.5 \log_{10} \text{ cfu/g}$:
 - ➔ the RTE food is **not able** to support growth of *Lm*.
 - ➔ RTE food classified in category 1.3

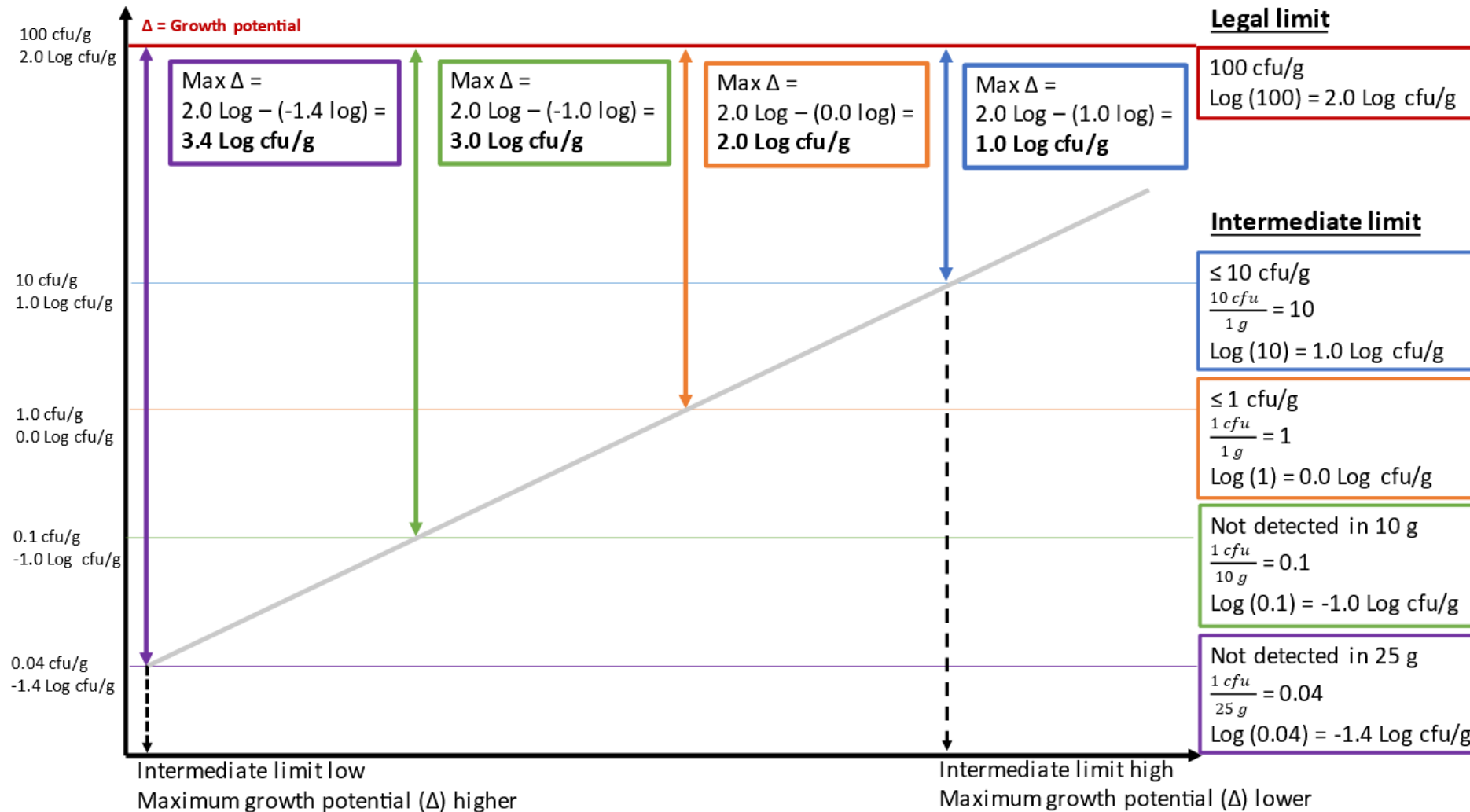
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BTSF Challenge test assessing the growth potential

Growth potential study is most appropriate to:

1. Determine, **under the conditions of the test**, the extent of the growth of *L. monocytogenes* (for category 1.2 products)
2. Determine whether the microbiological criterion of 100 cfu/g is respected during the shelf life of the product (combination of growth potential and initial level of contamination).

$$\log_{10}(N_0) + \Delta \leq 100 \text{ cfu/g}$$



BTSF Protocol EN-ISO 20976-1 + EURL *Lm* TGD

INTERNATIONAL
STANDARD

ISO
20976-1

First edition
2019-03

**Microbiology of the food chain —
Requirements and guidelines for
conducting challenge tests of food and
feed products —**

**Part 1:
Challenge tests to study growth
potential, lag time and maximum
growth rate**



EURL *Lm*
European Union Reference Laboratory for
Listeria monocytogenes
<http://eur-listeria.anses.fr>

EURL *Lm* TECHNICAL GUIDANCE DOCUMENT

**on challenge tests and durability studies for assessing shelf-life
of ready-to-eat foods related to *Listeria monocytogenes***

Version 4 of 1 July 2021 – Amendment 1 of 26 February 2026

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SF

+ Laboratory expertise

BTSF Protocol EN-ISO 20976-1 + EURL TGD

Steps to consider when designing a challenge test:

- Number and choice of batches
- Choice of strains
- Preparation of the inoculum
- Inoculation of the test units
- Storage conditions
- Measurement of the physico-chemical parameters
- Microbiological analysis
- Calculation of growth potential
- Application of results

N.B. These steps can be checked upon review of the challenge test report by an inspector

BTSF Challenge test assessing the growth potential

- In addition national conditions can be specified.
- E.g. Netherlands and Belgium use: 7°C, 7°C and 9°C (instead of 7°C, 7°C and 10°C)
- Applicable to foods sold in those countries irrespective of where they are produced.



BTSF Challenge test assessing the growth potential

Test report

- See also ISO 20976-1
- The results are specific for the food product tested (and strain used).

What can an inspector do?

- Check whether the challenge test has been carried out according to the EURL *Lm* TGD rules.
- Checklists available (EURL *Lm* CGD).



BTSF Limitations/benefits growth potential study

Benefits

- The calculation of the growth potential is based on the use of a simple formula
- The interpretation of the growth potential for Lm is easy.
The limit of $0.5 \log_{10}$ enables to determine whether the studied foodstuff supports or not Lm growth
- Experiments require less test units than experiments for the maximum growth rate

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BTSF Limitations/benefits growth potential study

Limitations

- Not possible to extrapolate the results obtained!
- Results are limited to the conditions used in the challenge test experimental design. If any of these are changed, another challenge test is required.
- Need to have information on the time/temperature profile to simulate the foreseeable storage conditions of the studied product
- Need to have incubator(s) with a temperature range allowing to reproduce with precision the defined temperature profile

BTSF Challenge test assessing the growth rate

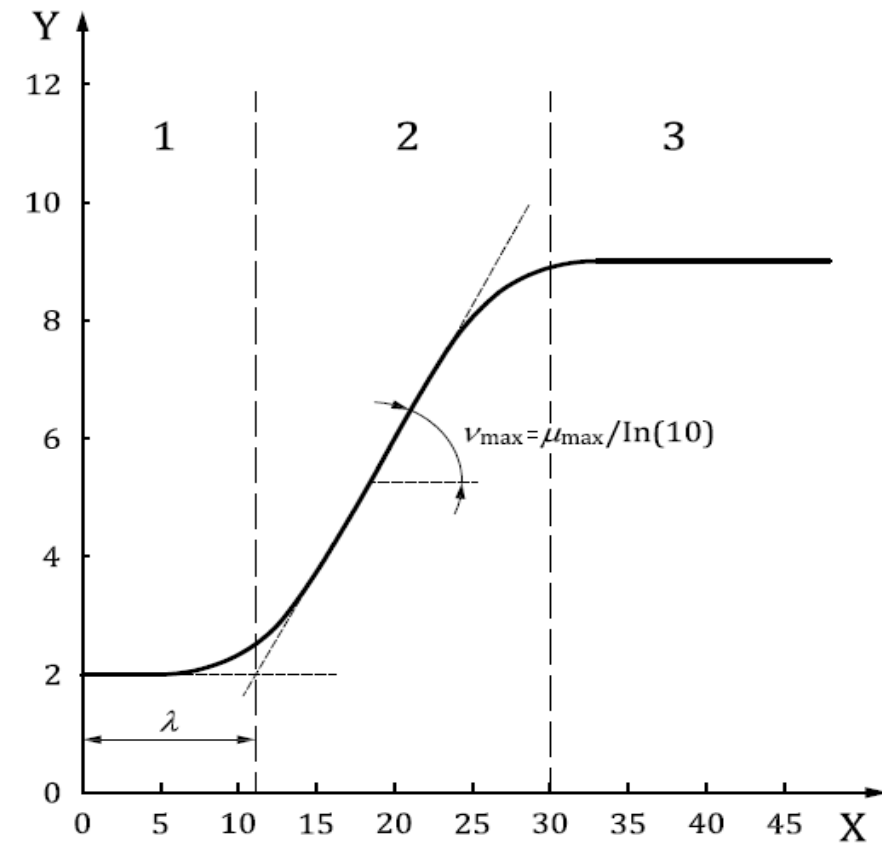
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BTSF Challenge test assessing the growth rate

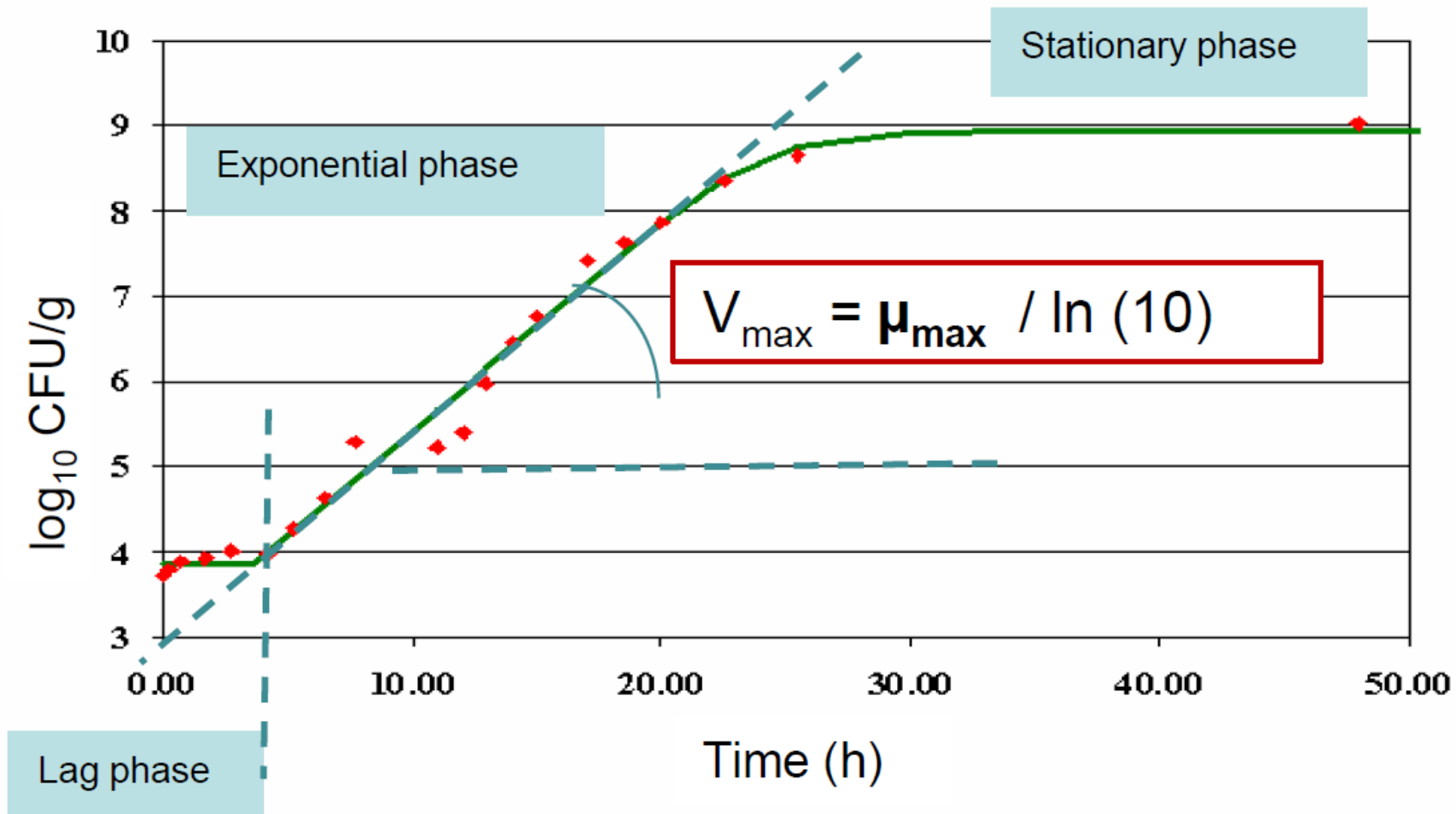
- The growth rate is defined as:

μ_{\max} = *Lm* growth rate in the exponential phase (2)

- Expressed in **natural logarithm** (ln) per unit of time (day or hour), the growth rate is noted μ_{\max} .
- or as V_{\max} when expressed in **decimal logarithm** (\log_{10}) per unit of time.
- To switch from one to another, the relation is : $\mu_{\max} = V_{\max} \times \ln(10)$
 $\mu_{\max} = V_{\max} \times 2,3$



BTSF Challenge test assessing the growth rate



BTSF Challenge test assessing the growth rate

- Such a study should consider:
 - Use of artificially contaminated food products
 - **Use of one *Lm* strain at the time**
 - **Food is stored at a constant temperature**
(usually between 6 and 10 °C)
 - Minimum of **eight experimental data points** distributed across all growth phases
- Main result is estimation of μ_{\max} or V_{\max}
- μ_{\max} or V_{\max} is used to calculate the growth rate at other temperatures
- Using growth rates at different temperatures the total growth during the shelf life of a product can be calculated

$$\text{growth rate}_{T^{\circ}} = \text{growth rate}_{CT} \cdot \frac{(T^{\circ} - T_{\min})^2}{(T_{CT} - T_{\min})^2}$$

BTSF Challenge test assessing the growth rate

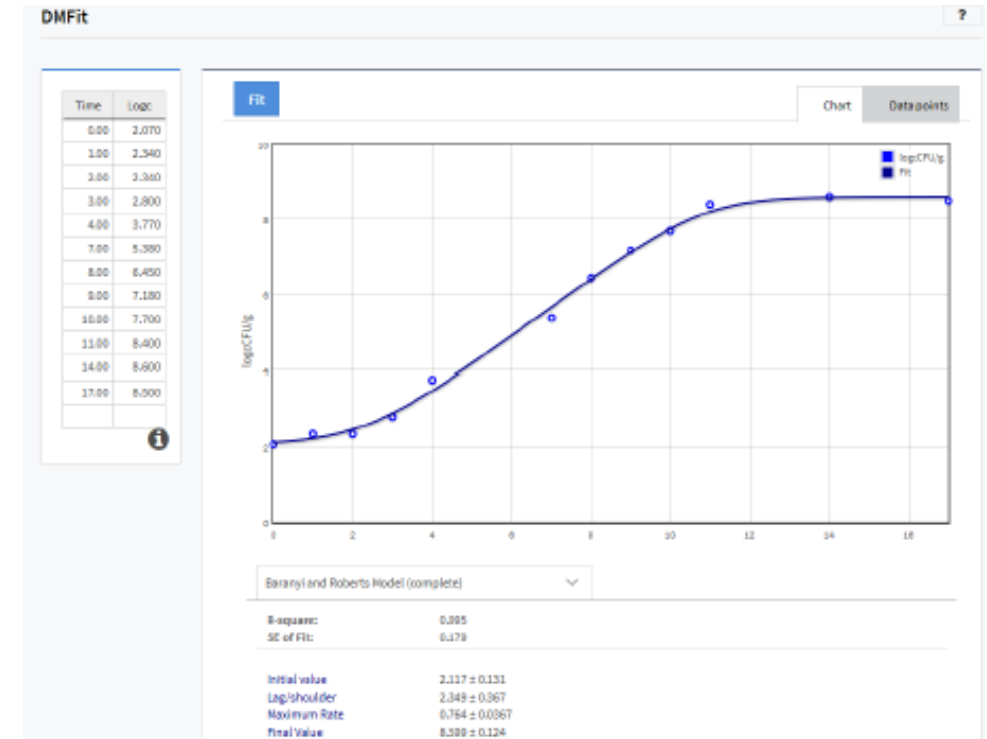
- The calculated growth is used to assess the increase of *Lm* in the food and to demonstrate that the 100 cfu/g limit will not be exceeded during the shelf life.
- The growth rate study is only relevant for foods belonging to the category 1.2 (foods **able** to support the growth of *Lm*).

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BTSF Protocol EN-ISO 20976-1 + EURL *Lm* TGD

Calculation of the maximum growth rate

- For each growth curve (one growth curve per batch and strain), the maximum growth rate (μ_{\max} or V_{\max}) is estimated by fitting a non-linear regression on all the experimental points of the growth curve.
- This fitting can be done by using free available predictive microbiological software, for example: DMFit from ComBase software (www.combase.cc) or Curve fitting from Sym'Previus (www.symprevius.eu).



BTSF Protocol EN-ISO 20976-1 + EURL TGD

Interpretation of results

- The maximum growth rate can be used to assess the increase in Lm during the shelf-life of the product under different storage temperatures.
- Based on the maximum growth rate determined in the study (μ_{\max} or V_{\max}) at a defined temperature the growth rate can be estimated at other temperature, using the following formula:

$$\text{growth rate}_{T^{\circ}} = \text{growth rate}_{CT} \cdot \frac{(T^{\circ} - T_{\min})^2}{(T_{CT} - T_{\min})^2}$$

Where:

Growth rate_{CT} = μ_{\max} or V_{\max} determined experimentally in the Challenge Test

T_{\min} = -2°C by default

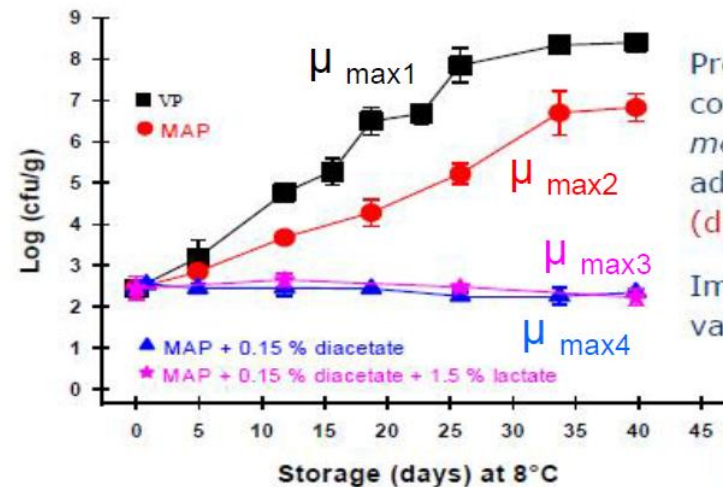
T_{CT} = temperature of the growth rate experiment

T° = temperature for which the growth rate is calculated.

BTSF Limitations/benefits growth rate study

Growth rate study is most appropriate to:

1. To assess the effect of factors impacting *Lm* growth (T° , pH, a_w , organic acids, MAP, ...)



Product characteristics and storage conditions determine if growth of *L. monocytogenes* can be prevented by addition of (di)acetate or if both (di)acetate and lactate are required

Important to prevent growth in various lightly preserved seafoods



DTU Food

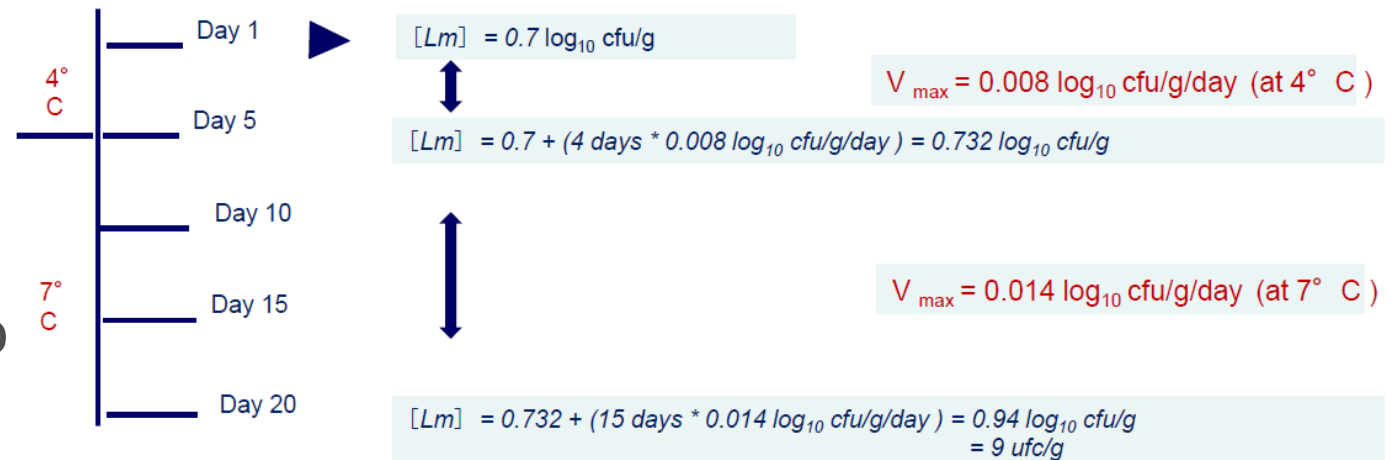
Mejlholm and Dalgaard (2007) - J. Food Prot. 70, 70-84

BTSF Limitations/benefits growth rate study

Growth rate study is most appropriate to:

2. To calculate L_m concentration at any stage of the shelf-life, under dynamic environmental conditions (T° , pH, aw ...) + to check compliance with the quantitative criterion at the end of shelf-life

Example for RTE foodstuff with a shelf-life of 20 days



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Conclusions

1. Designing, conducting and interpretation of a challenge test is complex and requires different (microbiological, analytical, processing,..) expertise.
2. Growth potential studies covers a specific storage condition (time and temperature) of the food.
3. Growth rate studies are only relevant for foods belonging to category 1.2.
4. Knowledge on challenge test is needed in order to check the (study) report

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Thank you!

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