Modelling in foods: Kinetics and Tools
IAFP Webinar

Organized by:
Microbial Modeling and Risk Analysis PDG

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Webinar Housekeeping

For best viewing of the presentation material, please click on ‘maximize’ in the upper right corner of the ‘Slide’ window, then ‘restore’ to return to normal view.

Audio is being transmitted over the computer so please have your speakers ‘on’ and volume turned up in order to hear. A telephone connection is not available.

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

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This webinar is being recorded and will be available for access by IAFP members at www.foodprotection.org within one week.
Facilitated Discussion

- Moderator
  - Marcel Zwietering
  - Wageningen University
  - marcel.zwietering@wur.nl

- Questions should be submitted via the Text Chat section at the bottom of the screen.
Modelling in foods: Kinetics and Tools
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Contact information for presenters

- Lihan Huang, Ph.D., CFS
  - USDA Agricultural Research Service
    - lihan.huang@ars.usda.gov
- Antonio Valero, PhD
  - University of Cordoba (SPAIN)
    - avalero@uco.es

- At the end: Keep your browsers open to complete the survey
Dr. Huang has earned a Ph.D. Degree in Food Science and Technology and is currently the Research Leader of the Residue Chemistry and Predictive Microbiology Research Unit at ERRC.

Prior to joining ERRC, Dr. Huang worked in the Research and Development Department of Campbell Soup Company and conducted research on thermal processing of low-acid foods in semi-rigid containers and aseptic processing of foods containing solid particulate.

He is the developer of the USDA Integrated Pathogen Modeling Program (IPMP-2013) and a new product called IPMP-Global Fit. He also serves as a Lead Scientist of a project.

Dr. Huang is a member of the International Association for Food Protection.
USDA Integrated Pathogen Modeling Program

A platform for easy and accurate data analysis in predictive modeling

Lihan Huang, Ph.D.
Research Leader
Residue Chemistry and Predictive Microbiology Research Unit
Eastern Regional Research Center
USDA Agricultural Research Service
Wyndmoor, PA
USDA Integrated Pathogen Modeling Program (IPMP)

What is it? Google it.

A suite of data analysis tools that contains 20+ most frequently used models

• Intelligent, interactive data analysis and model development
• Very easy-to-use graphical-user interfaces to guide every step
• Standardized data analysis and interpretation
Website to download

- IPMP is located in a secured USDA website
- A tutorial
- Download Instructions
- Compiled software (zipped file)
  - Windows 32 or 64 bit, Vista to Windows 10
Primary and secondary models

• **Primary model (time)**
  – Isothermal curves (growth and survival)
  – Growth rate and lag time
  – D value

• **Secondary model (temperature)**
  – Effect of temperature on growth rate and lag time
  – Effect of temperature on D values (z value)
Predictive Microbiology as An Inverse Problem

- $y = f(x, a, b)$
- $x$ is the independent variable
- $y$ is the dependent variable
- $a$ and $b$ are coefficients
- We know $x$ and $y$, but do not know $a$ and $b$
- We will find a way to identify $a$ and $b$ from $x$ and $y$
- This is an inverse problem
Predictive Microbiology as A Forward Problem

- $y = f(x, a, b)$
- $x$ is the independent variable
- $y$ is the dependent variable
- $a$ and $b$ are coefficients
- We know $a$ and $b$
- We want to know how $y$ changes with $x$
- This is a forward problem
The Traditional Approach: a 3-step process

Step 1 – Primary model
Data collection
- Isothermal 1
- Isothermal 2
- Isothermal 3
- Isothermal i
- Isothermal n

Step 2 – Secondary model
Data analysis
\[ \mu = g(T) \]
- \( \mu_1 \)
- \( \mu_2 \)
- \( \mu_3 \)
- \( \mu_i \)
- \( \mu_n \)

Step 3 - Predictions
Predictions
\[ y = f(t) \]
Forward problem
Primary Models for Growth

Isothermal growth curves

- Gompertz model
- Baranyi model
- Huang model
- Buchanan 3-phase (trilinear) model

We are interested in answering 3 questions from a growth curve

1. How long is the lag phase?
2. How fast can a microorganism grow?
3. What is the maximum cell density?
Primary Models for Survival (Inactivation)

- Linear model
- Weibull model
- Gompertz model

We are interested in answering 1 question from a survival curve

How fast does a microorganism die off?
Secondary Models

• What is the relationship between rate and temperature?

• What are the minimum, optimum, and maximum growth temperatures?
Techniques for Data Analysis

• Linear regression
• Nonlinear regression
• Typical inverse problems
Commercial Data Analysis and Statistical/Math Tools

- Very powerful
- Product-specific programming
- Not so user-friendly
- Training and learning
- Most are very expensive
- Difficult to learn and use
Some mysteries of predictive modeling

• Math is too hard
• I don’t know which model to choose
• I don’t know which model is better
• I don’t have a program to do it
• I don’t know how to program
Specific to Nonlinear Regression

- Initial guess values
- Improper initial guess values may not help in finding solutions (convergence)
- Graphical visualization helps finding suitable initial guess values
USDA - IPMP

• Based on a modern object-oriented computing language
• Advanced scientific computing libraries
• Modern graphical-user interface
• 20+ mathematical models
• Accuracy identical or equivalent to SAS and R
Effect of temperature on growth rate

Survival curves

Survival Models:
- Linear Model
- Gompertz Model
- Weibull Model
- Two/Three-Phase Linear Model

Secondary models - Temperature effect:
- Ratkowsky model
- Huang rate model
- Cardinal model
- Arrhenius-type model
IPMP 2013 Data Analysis

Fine-tuning capability

Pop up window
Click to adjust the spin box
Raw data
Preliminary curve
Slide to adjust parameter

Results of Data Analysis
2 6
3 7
4 8
5 9
6 10
7 10
8 10
9 10
Fine-tuned parameter

Fine-tuned curve

IPMP 2013 Data Analysis
Report Generation

Parameters

- Degree of freedom
- SSE
- MSE
- RMSE
- Residual stdev
- AIC (the smaller the better)
- Critical t-value

Error analysis

- Sum of squared errors
- Mean squared errors
- Root mean squared errors
- Akaike criterion

Raw data

- Lower and upper 95% confidence intervals for the expected value (mean)
- Lower and upper 95% confidence intervals for individual prediction

Predicted value

- Lower and upper 95% confidence intervals for the expected value (mean)
- Lower and upper 95% confidence intervals for individual prediction
Exponential growth and stationary phase

Cronobacter sakazakii in reconstituted infant formula
Lag phase and exponential growth

\[ \ln \text{cfu/g} \]

- t (h)

- raw data
- Huang
- Baranyi

*Listeria monocytogenes* in beef frankfurters
Lag phase, exponential growth, and stationary phase

Graph showing the growth of E. coli O157:H7 in beef, comparing raw data with models by Huang, Baranyi, and Gompertz.
Survival curves

$L. monocytogenes$ in ground beef
Temperature effect – Square-root Models

Cronobacter sakazakii in reconstituted infant formula
Cardinal model/Arrhenius-type model

Cronobacter sakazakii in reconstituted infant formula
Performance of IPMP
Comparison with Standard Statistical Packages
## Comparison with Standard Statistical Packages

### Huang Model

<table>
<thead>
<tr>
<th>Parameters</th>
<th>Results from R</th>
<th>Results from IPMP-2013</th>
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</thead>
<tbody>
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<td>Std. Err.</td>
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<td>8.93</td>
<td>0.220</td>
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<tr>
<td>$Y_{\text{max}}$</td>
<td>19.57</td>
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<td>$\mu_{\text{max}}$</td>
<td>0.518</td>
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<td>$\lambda$</td>
<td>2.47</td>
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### Baranyi Model

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<th>Parameters</th>
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<td>$h_0$</td>
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### Re-parameterized Gompertz Model

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<td>$\lambda$</td>
<td>3.50</td>
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</table>
IPMP for Data Analysis

- Simple data entry
- Choose models that suit your data
- Use graphical interfaces to adjust parameters for faster (almost guaranteed) convergence
- All data analysis and calculation are done behind the scene
- Fast and accurate
- Compare different models for the same set of data
- IPMP has significantly lowered the bar for those who want to work on predictive modeling
Thank you!

Lihan.Huang@ars.usda.gov
Prof. Antonio Valero has more than 10 years experienced managing projects, training courses, academic teaching and events in food and related sectors. Including national and international research projects related to predictive modeling and risk assessment.

Prof. Valero has participated in collaborative works with EFSA and has published over 70 peer-reviewed papers and book chapters. He has presented more than 80 communications in different congresses and symposia.

Prof. Valero is a member if the International Association for Food Protection.
BASELINE-APP

“A WEB-BASED SOFTWARE TOOL: PREDICTIVE MODELS AND SAMPLING PLANS”
Contents

- **Software features:**
  - Literature review and models development

- **Predictive models:**
  - Growth / inactivation
  - Models validation

- **Optimization of sampling plans:**
  - Attributes / concentration-based
  - Specific sampling plans

- **Derivation of Microbiological Criteria**
  - Case studies

- **Conclusions**

- **Questions**
Software features

- Free access online platform
- Graphical user-interface:
  - Tables
  - Graphs
- Flexibility: new models can be incorporated
- Comparison and Scenario Analysis studies

Free registration is available at www.baselineapp.com
The software tool allows:

1. **Predict growth and inactivation of different microorganisms in different food matrices.**

2. **Design and apply Sampling plans in different food categories.**

**Models Module**
- 75 predictive models implemented

**Sampling Plans Module**
- Help user to design efficient statistical sampling plans
- Smallest number of samples necessary
- While providing the largest confidence possible to detect a non-conforming lot
Literature review and models development

**Model types:**

**Primary models:**

- Growth
  - Gompertz
  - Baranyi and Roberts (1994)
  - Three linear phase model

- Inactivation
  - Lineal Model
  - Weibull

**Secondary models**

- Ratkowsky type
- Polynomial

\[
\ln(-\varphi_{max}) = a_0 + a_1(1/T) + a_2(1/pH) + a_3(1/b_w)
\]

\[
\ln(N(t)) = \ln(N_0) + \mu_{max}A(t) - \ln\left[1 + \frac{e^{\mu_{max}A(t)} - 1}{e^{N_{max} - N_0}}\right]
\]

\[
\sqrt{\mu_{max}} = b(T - T_{min})\sqrt{a_w - a_{w_{min}}}\sqrt{1 - 10^{pH_{min} - pH}}.
\]

Integration of the mathematical models into easy to use software tools
1. Identification of food matrices

- Seafood
- Plant products
- Milk / dairy products
- Egg / egg products
- Meat products
Literature review and models development

2. Identification of pathogens

Vibrio parahaemolyticus
Listeria monocytogenes
VTEC
Campylobacter
Salmonella
3. Identification of specific environmental conditions

**Minimum Water Activity for the Growth of Some Microorganisms**

- Cl. botulinum
- Salmonella
- Most Bacteria
- Most Yeasts
- Staphylococcus
- Most Molds
- Intermediate Moisture Foods
- Halophilic Bacteria
- Extreme Osmophiles e.g. some molds and yeasts
- Dehydrated Foods

**Water Activity Ranges of Some Foods**

- 1.0: Fresh Vegetables, Fruits, Meats, Poultry, Fish, Milk
- 0.9: Liverwurst, Cured Meats e.g. Ham, Certain Cheeses e.g. Swiss, Mozzarella
- 0.8: Salami, Some Dry Cheeses, Sugar Syrups, Flour, Cakes, Rice, Beans, Cereals
- 0.7: Sweet Brown Condensed Milk, Salt-Preserved Foods, Jams, Pepperoni, Honey, Marshmallows
- 0.6: Dried Fruits, Caramels

**Water Activity Minima**

- Microbial Growth: 0.6
- Dehydrated Foods: 0.6

**pH and Acidity Foods**

**High Acidity Foods**

- pH 3.0: lemons
- pH 4.0: pears
- pH 4.6: tomatoes

**Process Required**

- Boiling Water Bath
- Pressure Canning (Shorter Times)

**Low Acidity Foods**

- pH 5.0: carrots
- pH 6.0: chicken, peas, corn
- pH 7.0: hominy, shrimp

- **USDA recommends addition of acid to tomatoes to ensure proper acidity (pH below 4.6)**
4. Literature review of available predictive models

Predicting growth rates and growth boundary of *Listeria monocytogenes* — An international validation study with focus on processed and ready-to-eat meat and seafood

Ole Mejlhom, Annemarie Gunvig, Claus Borgsgaard, Jesper Blom-Hanssen, Lyndal Mellefont, Tom Ross, Françoise Leroy, Tony Else, Diana Visser, Paw Dalgaard

Extreme Heat Resistance of Food Born Pathogens *Campylobacter jejuni, Escherichia coli, and Salmonella typhimurium* on Chicken Breast Fillet during Cooking

Aurélie E.I. de Jong,1,2 Esther D. van Asselt,1,2 Marcel H. Zwietering,4 Maarten J. Nauta,1,2 and Rob de Jong1

Available online at www.sciencedirect.com

Available online at www.sciencedirect.com

Food Microbiology

Predictive models of growth of microorganisms in salted and cured meat products

Astryn Kajak, Danuta Kokożyn-Krajewska

Received 11 August 2004; accepted 22 September 2005

Correspondence should be addressed to Aurélie E.I. de Jong, aurélie.de.jong@wva.nl

Received 9 August 2011; Accepted 21 October 2011
Literature review and models development

4. Literature review of available predictive models

Electronic library catalogues:
ScienceDirect
Wiley online library
Pubmed
Springer
Revicien
Academic google
Grey literature

More than 100 scientific references meeting the proposed requirements were analyzed.
Selection criteria were previously defined.
Literature review and models development

5. Implementation of selected predictive models in the software

Growth Model

References
- Duffy et al., 1994

Primary Model
- Baranyi & Roberts, 1994

Microorganism
- Listeria monocytogenes

Food
- Cooked meat -with nitrate

Detail
- To highlight text...

Extra Information
- Time Unit
- Time Max

Back
6. Performance and visualization of predictive models

Predictive models: growth/inactivation

List Data

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Plot Data

Time 501

\[ y_1(t) \]

\[ x = 50.16, y = 4.92 \]

Points 100

Growth Models

Info Models

<table>
<thead>
<tr>
<th>Code</th>
<th>Food</th>
<th>Primary Model</th>
<th>Secondary Model</th>
<th>Microorganism</th>
<th>Nmax</th>
<th>Pmax</th>
<th>Lag</th>
<th>NT Time</th>
<th>Actions</th>
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</table>
Models validation

Model Information
- Code: 006018GxS
- Food: Oyster
- Primary: Gompertz, 1825
- Secondary: Legname and Nakovski
- Microorganism: Vibrio parahaemolyticus (pathogenic)

Conditions of validation
- Nº observations: 5
- Kinetic Parameter observed: \( \mu \text{ max} \)
- Environmental Factor compared: Temp

Observed data

<table>
<thead>
<tr>
<th>#</th>
<th>Temp [20,30]</th>
<th>( \mu \text{ max} )</th>
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Actions
- Accept
- Clean

Kwon, K.S., Min, K.J., Jung, Y.J.; Kwon, K.Y. Lee, J.K., Oh, S.W. 2008: A model of the effect of temperature on the growth of pathogenic and nonpathogenic Vibrio parahaemolyticus isolated from oysters in Korea. Food Microbiology 25, 0.05-0.44.
Models validation

Validation indexes

- SEP = 25 %
- RMSE = 0.03
- BF = 1.19
- AF = 1.88

Validation data

<table>
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<tr>
<th>Observ.</th>
<th>Predic.</th>
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<td>0.37</td>
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<tr>
<td>0.76</td>
<td>0.88</td>
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</tbody>
</table>

Conditions of validation

- N° observations: 5
- Kinetic Parameter observed: μ max
- Environmental Factor compared: Temp

References:

Data exportation
**SAMPLING PLAN MODULE**

**GENERIC MODULE**
- Presence/absence sampling plans
  - Two-class and three class
    - Binomial
    - Poisson
    - Negative Binomial

**PRACTICAL EXAMPLES**
- Concentration-based sampling plans
- Deriving an MC from a PO that is set as quantitative limit to the microbial concentration
- Deriving an MC from a PO that is set as the limit to the microbial prevalence
- Deriving an MC from an FSO for a product supporting growth of the pathogen between PO and FSO

**SPECIFIC SAMPLING PLANS**
- Development of fit-for purpose sampling plans for the specific food/risk combinations selected in BASELINE

**TOOLS**
- Creation of help-menus
- Additional information
- User-friendly reports containing the main results from the applied sampling plan

**BASELINE**

**European Commission**

**dtkfz.**

**UNIVERSIDAD DE CORDOBA**

**Q OPTIMUM QUALITY**

quality & innovation consultants
Optimization of sampling plans

- **Generic module:**
  - **Attributes:** Binomial, Poisson, Negative Binomial, Trinomial distributions
  - **Concentration-based:** Log Normal, Poisson-log normal distributions
Case studies

Derivation of Microbiological Criteria from established Performance Objectives

Case 1. Application of a PO / MC for concentration-based sampling plans

Smoked salmon contamination by *L. monocytogenes*.
Initial contamination of the product just after packaging: 1.5 log cfu/g
Storage conditions: 96 h at 4°C
Product formulation: 2ppm phenol + 3% salt

**PO = P (log cfu/g > 3) < 5% of the samples comprising the lot**

Which MC should be applied in order to comply with the selected PO?
Case studies

Journal of Food 106, 159-168.
Case studies

*L. monocytogenes* is able to grow up to 1.94 log cfu/g at the end of storage. We consider a standard deviation of the lot = 0.8 log cfu/g (solid food contamination assumed).

Distribution of *L. monocytogenes* ~ lognormal (1.94, 0.8)

PO = P (log cfu/g > 3 ) < 5 % of the samples comprising the lot
Case studies

1. Establish/decide on the maximum allowable concentration that should not be exceeded:
   - log cfu/g

2. Establish/decide on the maximum allowable percentage of units in the lot below the targeted concentration:
   - [0 - 99.99] %

3. Enter parameters corresponding to the concentration of the pathogen in the lot:
   - mean: 1.94
   - standard deviation: 0.8 [0 - 3]
Case studies

7. Please indicate the new value for mean: 1.5

8. Calculation of the percentage of units below targeted concentration: 96.9603 [0 - 99.99]%

9. Does the lot characteristics fulfil with the targeted objective? Yes
Case studies

mean: 1.5 log cfu/g
sigma: 0.8 log cfu/g
m: 2 log cfu/g
n: 5 samples
c: 0 samples

$P_{acc} = 0.21$
### Criteria Interpretation Sampling plan

<table>
<thead>
<tr>
<th>n</th>
<th>c</th>
<th>m</th>
<th>M</th>
<th>Satisfactory</th>
<th>Unsatisfactory</th>
<th>Attributes (two classes)</th>
</tr>
</thead>
<tbody>
<tr>
<td>10</td>
<td>0</td>
<td>2</td>
<td>NA</td>
<td>0 samples above 100 cfu/g</td>
<td>≥ 1 sample above 100 cfu/g</td>
<td></td>
</tr>
</tbody>
</table>

\[ P_{acc} = 0.05 \]
Case studies

Derivation of Microbiological Criteria from established Performance Objectives

Case 2. Decision support tools for derivation of MC in foods. Application to *Escherichia coli* O157:H7 in leafy greens

- Knowledge of expected prevalence/concentration in the studied food product
- Processing conditions
- Expected behaviour throughout the production chain
- Expected consumer handling and storage
Case studies

1. Is the expected prevalence of the pathogen considered high in the raw material and/or intermediate food products?  **No**

6. Is the pathogen normally present in the raw material and/or intermediate food products at low levels, i.e. less than 10 cfu/g or ml?  **Yes**

7. Could cross-contamination or recontamination occur during handling and processing?  **Yes**

3. Can the pathogen grow/survive during storage up to consumption level?  **Yes**

4. Is it possible to apply a bactericidal treatment at consumption level that produces non significant risk?  **Yes**

**GROUP 1:** Very low concentration (<1 cfu/g) non detectable by conventional analytical methods (quantitative)

**GROUP 2:** Low concentration (<10 cfu/g) detectable but not countable (which would be representing censored data)
**Case studies**

Assumed mean concentration = 1 cell/100g = $-2 \log_{10} \text{cfu/g}$
Standard deviation = $0.8 \log_{10} \text{cfu/g}$

Poisson-log normal distribution (-2; 0.8) $\log_{10} \text{cfu/g}$
Non detectable levels by conventional techniques

<table>
<thead>
<tr>
<th>Qualitative Sampling Plans</th>
<th>Concentration-based Sampling Plans</th>
<th>Generic Module</th>
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</thead>
</table>

<table>
<thead>
<tr>
<th>Distribution</th>
<th>Inputs</th>
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<tr>
<td>2</td>
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<tr>
<td>3</td>
<td>class counts</td>
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<table>
<thead>
<tr>
<th>mean</th>
<th>standard deviation</th>
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<td>-2</td>
<td>0.8</td>
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</table>

<table>
<thead>
<tr>
<th>m</th>
<th>n</th>
<th>c</th>
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<tbody>
<tr>
<td>-1.398</td>
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<table>
<thead>
<tr>
<th>samples</th>
<th>weight</th>
<th>g</th>
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</thead>
<tbody>
<tr>
<td></td>
<td>25</td>
<td></td>
</tr>
</tbody>
</table>

Variable sampling plans are applied when frequency distributions of microorganisms are known, which assume that the measurements made on a series of samples follow a normal distribution with approximately 95% of all test values within +/-2 standard deviations from the mean.
Variable sampling plans are applied when frequency distributions of microorganisms are known, which assume that the measurements made on a series of samples follow a normal distribution with approximately 95% of all test values within +/-2 standard deviations from the mean.
Sampling Plan Report

Example II

Deriving a Microbiological criteria from a Performance Objective that is set as a concentration value of the pathogen

Setting the PO: "the maximum frequency and / or concentration of a hazard in a food at a specified step in the food chain before consumption that provides or contributes to an Food Safety Objective or an Appropriate Level Of Protection, as applicable"

Input Data

» Maximum allowable concentration that should not be exceeded: 3 log cfu/g
» Maximum allowable percentage of units in the lot below the targeted concentration. 95 % [0 - 99.99]
» Mean corresponding to the concentration of the pathogen in the lot: 1.5 log cfu/g
» Standard Deviation corresponding to the concentration of the pathogen in the lot: 0.8 [0 - 3]

Results

» Percentage of units below targeted concentration (PO): 96.960 %

Sampling Plan Data

» m, microbiological limit log cfu/g: 2 log cfu/g
» n, number of units comprising the sample to be taken: 10 samples
» c, allowable number of sample units giving values below m: 0 samples

![Graph 1](Highcharts.com)

![Graph 2](Highcharts.com)
Specific sampling plans
Conclusions

The Baseline software tool:

- predicts microbial behavior of different pathogens and food matrices with validated models
- makes possible the addition of new models as well as data are being gathered
- provides an easy derivation of Microbiological Criteria and Sampling plans from previously established risk-based metrics
- offers a tools menu to perform scenario analyses
- includes decision-support system to help non experienced users to choose the suitable option
THANK YOU FOR YOUR ATTENTION!!

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Questions

- Questions
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- Keep your browsers open to complete the survey !!
Next webinar

- Dose response for *Listeria monocytogenes*
- Prof. Dr. Fernando Pérez Rodríguez (Cordoba)
- Dr. Régis Pouillot (formerly FDA)

- Thursday June 1st, 2017
- 10:00 -11:00 am U.S. Central Time

- Keep your browsers open to complete the survey !!