LISTERIA MONOCYTOGENES IN KANT-EN-KLARE LEVENSMIDDELEN: BORGING VAN DE HOUDBAARHEIDSTERMIJN

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INTRODUCTION

Challenge test ⟺ Durability study

- Artificially inoculated
- Defined storage conditions (manufacturer, retail and consumer)

- Naturally present micro-organisms
- More realistic but:
  - implementation is limited due to low prevalence and low contamination levels
  - contamination is heterogeneously distributed in the food

➔ *Listeria monocytogenes*

INTRODUCTION: *L. MONOCYTOGENES*

Growth characteristics

Parameter	Minimum	Optimal	Maximum
Temperature (°C)	0	30-37	45
pH	4.4	7.0	9.4
a_w	0.92	0.99	> 0.99
Salt (% in water phase)	< 0.5	0.7	12 - 16

Atmosphere: facultative anaerobe, growth in presence and absence of oxygen

Thermal inactivation $D_{60°C}$: 2.5 – 4.0 min
$D_{65°C}$: 0.75 min
$D_{70°C}$: 0.1-0.3 min

INTRODUCTION: *L. MONOCYTOGENES*

- Important for food industry as *Listeria* is able to grow at refrigerated temperatures and is able to persist in food-processing areas and equipment
- Cause of listeriosis
- Susceptible population: YOPI's
- Specific regulation for RTE-foods
- What is RTE?

INTRODUCTION: EU 2073/2005

RTE-food: food intended by the producer or the manufacturer for direct human consumption without the need for cooking or other processing effective to eliminate or reduce microorganisms to an acceptable level of concern

I. RTE foods for infants and for medical purposes
   ➔ absence in 25 g

II. RTE foods **able** to support growth, others than those belonging to category I
   ➔ absence in 25 g (before the food has left the immediate control of the food producer)
   ➔ < 100 CFU/g (products place on the market during their shelf-life)

III. RTE food **unable** to support growth, others than those belonging to category I
   ➔ < 100 CFU/g (products place on the market during their shelf-life)

INTRODUCTION: EU 2073/2005

Always belonging to category III

- pH ≤ 4.4
- a_w ≤ 0.92
- pH ≤ 5.0 and a_w ≤ 0.94
- Shelf life less than five days

Other products belonging to category III should be scientifically proven

- Characteristics of the product (pH, a_w, salt, concentration of preservatives etc.)
- Available scientific literature and research data
- Predictive mathematical modelling
- Challenge testing

INTRODUCTION

	Growth rate	Growth potential
Strains	Two strains in monoculture	Cocktail of minimally two strains
Temperature	Constant	Defined temperature profile (taking into account reasonably foreseen abuse at consumer stage)
Number of inoculated samples	Min. 15 for each growth curve	Min. 9 (for one batch in triplicate)
Advantages	Extrapolation to other temperatures is possible	Easy Cheaper
Disadvantages	Only valid for the specific product Labour intensive Expensive	Only valid for the specific product under the specific temperature profile Intermediate points are recommended

STUDIES ON GROWTH POTENTIAL

1. **Description of the product (group)**
   - FBO should have an idea on the variability on product characteristics
   - FBO should identify the opportunities for contamination
   - Homogeneity of the food product should be considered

Important for the inoculation procedure




STUDIES ON GROWTH POTENTIAL

2. Compiling a data base

FBO should build up a database with measured values on the most important physico-chemical characteristics

- pH
- a_w
- salt
- dry matter
- if applicable: type and concentration of preservatives
- time-temperature profiles
- packaging concept

STUDIES ON GROWTH POTENTIAL

3. Evaluating growth potential in the food based on literature data and/or predictive models

Freeware software packages:

- **ComBase**: based on a large database of studies (not necessarily peer reviewed)
- **MRV**: based on the ComBase database
- **Food Safety and Spoilage predictor**: product based and peer reviewed data
- **DMRI**: product based and peer reviewed data

Background knowledge on the principles of developing predictive models as well as on food microbiology is essential

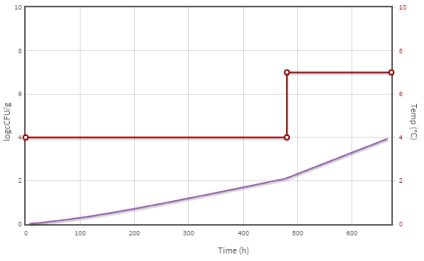
STUDIES ON GROWTH POTENTIAL

3. Evaluating growth potential in the food based on literature data and/or predictive models

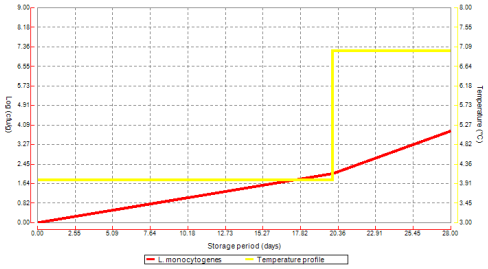
Case study: meat product stored in MAP

- pH: 6.44
- a_w : 0.97
- MAP: 60% CO_2
- T-profile: 20 days at 4°C – 8 days at 7°C

COMBASE



FSSP



STUDIES ON GROWTH POTENTIAL

3. Evaluating growth potential in the food based on literature data and/or predictive models

Case study: meat product stored in MAP

Important to be critical on the outcome of a predictive model

Other preservatives might be present: lactic acid, acetic acid,…

➔ If the FBO has no database with these data, it has no advantage to use predictive models

STUDIES ON GROWTH POTENTIAL

4. Performing a challenge test

- Protocol is described in the EU RL technical guidance on *L. monocytogenes*
- Some Member States detailed the protocol by national guidelines
- Should be performed by an experienced laboratory

➔ The producer is responsible for the challenge test protocol
➔ Discussion between FBO and experienced lab is necessary
➔ Challenge test is no routine analysis

➔ Some points of attention

CHALLENGE TEST IN LABORATORY

Selection of the strains

- Well characterized: knowing the cardinal values
- Using a cocktail

Standardization of the test inoculum

- Adaptation to cold temperatures (if needed)
- Essential to be able to inoculate at sufficiently low inoculum levels

CHALLENGE TEST IN LABORATORY

Inoculation procedure

- As soon as possible after production
- Different inoculum for each batch
- Volume should not exceed 1% of the mass of the test unit
- Standard deviation on the inoculum level should not exceed 0.5 log units
- Inoculation at the spot where the contamination can occur:

CHALLENGE TEST IN LABORATORY

Inoculation procedure



- Inoculation at the spot where the contamination can occur:
  - In depth for homogeneous food or mixed food
  - At the surface to mimic contamination at specific parts (e.g. rind of a cheese,…)
  - Layered food: at the interfaces (e.g. sliced ham, …)

→ Repacking often necessary

CHALLENGE TEST IN LABORATORY

Packaging

For MAP products the gas concentration is important

- Inoculated through septum: control of leakage
- Repacked samples:
  - Initial CO_2 concentration
  - Gas/product ratio
  - Packaging with similar gas barrier properties

➡ Headspace analyses on each test unit (inoculated and non-inoculated) to check for leakage

CHALLENGE TEST IN LABORATORY

Storage conditions

Fase in de keten	Informatie over de opslagtijd beschikbaar		
	Ja	Nee	
		houdbaarheid ≤ 21 dagen	houdbaarheid > 21 dagen
Producent	Gemiddelde opslagtijd	1/3 houdbaarheidsperiode	7 dagen
Retail	Gemiddelde opslagtijd	1/2 resterende tijd	1/2 resterende tijd
Consument	Resterende tijd	1/2 resterende tijd	1/2 resterende tijd

Temperature:

Producer and retail: 7°C or measured data

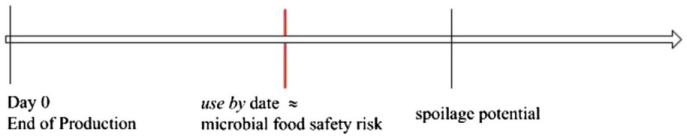
Consumer: 9°C

Source: NVWA informatieblad 85

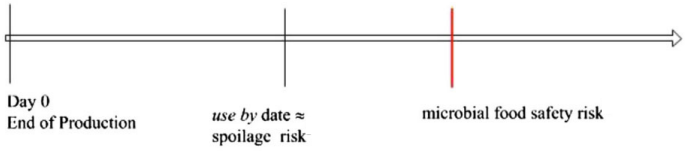
CHALLENGE TEST IN LABORATORY

How to determine shelf life?

Group 1 : *use by* food products for which the shelf-life date is limited by food safety parameters



Group 2 : *use by* food products for which the shelf-life date is limited by quality parameters



Van Boxstael et al. (2013)

CHALLENGE TEST IN LABORATORY

Microbial analyses

- Mandatory: *L. monocytogenes* detection and/or enumeration
- Recommended: specific spoilage organism

⇨ According to (inter)national standards

⇨ Under accreditation

CHALLENGE TEST IN LABORATORY

Calculating growth potential:

Batch	Concentration Day 0	Concentration Day end	Growth potential	Growth potential product
1	2.59	4.30		
	2.23	4.48		
	2.45	4.30		
2	2.18	3.54		
	2.11	3.74		
	2.11	3.79		
3	2.59	5.51		
	2.46	4.44		
	2.48	4.39		

CHALLENGE TEST IN LABORATORY

Calculating growth potential: according EU

Batch	Concentration Day 0	Concentration Day end	Growth potential	Growth potential product
1	2.59	**4.30**	4.30 – 2.45 = 1.85	1.96
	2.23	4.48		
	2.45	4.30		
2	2.18	3.54	3.74 – 2.11 = 1.63	
	2.11	**3.74**		
	2.11	3.79		
3	2.59	5.51	4.44 – 2.48 = 1.96	
	2.46	**4.44**		
	2.48	4.39		

CHALLENGE TEST IN LABORATORY

Calculating growth potential:

Batch	Concentration Day 0	Concentration Day end	Growth potential	Growth potential product
1	2.59	4.30		
	2.23	4.48		
	2.45	4.30		
2	2.18	3.54		
	2.11	3.74		
	2.11	3.79		
3	2.59	5.51		
	2.46	4.44		
	2.48	4.39		

CHALLENGE TEST IN LABORATORY

Calculating growth potential: according NVWA

Batch	Concentration Day 0	Concentration Day end	Growth potential	Growth potential product
1	2.59	**4.30**	4.30 – 2.45 = 1.85	3.03
	2.23	4.48		
	2.45	4.30		
2	2.18	3.54	3.74 – 2.11 = 1.63	
	2.11	**3.74**		
	2.11	3.79		
3	2.59	**5.51**	5.51 – 2.48 = 3.03	
	2.46	4.44		
	2.48	4.39		

If at day end the difference between the highest and lowest value > 0.5

Highest value should be used to determine growth potential

CHALLENGE TEST IN LABORATORY

Calculating growth potential:

Batch	Concentration Day 0	Concentration Day end	Growth potential	Growth potential product
1	2.59	4.30		
	2.23	4.48		
	2.45	4.30		
2	2.18	3.54		
	2.11	3.74		
	2.11	3.79		
3	2.59	5.51		
	2.46	4.44		
	2.48	4.39		

CHALLENGE TEST IN LABORATORY

Calculating growth potential: suggestion FMFP-UGent

Batch	Concentration Day 0	Concentration Day end	Growth potential	Growth potential product
1	2.59	4.30	4.48 – 2.23 = 2.25	3.05
	2.23	**4.48**		
	2.45	4.30		
2	2.18	3.54	3.79 – 2.11 = 1.68	
	2.11	3.74		
	2.11	**3.79**		
3	2.59	**5.51**	5.51 – 2.46 = 3.05	
	2.46	4.44		
	2.48	4.39		

Always consider the highest value at Day end and lowest value at Day 0 ⇨ **WORST CASE**

CHALLENGE TEST IN LABORATORY

Interpretation of results:

- Target value should always be: 'absence in 25 g'
- Tolerance value at the end of the manufacturing process is related to the growth potential

Growth potential (log CFU/g) during shelf life	Tolerance value at the end of the manufacturing process
Negative	< 100 CFU/g
Between 0.00 and 0.49	< 100 CFU/g
Between 0.50 and 0.99	< 10 CFU/g
Between 1.00 and 1.99	Absence in 1 g
Between 2.00 and 2.99	Absence in 10 g
More than 3.00	Absence in 25 g

GENERAL CONSIDERATIONS

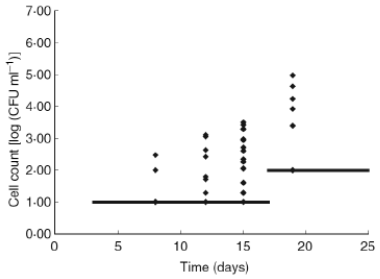
End responsibility of conducting challenge test

- The FBO should define its product & production process and inform the consultant or lab having to conduct the study
- A representative product or batch should be defined or tailor made
- It should be described to which extent the results can or cannot be extrapolated to other food types

GENERAL CONSIDERATIONS

The inoculum level

- Too high inoculum (> 1000 CFU/g)
  - ➔ not realistic and overestimation of growth
- Too low inoculum (< 100 CFU/g)
  - ➔ increasing variability of growth



Growth in cooked ham stored at 7°C
Inoculum level: 1 CFU/15g
Samples: 50 each day of analysis
—— Detection level

François et al., 2006

GENERAL CONSIDERATIONS

Interpretation on the time temperature profile

Supply chain: internal storage, distribution to retail shops and consumer's home

BUT: what about

- food sold to other FBO's for further portioning or packaging
- food service operations (e.g. hotels, restaurants, catering,…)

Overall shelf life with minimum and maximum temperatures
Stages of post contamination (e.g. slicing, repacking,…)

COMMUNICATION BETWEEN VARIOUS ACTORS IS ESSENTIAL

GENERAL CONSIDERATIONS

Interpretation on the time temperature profile

Distribution: - temperature usually well controlled
- imposed temperatures can differ from country to country

Consumer:
- temperatures in household refrigerators may vary a lot
- shelf-life labels are not always respected or understand well by the consumer
- shopping frequency will influence the storage time in this phase

CONCLUSION

Important for each FBO:
- to inventory all necessary data
- to have thorough knowledge on their products

⇒ Decide whether a challenge test is necessary

Performing a challenge test on a food product can not be considered as a routine analyses and requires an extended preparation