International Association for Food Protection®

The Challenge of Conducting Challenge Tests

- **Hélène Bergis, Presenter**

ANSES: French Food Safety Agency

- **Paul in 't Veld, Presenter**

Netherlands Food and Consumer Product Safety Authority

- **Florence Postollec, Presenter**

ADRIA

- **Mariem Ellouze, Moderator**

Nestlé Research Center

Sponsored by the IAFP Foundation

Organized by the IAFP Microbial Modelling and Risk Analysis PDG


International Association for Food Protection®

Webinar Housekeeping

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Microbial Modelling and Risk Analysis PDG

Chair: Bala Kottapalli, Conagra Brands

Vice Chair: Panagiotis Skandamis, Agricultural University of Athens

Mariem Ellouze

Senior specialist Microbial Risk Assessment,

Food Safety Microbiology Group
Food Safety Research Department
Institute of Food Safety and Analytical Sciences
Nestlé Research, Lausanne, Switzerland

Nestlé | Research and Development

- Project Management
- Technical assistance
- Chair of ICPMF
- Member of ISO 20976 (Challenge Tests for growth and inactivation)
- Project Leader of ISO PWI 23691 Determination and use of cardinal values in predictive microbiology

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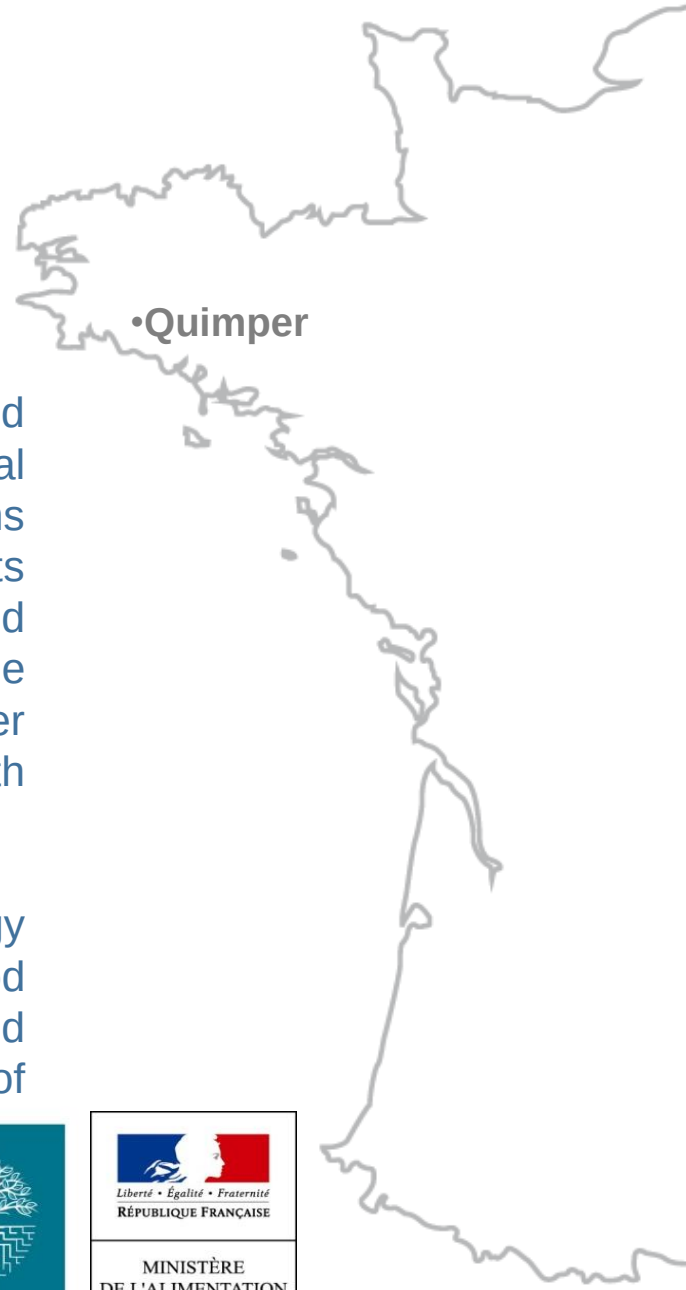
Florence Postollec
Project manager
ADRIA - UMT ACTIA19.03 ALTER'iX, France



For the past 14 years, she is collaborating with the Mafart Team on risks associated to foodborne sporeformers within the frame of UMT ACTIA competitive national cluster. This collaboration, based on shared Research & Developpement axis, aims at increasing knowledge and expertise to better mitigate sporeformer contaminants involved in food safety and spoilage issue. Her main interest relies in biodiversity and behaviour heterogeneity induced after stress exposure. She is involved in the developpement of applied scientific projects or services related to sporeformer hazard identification, process or shelf-life optimization in close collaboration with food industrials.

ADRIA, an independent and non-profit organization, is a leading Food Technology Institute in food safety & quality that provide technical support and services to food industries and suppliers. Our lab and experts are recognized by the French Food Ministry (DGAL) for the validation of challenge test studies for the determination of food shelf-life.

ADRIA

ITAI

Liberté • Égalité • Fraternité
RÉPUBLIQUE FRANÇAISE

MINISTÈRE
DE L'ALIMENTATION
DE L'AGRICULTURE
ET DE LA PÊCHE

CHALLENGE TESTING & STANDARDISATION, RECENT DEVELOPMENTS

Florence POSTOLLEC
florence.postollec@adria.tm.fr

International Association for Food Protection webinar

13 september 2019

STANDARDIZATION

- Process of developing and implementing technical guidelines based on a consensus

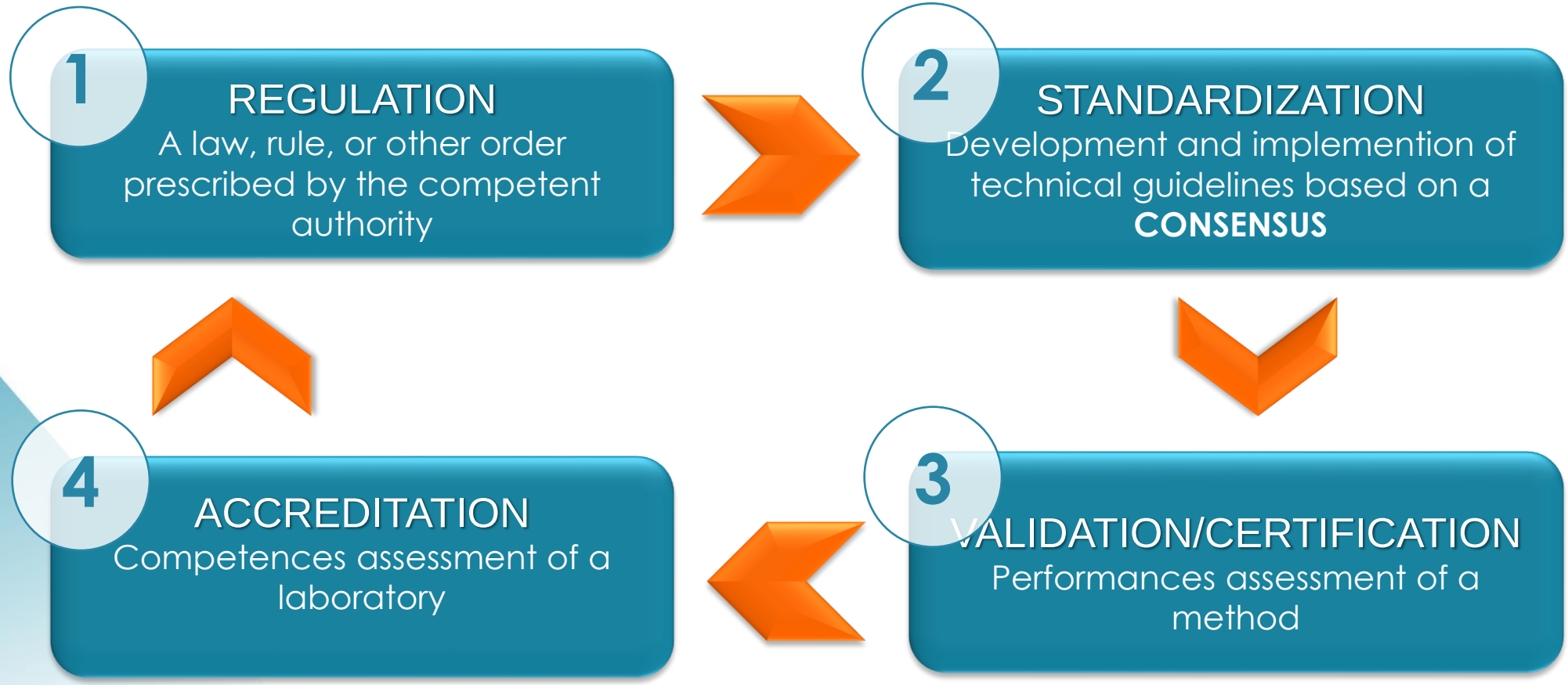
Optimize efforts & time

Help in Microbial Risk Assessment

Ensure International Recognition

Facilitate Food Trade

A PROCESS AS A WHOLE



ISO STANDARDS

- International Standardization Organization: independent, non governmental international organization with a membership of 164 national standards bodies
- ISO standards are developed within several Technical Committees (TC), Sub Committees (SC) and Working Groups (WG)

  → ISO TC34/SC9 Microbiology of the food chain
- Liaisons with various bodies and organization

  →AOAC International, CAC, EC, ICMSF, IDF, IUMS, WHO …

More info on www.iso.org

ISO TC34 / SC9 / WG19, CHALLENGE TESTS

- Context: On the application of general principles of food hygiene, it is the responsibility of the Food Business Operators (FBO) to control microbial risks in foods. Therefore, the FBO shall conduct studies in order to investigate compliance with the criteria throughout the production and storage processes.

In the framework of Microbial Risk Assessment (MRA), several complementary approaches are developed to estimate food safety and food quality risks posed by pathogen or spoilage microorganisms in the food chain. MRA is adopted by regulators under the auspices of the international agency for setting food standards

ISO TC34 / SC9 / WG19, CHALLENGE TESTS

- Creation date: 2014
- Secretariat: AFNOR (France),

Stéphanie Tiprez (Secretary) & Florence Postollec (Convenior)

- Objectives: to provide technical rules and calculations approaches to investigate the ability of inoculated micro-organism of concern to grow or survive in the raw materials, intermediate or end-products under different reasonably foreseeable food processes, storage and use conditions.
- Members: experts from food industry, food technology institute, food testing laboratory, research center and regulatory bodies + support team, liaison representative, document monitor & technical program manager to ensure smooth & efficient development

ISO TC34 / SC9 / WG19, CHALLENGE TESTS

- Deliverable: 3 standards on microbiology of the food chain

ISO 20976-1:2019 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

ISO PWI 20976-2 Requirements and guidelines for conducting challenge tests of food and feed products – **part 2**: challenge tests to study inactivation potential and kinetics parameters

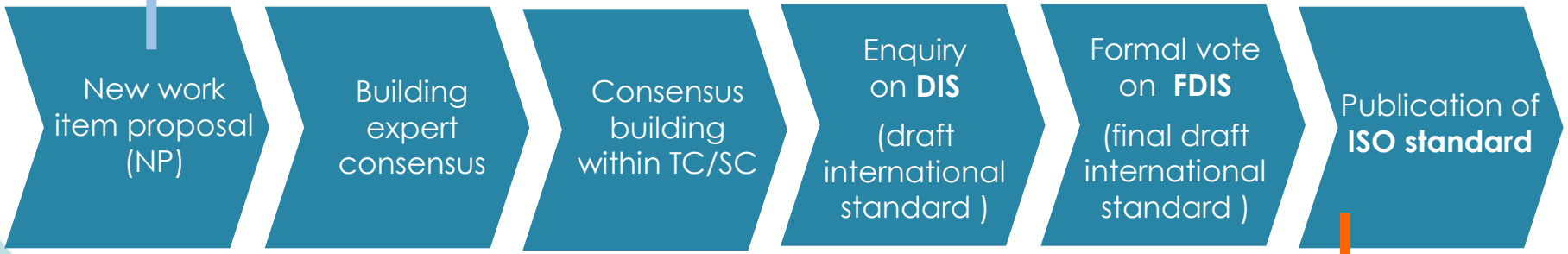
ISO PWI 23691 Determination and use of cardinal values in predictive microbiology

ISO TC34 / SC9 / WG19, CHALLENGE TESTS

- Standards development process in 6 steps

ISO PWI 20976-2 — Requirements and guidelines for conducting challenge tests of food and feed products – part 2: inactivation

ISO PWI 23691 — Determination and use of cardinal values

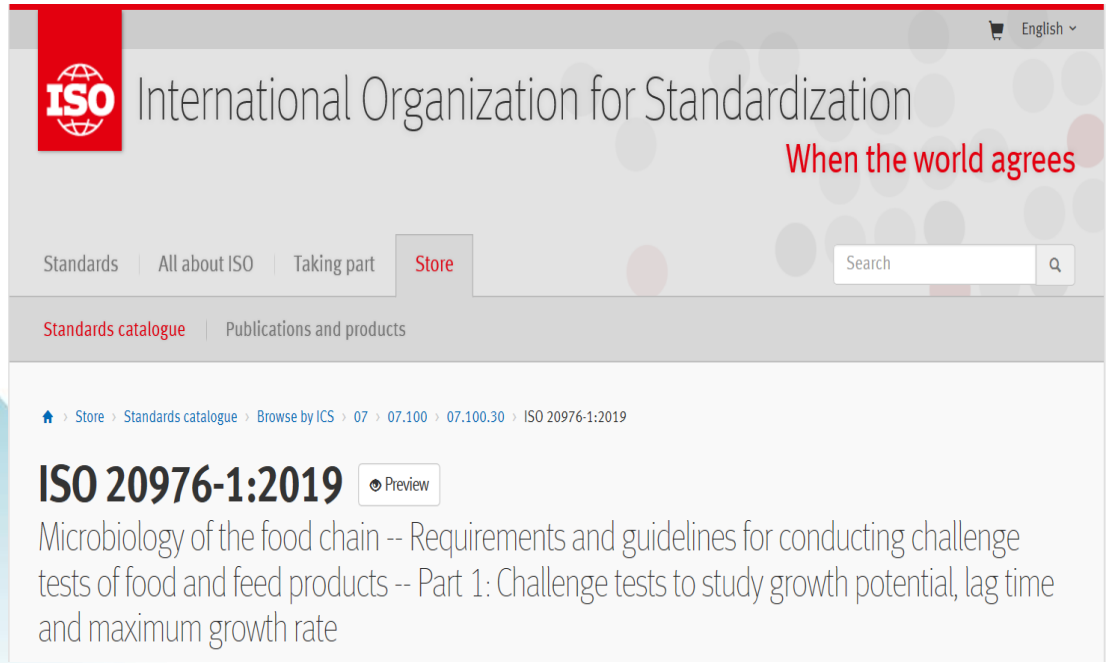


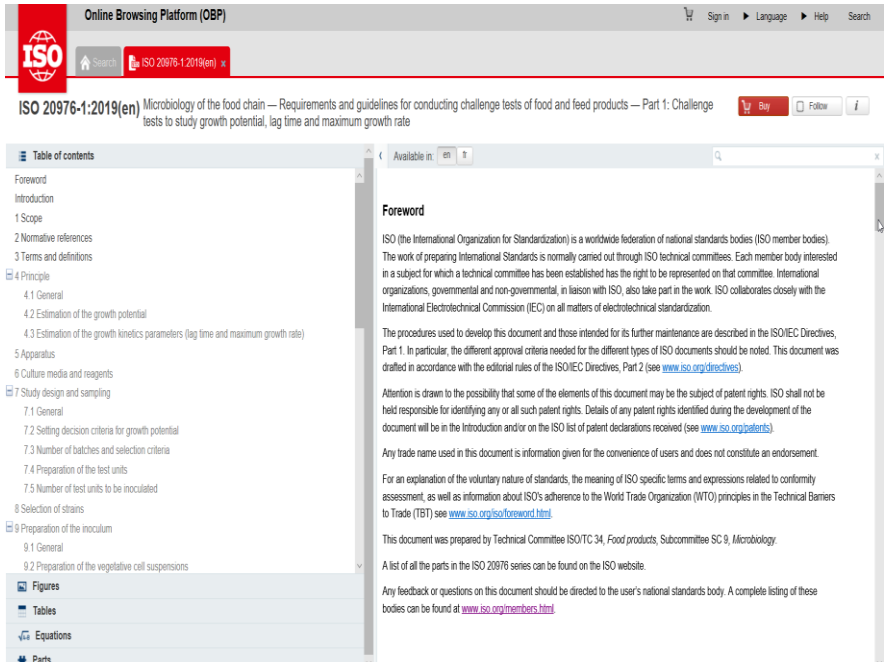
Requirements and guidelines for conducting challenge tests of food and feed products – part 1: growth — ISO 20976-1:2019

ISO 20976-1:2019

Requirements and guidelines for conducting challenge tests of food and feed products – part1: challenge tests to study growth potential, lag time and maximum growth rate

- Publication: in march 2019





ISO 20976-1:2019

- Scope: this document specifies protocols for conducting microbiological challenge tests for growth studies on vegetative and spore-forming bacteria in raw materials and intermediate or end products

- Principle

✓Growth POTENTIAL studies : Validate the specific food characteristics and conditions applied. → When microbiological criteria are not fulfilled or conditions are changed, a new growth potential study is carried out

✓ Growth KINETICS studies : Estimate and validate the microbiological food shelf-life. → Tests particularly suitable for food innovation…. more informative but as well more complex than growth potential studies

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Training, audit & advices

- Training & events
- Advices, regulatory support
- HACCP, BRC, IFS, ISO 22000 Audits

Food & pack solutions

- Functional ingredient characterization
- Food packaging
- Food innovation

Food safety & quality

- Molecular typing
- Risk assessment on pathogens & spoilage microflora
- Method validation studies in food microbiology

UMT ALTER'IX
FOOD SPOILAGE BY SPORE-FORMING BACTERIA & MOULDS

Scientific developments & valorization

- Phenotypic & molecular characterization
- Risk assessment on sporeforming complex microflora
- Development of generic predictive microbiology approaches

ADRIA

Hélène Bergis

Research Engineer at the French Food Safety Agency (ANSES)

European Reference Laboratory Lead for *Listeria monocytogenes*.

Involved in trainings to the competent European authorities

Performs audits of the laboratories recognized by the French authorities to perform *L. monocytogenes* challenge tests.

anses
French agency for food, environmental and occupational health & safety
Investigate, evaluate, protect

EURL Lm
European Union Reference Laboratory
for *Listeria monocytogenes*

Listeria monocytogenes and challenge testing

Hélène Bergis
EURL for Listeria monocytogenes

European Regulation framework

- Regulation (EC) No 2073/2005 lays down microbiological criteria for certain micro-organisms and the implementing rules to be complied with by Food Business Operators (FBOs)

  - Article 3 ‘General requirements‘

  “ FBOs shall ensure that foodstuffs comply with the relevant **microbiological criteria set out in Annex I** .”

European regulatory framework

Food safety criteria for RTE foods / Lm (**Annex I** of Regulation (EC) 2073/2005)

Food category	Sampling plan		Limits m = M	Stage where the criterion applies
	n	c		
1.1 RTE foods intended for infants and RTE foods for special medical purposes	10	0	Abs. in 25 g	Products placed on the market during their shelf-life
1.2 RTE foods **able** to support the growth of *L. monocytogenes* other than those intended for infants and for special medical purposes	5	0	100 cfu/g	Products placed on the market during their shelf-life
	5	0	Abs. in 25 g	Before the food has left the immediate control of the food business operator who has produced
1.3 RTE foods **unable** to support the growth of *L. monocytogenes* other than those intended for infants and for special medical purposes	5	0	100 cfu/g	Products placed on the market during their shelf-life

European regulatory framework

Annex II of Regulation (EC) 2073/2005 specifies the elements to be included in the studies conducted by the FBOs to investigate the compliance with the defined criteria throughout the shelf-life.

✓ Characteristics of the product: physico-chemical characteristics, preservatives content, type of packaging, process, foreseen shelf-life

✓ Available scientific literature and research data regarding the growth and survival characteristics of the micro-organisms of concern

✓ And when necessary,

- Predictive microbiology
- **Challenge-tests**
- Durability studies

European Guidance Documents on Shelf-life studies

Guidance document on Lm shelf-life studies for RTE foods, under EC Reg. 2073/2005	Technical Guidance Document for conducting shelf-life studies on Lm in RTE foods	Guidance Document to evaluate the competence of labs implementing challenge tests & durability studies related to Lm in RTE foods
2008	2014	2018
DG Santé	EURL Lm	EURL Lm
FBOs / CAs	Laboratories	CAs / NRLs or organizations

Standard EN ISO 20976-1 & Technical Guidance Document

anses
French agency for food, environmental and occupational health safety
Food safety laboratory
Maisons-Alfort, France

EURL Lm
European Union Reference Laboratory for
Listeria monocytogenes

EURL *Lm* TECHNICAL GUIDANCE DOCUMENT

for conducting shelf-life studies on *Listeria monocytogenes* in ready-to-eat foods

Version 3 of 6 June 2014 – Amendment 1 of 21 February 2019

Annie Beaufort, Hélène Bergis, Anne-Laure Lardeux, Unit Modelling of Bacterial Behaviour, Bertrand Lombard, Manager EU Reference Laboratory for *Listeria monocytogenes* Anses-Food Safety Laboratory, Maisons-Alfort, France

In collaboration with representatives of 10 National Reference Laboratories (NRLs) for *Listeria monocytogenes* and 1 associated National Reference Laboratory for *Listeria monocytogenes*:

- Marie Polet and Nadine Botteldoorn, Scientific Institute of Public Health, Belgium;
- George Papageorgiou, State General Laboratory, Cyprus;
- Jens Kirk Andersen and Jeppe Boel, National Food Institute, Danish Technical University, Denmark;
- Bernadette Hickey, Dairy Science Laboratory, Republic of Ireland;
- Vincenza Prencipe, Istituto Zooprofilattico Sperimentale dell'Abruzzo e del Molise "G. Caporale", Italy;
- Wilma Jacobs-Reitsma, National Institute for Public Health and the Environment (RIVM), The Netherlands (NL-NRL);
- Ife Fitz-James, Netherlands Food and Consumer Product Safety Authority (NVWA), The Netherlands (associated NL-NRL);
- Celcidina Maria Pires Gomes, Instituto Nacional De Investigação Agrária e Veterinária (INIAV), Portugal;
- Lenka Cabanova, State Veterinary and Food Institute, Slovakia;
- Cristina Acebal Sarabia, Institute for Hygiene and Veterinary Public Health, Spain;
- Taran Skjerdal, Norwegian Veterinary Institute, Norway.

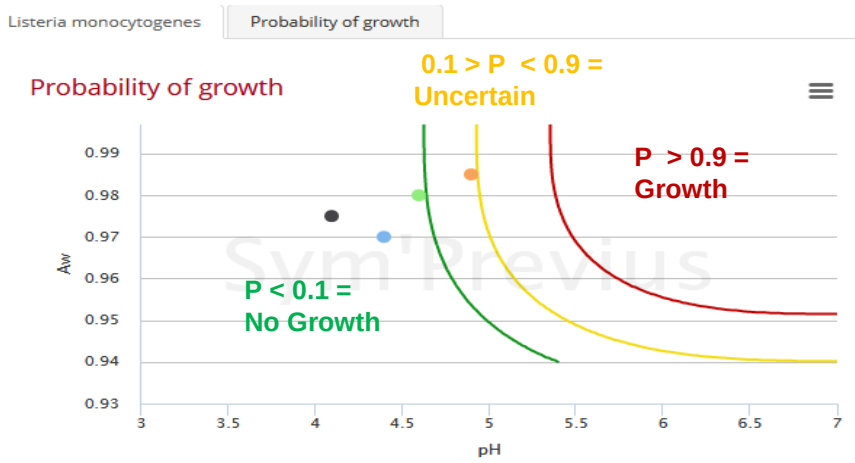
And in collaboration with a representative of another laboratory: Gail Betts, Campden & Chorleywood Food Research Association, Gloucestershire, United Kingdom (for the UK-NRL).

Standard EN ISO 20976-1 vs Technical Guidance Document

	EN ISO 20976-1	EURL Technical Guidance
Scope	Protocols for challenge tests with vegetative, spore forming bacteria in raw material, intermediate or end product	Protocols for challenge tests & Durability study **on Lm in RTE foods**

Standard EN ISO 20976-1 vs Technical Guidance Document

Growth potential	EN ISO 20976-1	EURL Technical Guidance
Number of batches	**Minimum of 3 batches** Single batch clearly justified: evaluating impact of new formulation of food; using a batch to represent the worst case conditions	Determined according a growth / no growth module of a predictive µbiological software: • **3 batches if growth probability >10%** • **1 batch if growth probability < 10%**
Selection of the batches	**Be representative of the variability** of the production process	

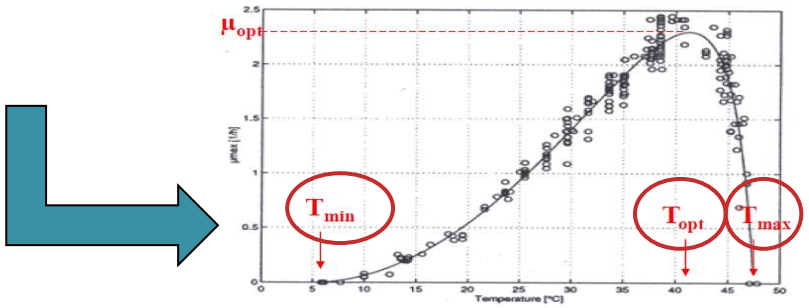


Standard EN ISO 20976-1 vs Technical Guidance Document

	EN ISO 20976-1	EURL Technical Guidance
Number of test units / sampling point	Refer to Annex B (**normative**) **Min. 1** test unit per sampling point	**Min. 3** test units per sampling point
Number of sampling points	**5 sampling points**	**Day 0 and Day end**, but recommended to use intermediate points

Standard EN ISO 20976-1 vs Technical Guidance Document

	EN ISO 20976-1	EURL Technical Guidance
Selection of strains	**Strains to be characterised** (biochemically/ serologically/ genetically) Strains isolated from food matrix, product environment or clinical /food environment outbreaks, preferable to culture type collections **Whenever possible use strains where cardinal values are determined**.	**At least 2 strains** : **1 with known growth characteristics** (EURL Lm set of 25 *Lm* selected fast strains), others from foods, environment, outbreak, collection



ISO PWI 23691: Determination and use of cardinal values in predictive microbiology

Standard EN ISO 20976-1 vs Technical Guidance Document

	EN ISO 20976-1	EURL Technical Guidance
Inoculation of the test units	i) **Inoculation level of at least 5 times the quantification limit of the enumeration and not > 10^4 cfu/g.** ii) when the inoculum conc. is low the limit of quantification can be lowered by increasing the number of plates. iii) Quality of the inoculation checked at Day 0 : **standard deviation < 0,3 log cfu/g**	Contamination target **around 100 cfu/g** Recommended to **lower the limit of quantification of 10 cfu/g** by using 1ml over 3 x 90mm plates or 1 ml onto 140mm plate.

Standard EN ISO 20976-1 vs Technical Guidance Document

EN ISO 20976-1

EURL Technical Guidance

Storage of the test units

T° for storage should allow growth of target microorganisms and be as close as possible to reasonable foreseeable food storage conditions

Table of time temperature combinations for each stage of the cold chain : manufactuer – retail- consumer

Stage of cold chain	Storage (incubation) temperature			Storage (incubation) duration			
						Shelf life ≤ 21 days	Shelf life > 21 days
From manufacture until the arrival to the display cabinet	Temperature justified by detailed information*	Or if not known	~~8~~ 7°C	Duration justified by detailed information	Or if not known	One third of the total shelf life of the product	7 days
Retail: Display cabinet	Temperature justified by detailed information**	Or if not known	~~12~~ 7°C	Duration justified by detailed information	Or if not known	One third of the total shelf life of the product	½ (shelf life – 7 days)
Consumer storage	Temperature justified by detailed information**	Or if not known	12°C	Duration justified by detailed information	Or if not known	One third of the total shelf life of the product	½ (shelf life – 7 days)

* Temperature justified by detailed information: the 95th percentile of the FBO's data observation.

** Temperature justified by detailed information: the ~~75th~~ 95th percentile of the observations for the country where the stage of the cold chain is located.

Standard EN ISO 20976-1 vs Technical Guidance Document

	EN ISO 20976-1	EURL Technical Guidance
Results	Calculation of Growth potential **(Δ)** for each batch **$\Delta = \log_{max} - \log_{initial}$** Growth potential of the product : highest growth potential values from all the batches. Reject CT if at time zero, **std dev. > 0.3 log cfu/g**	Calculation of Growth potential **(δ)** for each batch **$\delta = \text{median log}_{end} - \text{median log}_{initial}$** Idem Study unacceptable, if at Day 0, standard deviation > 0.5 log cfu/g

Standard EN ISO 20976-1 vs Technical Guidance Document

	EN ISO 20976-1	EURL Technical Guidance
Exploitation of the results	Suspected outliers shall be investigated If log initial is the highest value from all the test units sampled, growth potential = 0	**Use to classify RTE foods** • when $\delta > 0.5$: food classified to be **able to support** Lm growth (cat 1.2 of Reg. (E.C) 2073/2005) • when $\delta \leq 0.5$: food classified to be **unable to support** Lm growth (cat. 1.3 of Reg.(EC) 2073/2005) **Use to quantify growth of Lm** in RTE foods of cat. 1.2 **Use to calculate [Lm] at the end of the shelf-life**, if [Lm] at the production is known

European perspectives

Revision of Guidances on Lm shelf-life studies for harmonisation

EN ISO 20976-1 : 2019

EURL Lm Technical Guidance Document for conducting shelf-life studies on Lm in RTE foods

EURL Lm

EURL Lm Guidance Document to evaluate the competence of labs implementing CT, durability studies

Guidelines for conducting challenge tests of food and feed products - Part 1 : Challenge to study the growth potential, lag time and the maximum growth rate

DG SANCO Guidance Document on Lm shelf-life studies for RTE foods, under Reg. (EC) N° 2073/2005

European perspectives

- Investigate the storage temperature at consumer level based on a review of available **data on domestics refrigerators in Europe**

Table 3 of the EURL Lm Technical Guidance document on the storage conditions throughout the cold chain

Stage of cold chain	Storage (incubation) temperature			Storage (incubation) duration			
						Shelf life ≤ 21 days	Shelf life > 21 days
From manufacture until the arrival to the display cabinet	Temperature justified by detailed information*	Or if not known	~~8~~ 7°C	Duration justified by detailed information	Or if not known	One third of the total shelf life of the product	7 days
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* Temperature justified by detailed information: the 95th percentile of the FBO's data observation.

** Temperature justified by detailed information: the ~~75th~~ 95th percentile of the observations for the country where the stage of the cold chain is located.

European perspectives

- **Development (by EURL Lm with WG of 6 NRLs and 3 CAs) of a "European Training Support" on food shelf-life studies related to *Listeria monocytogenes* (to be finalized in 2019)**

  - Train staff of MS's competent authorities involved in inspection of RTE foods regarding the *Listeria monocytogenes* criteria, and in charge of the evaluation of shelf-life studies

  - Provide to CAs the information and tools useful to make sure that shelf-life studies, implemented to justify the quantitative criteria for Lm in RTE foods, are satisfactory

Paul in ‘t Veld

Netherlands Food and Consumer Product Safety Authority (NVWA)

- Working for the competent authority in The Netherlands
- PhD in food microbiology
- Chair of ISO WG3 on method validation and expert in ISO WG19 on challenge testing
- Involved in evaluation of studies on the growth of *Listeria monocytogenes* in RTE foods.

Nederlandse Voedsel- en Warenautoriteit
Ministerie van Landbouw, Natuur en Voedselkwaliteit

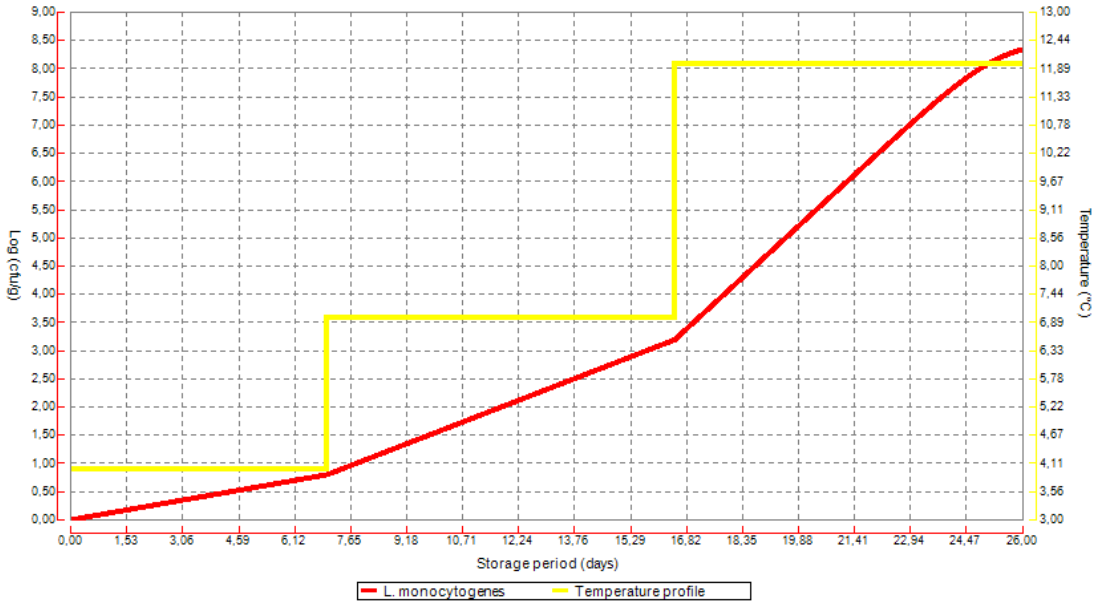
Conducting Challenge Tests for *Listeria monocytogenes*, a real challenge

Paul in 't Veld

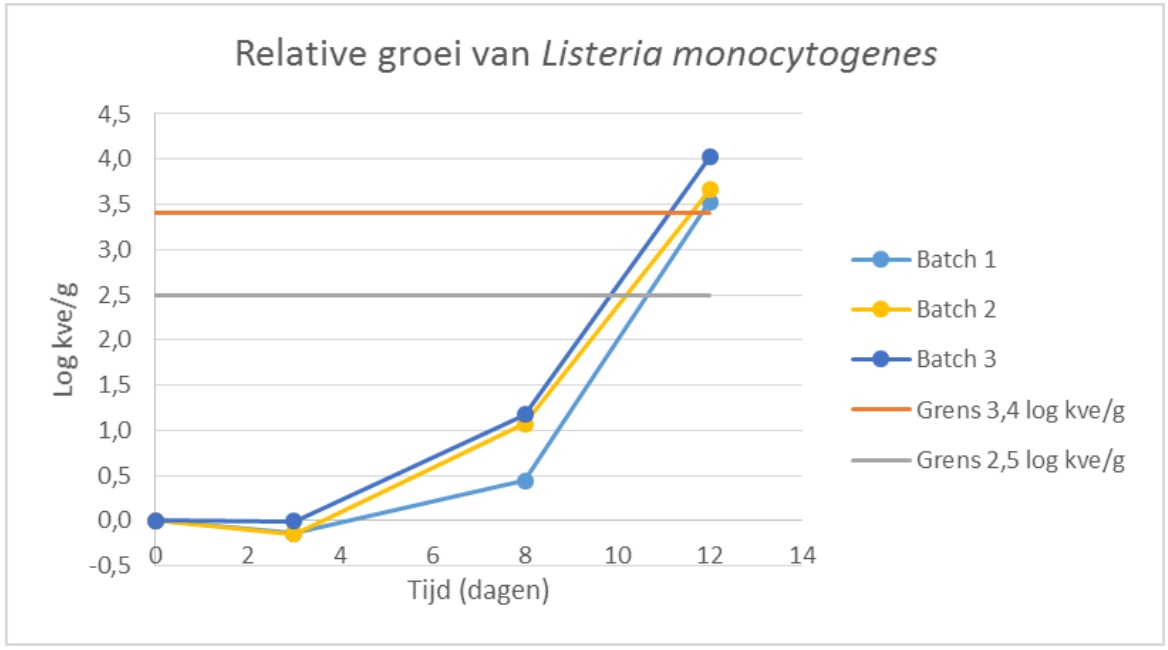
Netherlands Food and Consumer Product Safety Authority (NVWA)

How to conduct (additional) studies

Modelling

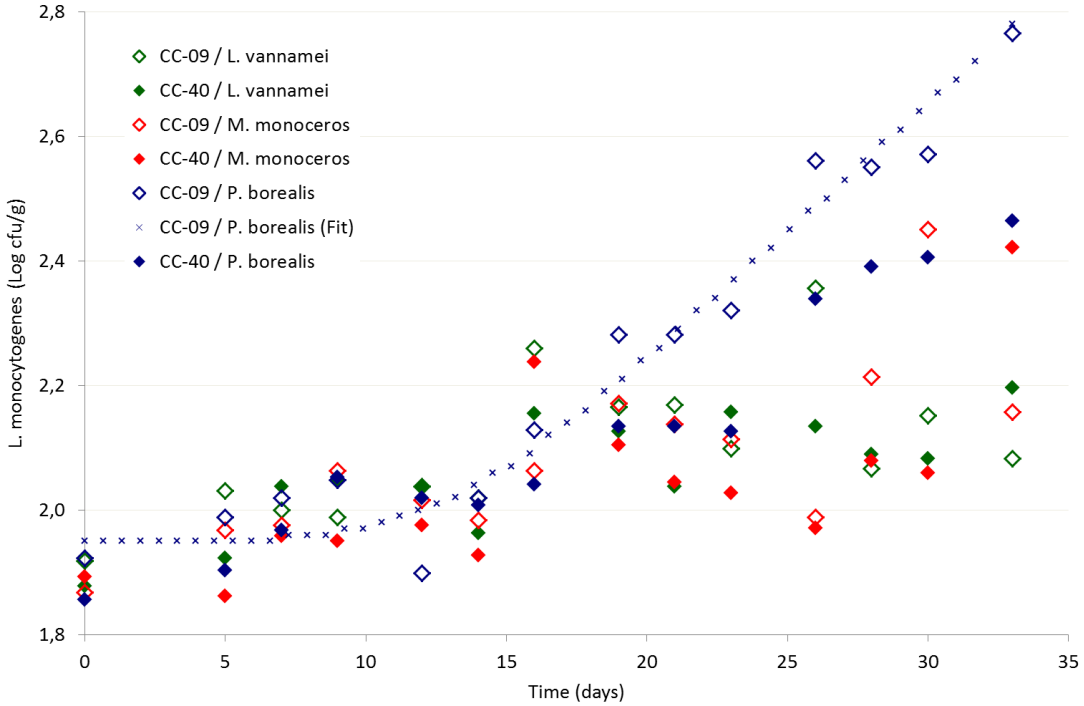


Challenge testing



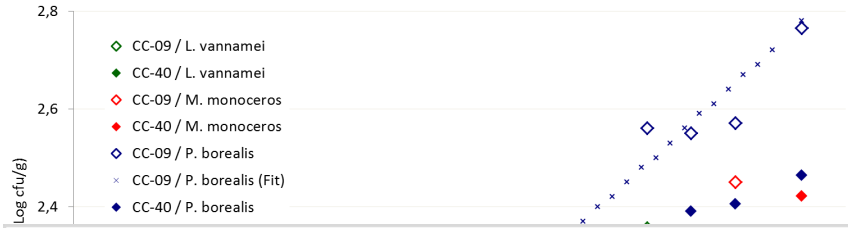
Challengetesting

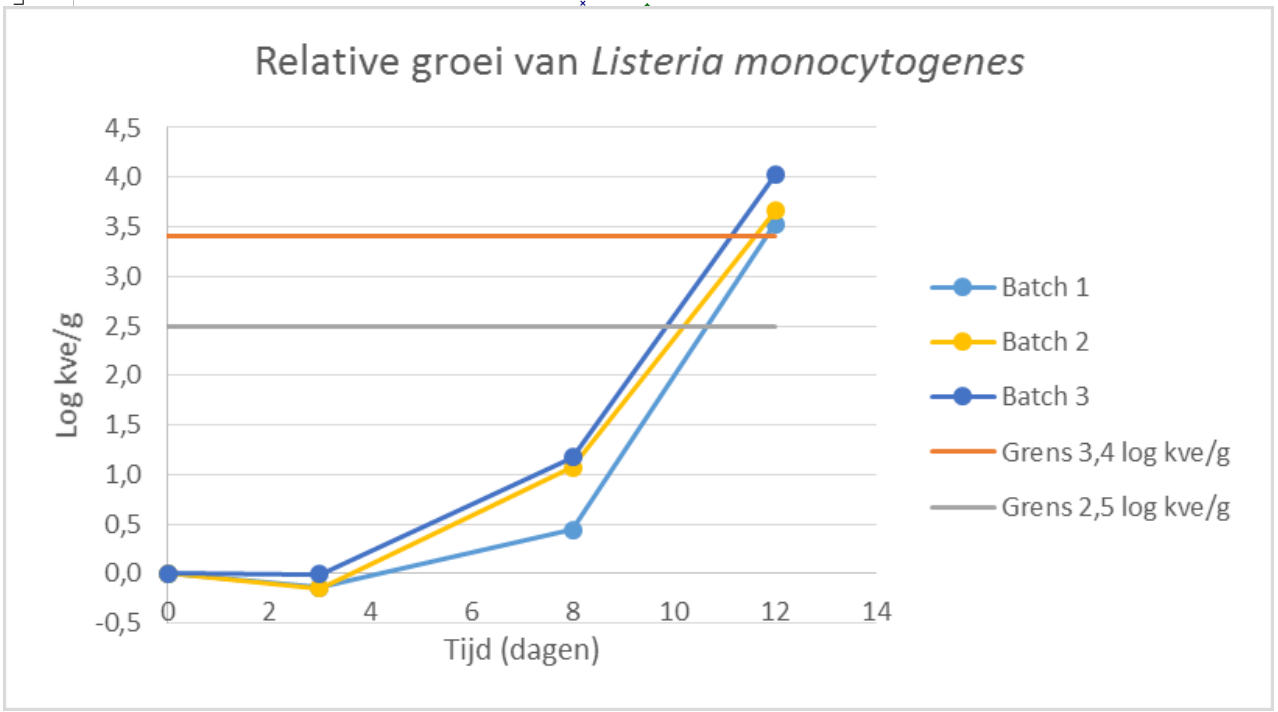
- Two types of experimental design:
  - **Maximum growth rate determination**



Challengetesting

- Two types of experimental tests:
  - Maximum growth rate determination
  - **Determination growth potential**





Growth potential determination

Example of factors influencing a challenge test:

- Test of a single product or a group of products
- Selection of batches to be tested
- Selection of strains and culturing them
- Inoculation of product
- Temperature profile for incubation of a product
- Intermediate points for testing
- Calculation of the growth potential
- Linking the growth potential to initial contamination
- What to do when product is changed
- Cooperation between FBO and lab

Test of a single product or a group of products

- FBO can have many products
- Necessary to test each product?
- How to group products?
- Use of physico-chemical characteristics (including preservatives) and production process

Inoculation of product

- Depending on the type of product and where (re)contamination can occur.
- Take of care of maintaining MAP conditions.
- Keep original packaging materials as far as possible

Temperature profile for incubation of a product

- Recently revised criteria at EU level.
- Consumer stage still high temperature.
- Possibility to deviate once dat submitted by FBO.
- Take into account national requirements,
  e.g. NL + BE: 7 °C, 7 °C, 9 °C

Table 3: Flow diagram of storage conditions throughout the cold chain

Stage of cold chain	Storage (incubation) temperature			Storage (incubation) duration			
						Shelf life ≤ 21 days	Shelf life > 21 days
From the manufacture until the arrival to the display cabinet	Temperature justified by detailed information*	Or if not known	~~8~~ 7°C	Duration justified by detailed information	Or if not known	One third of the total shelf life of the product	7 days
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* Temperature justified by detailed information: the 95th percentile of the FBO's data observation

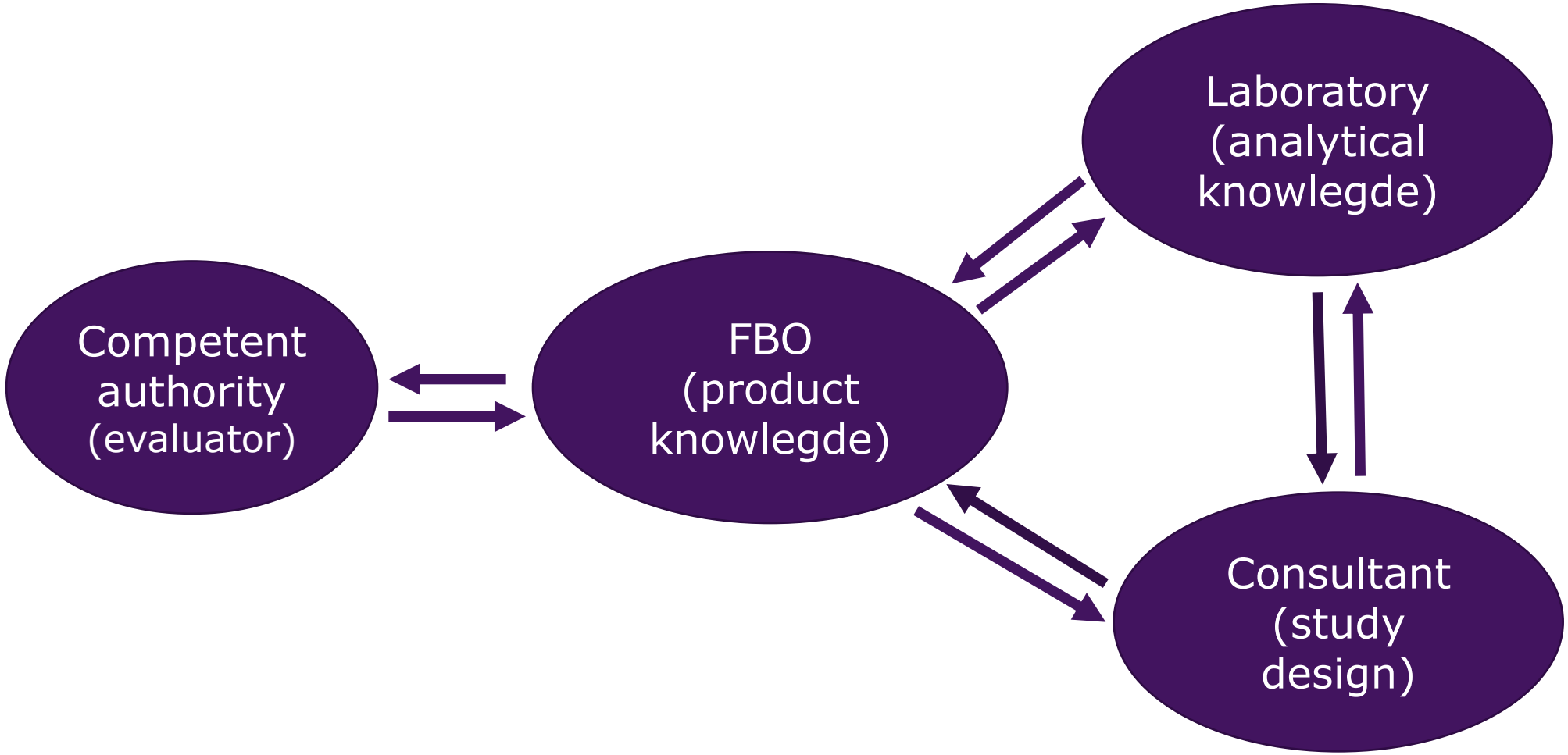
** Temperature justified by detailed information: the 95th percentile of the observations for the country where the stage of the cold chain is located.

Calculation of the growth potential

- Is the use of the median always justified?

Day	cfu/g	Log cfu/g	Difference based on median	Growth potential	Difference based on maximum	Growth potential
0	90	1,95	2,15 - 1,98 = 0,17	**0,17**	2,15 - 3,70 = 1,55	**1,55**
	95	1,98				
	95	1,98				
42	55	1,74				
	150	2,15				
	5000	3,70				

Cooperation between FBO and lab



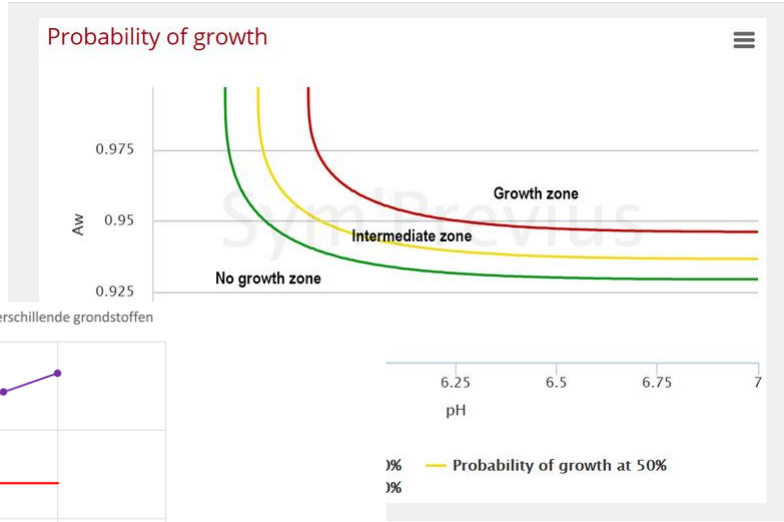
Maximum growth rate

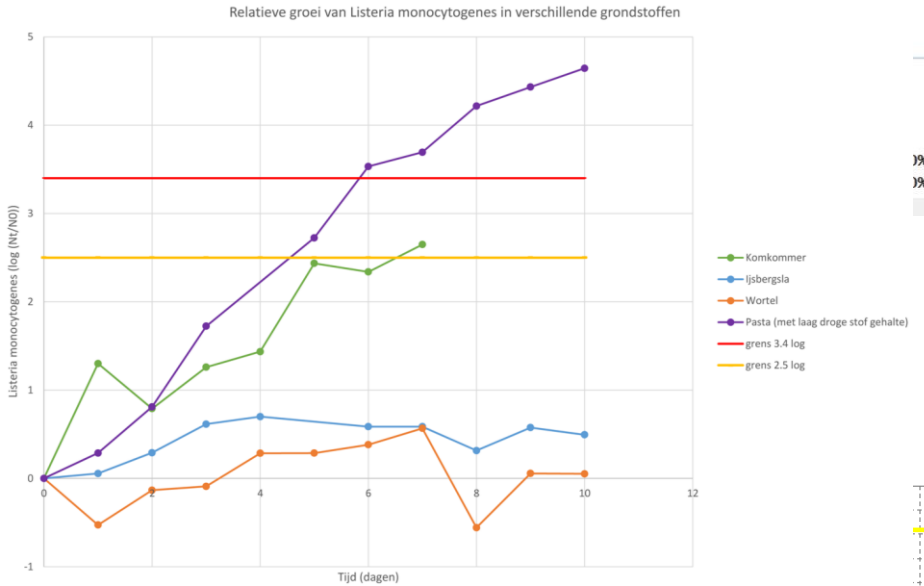
- Basis the same as for determination growth potential.
- Advantage:
  - More flexible: results are independant from temperature during storage and shelf life duration.
  - Tests are done at constant temperature, e.g. 8 °C.
- Disadvantage:
  - More expensive: strains have to be tested individually.
  - Need to consider 95% confidence limits
  - More difficult calculations are needed.

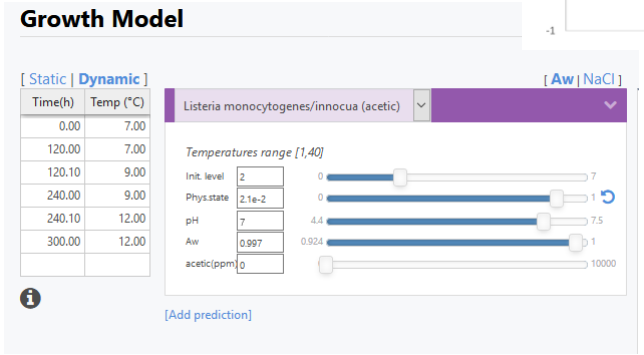
How to conduct additional studies

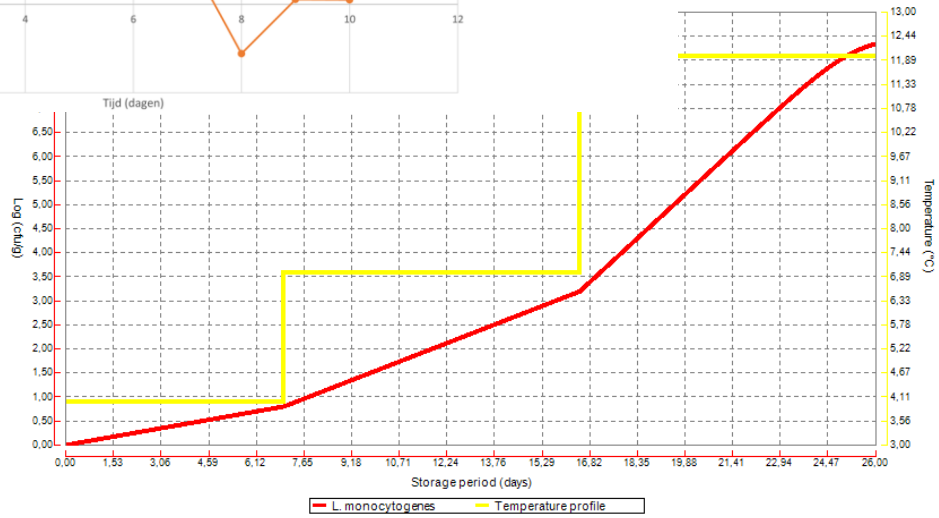
Modelling:

- How to select a (generally available) model?
- Use of lag fase in model?
- Differences between models









How to select a (generally available) model?

- FSSP, Combase, PMP, Symprevious,….
- Representative for the product Parameters in the model (a_w versus salt + dry matter content)
- Misuse of models (working outside their limits)
- Use of confidence intervals in the interpretation?

- Use of models for composite food products (interaction between components)

Multistate outbreak of Listeria monocytogenes infections linked to whole apples used in commercially produced, prepackaged caramel apples: United States, 2014-2015.

Angelo KM[1], Conrad AR[1], Saupe A[2], Dragoo H[3], West N[4], Sorenson A[5], Barnes A[6], Doyle M[7], Beal J[7], Jackson KA[1], Stroika S[1], Tarr C[1], Kucerov S[1], Gould LH[1], Wise M[1], Jackson BR[1].

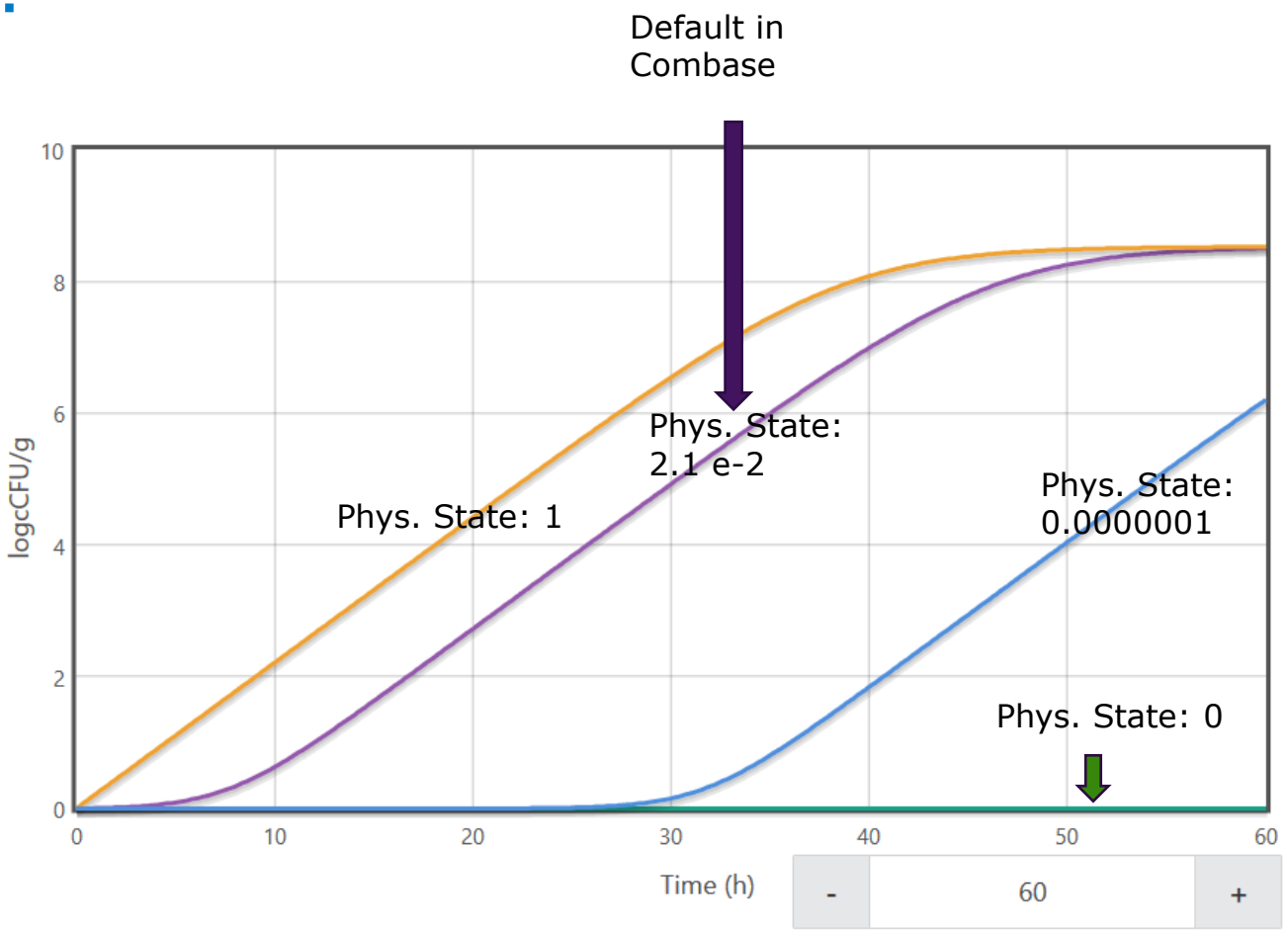
Author information

Abstract

Whole apples have not been previously implicated in outbreaks of foodborne bacterial illness. We investigated a nationwide listerio

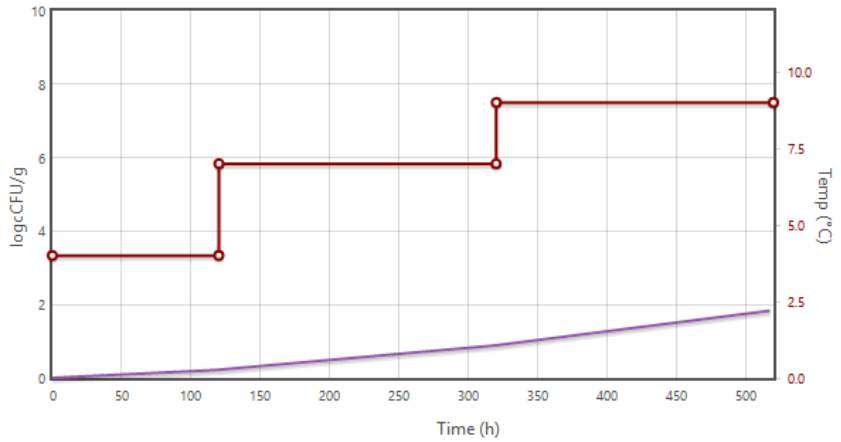
Use of lag fase in model?

- Guidance document does not prohibit the use of a lag-phase, but what is realistic?
- The lag-phase default value in Combase is not directly linked to stress conditions.
- Authorities in NL prohibit use of lag-phase in models.

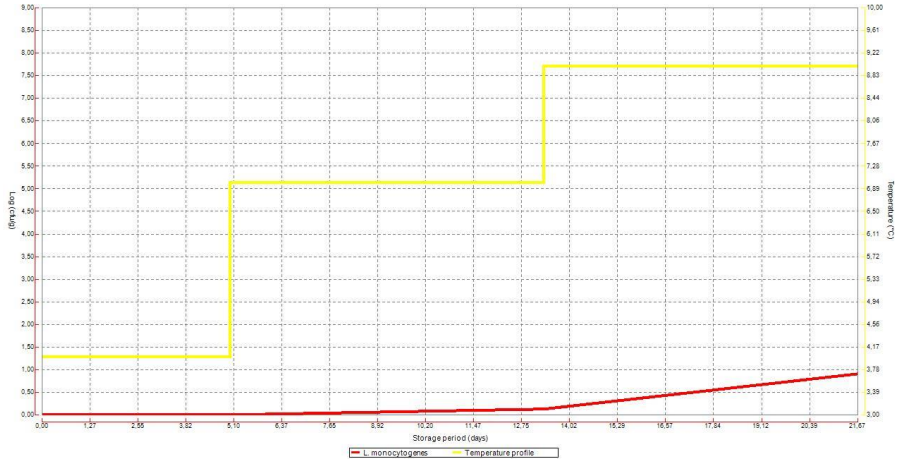


Differences between predictions

- Different models will give different predictions.
- What is the truth?
- FBO will try to use the most favourable model.
- Use different models!!!



Combase:



FSSP

Conclusion

- Performing a study is complex
- Challenge testing involves various areas of experitise
- Knowlegde for the evaluation of studies is not widely available at the Competent Authorities due to its complexity

- Modelling seems an easy solution but has limitations.

Carefully plan a study

Questions?

Questions should be submitted to the presenters during the presentation via the **Questions section** at the right of the screen.

Slides and a recording of this webinar will be available for access by IAFP members at www.foodprotection.org within one week.

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