

# **Guidelines for Setting Shelf Life of Chilled Foods in Relation to Non-proteolytic *Clostridium botulinum***



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# Guidelines for Setting Shelf Life of Chilled Foods in Relation to Non-proteolytic *Clostridium botulinum*

## Introduction

The primary responsibility for food safety rests with the food business operator (FBO). General implementation of procedures based on HACCP principles, together with the application of good hygiene practice, should reinforce FBOs' responsibility.

This document has been developed by a number of FBOs, trade bodies, associations and laboratories in the UK and Australia to provide guidance in relation to non-proteolytic *Clostridium botulinum* and:

- Shelf life establishment for chilled foods by FBOs, and
- On challenge testing foods by laboratories.

The guidance provides information to enable FBOs to consider:

- How to establish shelf life in relation to non-proteolytic *Clostridium botulinum*
- What needs to be considered and what actions need to be taken to determine whether challenge testing is appropriate before contacting a laboratory,
- What issues the laboratory should take into consideration for challenge testing to be carried out appropriately and give valid scientific data, and
- How to use these data to establish safe shelf life with respect to non-proteolytic *Clostridium botulinum*

The guidance is designed to also ensure that sufficient information is provided by FBOs and laboratories to arrive at valid decisions and to support FBOs when challenged by Competent Authorities.

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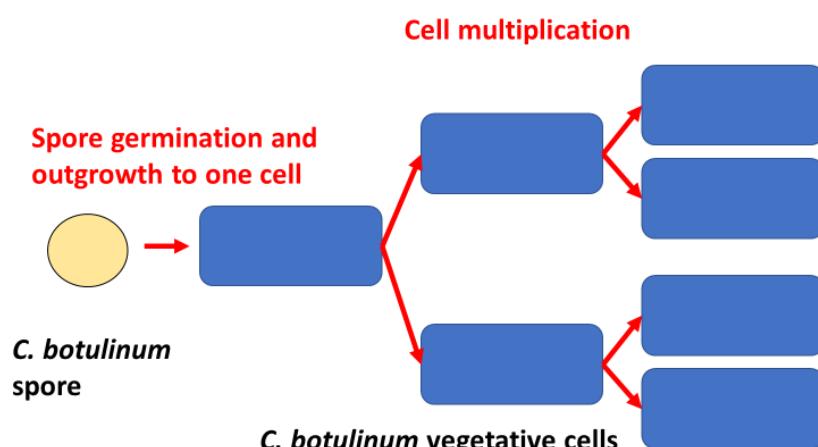
## **1. *Clostridium botulinum*: Key Facts**

*Clostridium botulinum* (*C. botulinum*) is a bacterium that produces spores that survive for an extended period under harsh conditions, and may survive some heat processes applied by the food industry. The spores are ubiquitous in the natural environment, being present in soil, for example. Spores can germinate and outgrow to form new vegetative cells that produce botulinum neurotoxin and/or multiply (Figure 1). Foodborne botulism is an intoxication caused by consumption of botulinum neurotoxin that has been pre-formed by *C. botulinum* in the food. Thus, botulinum neurotoxin is the hazard. Botulinum neurotoxin is the most powerful toxin known, 30 ng of which can be fatal to a human. Although tests in laboratory medium indicate that neurotoxin is formed in late exponential/early stationary phase, there is evidence from challenge tests that botulinum neurotoxin may be formed in food without a measured increase in bacterial viable count (Bell & Kyriakides 2000<sup>1</sup>, Brown & Gaze 1990<sup>2</sup>, Brown *et al* 1991<sup>3</sup>, Hyttiä *et al* 1999<sup>4</sup>). Possible explanations include that the measured viable count fails to recognise prior cell growth and subsequent death, and/or the cells have been metabolically active and formed neurotoxin but the viable count has not increased. Guidance documents produced in the USA/Canada for challenge test studies emphasise the importance of verifying that neurotoxin formation can be prevented (Doyle 1991<sup>5</sup>, NACMCF 2010<sup>6</sup>, Health Canada Food Directorate 2010<sup>7</sup>, NACMCF 1992<sup>8</sup>), and one recently stated that “detection of toxins is measured rather than growth, as toxin can be produced without an increase in numbers” (NACMCF 2010<sup>6</sup>). Most published challenge tests measure neurotoxin formation rather than viable count. Although ACMSF/FSA documents refer to the prevention of growth and neurotoxin production (FSA, 2017<sup>9</sup>), it is generally accepted that it is sufficient to demonstrate the prevention of neurotoxin production, as stated above.

There are two main types of *C. botulinum*: psychrotrophic (also known as non-proteolytic), which is capable of neurotoxin formation during cold storage, and mesophilic (also known as proteolytic) which require higher temperatures to form neurotoxin. Strains of psychrotrophic (non-proteolytic) *C. botulinum* form botulinum neurotoxin of types B, E or F, while strains of mesophilic (proteolytic) *C. botulinum* form botulinum neurotoxin of types A, B, and/or F. Foodborne botulism is most commonly associated with botulinum neurotoxin of types A, B, or E. Reviews have been published on *C. botulinum* (Hauschild 1989<sup>10</sup>, Bell & Kyriakides 2000<sup>1</sup>, Peck 2009<sup>11</sup>, Johnson 2013<sup>12</sup>, Peck 2014<sup>13</sup>, Peck *et al* 2008<sup>14</sup>).

The minimum temperature at which non-proteolytic *C. botulinum* forms neurotoxin is 3°C, while for proteolytic *C. botulinum* this is 10-12°C. Thus, in correctly stored chilled foods (maximum of 8°C), there is the potential for non-proteolytic *C. botulinum* but not proteolytic *C. botulinum* to form neurotoxin (Peck 2006<sup>15</sup>, Peck *et al* 2008<sup>14</sup>, Lindström *et al* 2006<sup>16</sup>). Note this guidance focuses on non-proteolytic *C. botulinum*, but if the storage temperature may reach or exceed 10°C, then the risk of production of botulinum neurotoxin from proteolytic *C. botulinum* must be considered and included in the risk assessment. Note: fermented products (e.g. cheese maturation, yogurt production, fermented meat, etc.) rely on holding at higher temperatures to result in sufficiently rapid pH and water activity ( $A_w$ ) decline, to assure safety.

**Figure 1: *Clostridium botulinum* cell cycle**



## **2. Definitions (see also Figure 1)**

Definitions are adapted from various sources including ISO<sup>17</sup>, EURL<sup>18</sup> and legislation<sup>19</sup>.

**Bacterial spore:** resistant form of bacteria which is dormant until it germinates

**Challenge test:** study of the fate of microorganisms (or their spores) artificially inoculated in a food, including the formation of neurotoxins

**Exponential growth phase:** growth phase in which the bacterial cells are multiplying exponentially. A period within the growth cycle where the viable count is said to double, i.e. 1, 2, 4, 8, 16 etc.

**Germination:** mechanism by which a bacterial spore starts becoming a vegetative cell

**MLD<sub>50</sub>:** median lethal dose of botulinum neurotoxin required to kill half the members of a tested mice population after a specified test duration. Typically, 10 pg botulinum neurotoxin is equivalent to approximately 1 MLD<sub>50</sub>. The human lethal dose is approximately 30 ng or 3,000 MLD<sub>50</sub>

**Organising laboratory:** laboratory with responsibility for managing the challenge tests

**Outgrowth:** steps in the transition of a germinated spore to the first cellular division of a vegetative cell (includes cell wall synthesis, cellular elongation)

**pH:** a measure of the acidity or alkalinity of a food. The pH 7 is defined as neutral. Values of pH less than 7 are considered acidic, and those with greater than 7 are considered basic (alkaline)

**Sampling:** selection of one or more unit or portions of food such that the units or portions selected are representative of that food

**Sampling point (date):** selected date to collect data in the study design

**Shelf life:** either the period corresponding to the period preceding the ‘use by’ or the minimum durability date<sup>19</sup>. In practice this means the period during which the product maintains its microbiological safety and sensory qualities at a specific storage temperature. It is based on identified hazards for the product, heat or other preservation treatments, packaging method and other inhibitory or inhibiting factors that may be used

**Stationary phase:** phase in which the bacterial population is at its maximum level; the number of bacterial cells dying and being produced is at equilibrium

**Test portion:** measured (volume or mass) amount of the representative sample taken from the test unit for use in the analysis

**Test unit:** measured (volume or mass) amount of the food used for inoculation

**Vegetative cell (of *C. botulinum*):** bacterial form which is capable of forming botulinum neurotoxin and/or multiplying under favourable environmental conditions

**Water activity (A<sub>w</sub>):** a measure of the water in a food that is available to microbes. It is not the same as the water content of the food, as some of the water in food can be bound to other molecules. Only unbound water can support the growth of microbes. The water activity scale extends from 0 to 1.0 (pure water) but most foods have a water activity level in the range of 0.2 for very dry foods to 0.99 for moist fresh foods

### **3. Factors for Food Business Operators to consider regarding setting shelf life in relation to non-proteolytic *Clostridium botulinum***

Setting shelf life requires FBOs to carefully consider a wide variety of factors and hurdles – raw material quality, hygienic processing, temperature, water activity ( $A_w$ ), acidity, packaging atmosphere – to determine how to control microbiological growth and/or the development of conditions that can lead to foodborne illness or spoilage. The appropriate choice and combination of these factors enables the optimum shelf life to be set for a food in combination with a food's usage conditions.

The following section provides information on the establishment of shelf life.

In some cases, where necessary, non-proteolytic *C. botulinum* challenge testing may be part of the approach to data generation. However, before challenge testing is commissioned by the customer they must discuss the proposed work in detail with the organising laboratory and understand the following:

- Why a challenge test is needed, and if other options (e.g. modelling, risk assessment, literature review) are more appropriate
- Whether the proposed approach is sufficiently scientifically rigorous to provide valid information
- How generated data will answer the question asked and that these data can be used in risk management decisions (e.g. converted to shelf life)
- How the project maximises value for money in terms of the number of samples to be tested and the wider applicability of results to products

#### **Q1 How could the shelf life of my product be extended, e.g. beyond 10 days?**

The Advisory Committee for the Microbiological Safety of Foods (ACMSF<sup>20</sup>) recommends in relation to vacuum and modified atmosphere packed chilled foods that in addition to chill temperatures (3-8°C) which should be maintained throughout the food chain, the following controlling factors should be used singly or in combination to prevent growth and neurotoxin production by non-proteolytic *C. botulinum* in chilled foods with a shelf life of more than 10 days:

- A heat treatment of 90°C for 10 minutes or equivalent lethality at the coolest point in the food, or
- A pH of 5.0 or less throughout the food and throughout all components of complex foods, or
- A minimum salt level of 3.5% in the aqueous phase throughout the food and throughout all components of complex foods, or
- An  $A_w$  of 0.97 or less throughout the food and throughout all components of complex foods, or
- A combination of heat and preservative factors which can be shown consistently to prevent growth and neurotoxin production by non-proteolytic *C. botulinum*

Although ACMSF/FSA documents refer to the prevention of growth and neurotoxin production (FSA 2017<sup>9</sup>), it is generally accepted that it is sufficient to demonstrate only the prevention of neurotoxin production, as foodborne botulism is an intoxication with botulinum neurotoxin (the hazard). Guidance documents for challenge test studies produced in the USA/Canada emphasise the importance of demonstrating that neurotoxin formation can be prevented (Doyle 1991<sup>5</sup>, NACMCF 2010<sup>6</sup>, Health Canada Food Directorate 2010<sup>7</sup>, NACMCF 1992<sup>8</sup>). If viable counts are carried out, then a significant increase in viable count should be taken to indicate a hazardous scenario even when neurotoxin formation is not detected. Importantly, however, a failure to measure an increase in viable count does not prove that neurotoxin has not been formed.

Guidance on considerations in relation to non-proteolytic and proteolytic *C. botulinum* and cheese has been published by the Specialist Cheesemakers Association:

<http://www.specialistcheesemakers.co.uk/media/download.aspx?MediaId=151>

See Appendix 1 for a worked example.

**Q2 How can I defend an existing product's shelf life if it is beyond 10 days, and what options do I have to extend shelf life beyond 10 days?**

HACCP implementation must be in place from New Product Development onwards to ensure that relevant hazards and their necessary controls have been identified from the outset.

It is important that each food type is made consistently so that the inherent characteristic (e.g. thermal process received, pH,  $A_w$ ) do not vary significantly. If consistency is not achievable, the worst possible case should be assessed as below.

When defending or extending a product's shelf life, information should be available covering the following elements:

**a) Risk Assessment to consider parameters including:**

Recipe control	pH, $A_w$ , salt, preservatives, moisture content
Shelf life	desired duration and storage conditions (temperatures) and how these affect the types of pathogens that could grow or produce neurotoxin
Ingredients	sourcing location, dried or fresh, published risk data associated with ingredients, e.g. Barker <i>et al</i> 2016 <sup>21</sup>
Hygiene measures	including area segregation, cleaning and disinfection regime, i.e. appropriate use of biocides
Process	type (e.g. thermal, high pressure) process duration, thermal profile, aseptic or clean fill, pre- or post-process packing

**b) Modelling likelihood of microbial activity using Risk Assessment data**

Free online software such as ComBase ([www.combase.cc](http://www.combase.cc)) can provide data on the likelihood of microbial growth, but does not give an indication of neurotoxin production. A prediction of growth can be taken as indicative of neurotoxin formation, however a prediction of no increase in viable count may not correlate to an absence of neurotoxin formation. Therefore, predictive models should be used with caution, and expert advice taken on interpreting the results. It is noted that there are several reports in the scientific literature that neurotoxin formation by *C. botulinum* can occur in the absence of an increase in viable count (e.g. Brown & Gaze 1990<sup>2</sup>, Brown *et al* 1991<sup>3</sup>, Hytyiä *et al* 1999<sup>4</sup>, Bell & Kyriakides 2000<sup>1</sup>). Possible explanations include (i) the measured viable count fails to recognise prior cell growth and subsequent death, and/or (ii) the cells have been metabolically active and formed neurotoxin although the viable count has not increased. A failure to measure (or predict) an increase in viable count does not therefore prove that neurotoxin has not been (or will not be) formed.

**c) Shelf Life Studies**

Shelf life studies involve storing non-inoculated foods under expected conditions and assessing their microbiological, organoleptic, chemical and physical quality. This should be done prior to production and by way of ongoing monitoring during production runs. This provides valuable information on product quality, but is not primarily concerned with microbiological safety, and certainly not with safety with respect to *C. botulinum*.

**d) Risk Review**

Review of data from Risk Assessment, modelling, and literature data from refereed publications to determine whether the pathogen could produce neurotoxin over the intended shelf life and storage conditions, including taking account of variation in pH and/or  $A_w$  over product life. If the risk review shows

that controlling factors for non-proteolytic *C. botulinum* are not in place then a challenge test should be considered.

Controlling factors in addition to chilled storage (at  $\geq 3^{\circ}\text{C}$  to  $8^{\circ}\text{C}$  max):

- $\leq 10$  days shelf life
- pH 5.0 max throughout the food, or
- $A_w$  0.97 max throughout the food, or
- Salt (NaCl) 3.5% minimum throughout the aqueous phase of the food, or
- A combination of heat and preservative factors which can be shown consistently to prevent growth and neurotoxin production by non-proteolytic *C. botulinum*, e.g. combination of pH/ $A_w$ /NaCl/preservatives, the SUSSLE Process giving the SUSSLE Shelf Life
- Thermal process  $90^{\circ}\text{C}$  for 10 mins equivalent (e.g. 42 days max is indicative in foods where it is suspected to contain lysozyme (Fernandez & Peck 1999<sup>22</sup>)

Additional but sometimes less easily quantifiable factors that can play a role in control of non-proteolytic *C. botulinum*, e.g.:

- Competition from other microorganisms
- Nitrate and/or nitrite
- Other permitted preservatives

#### e) Challenge Testing

The Risk Review can inform where challenge testing can provide further data required to establish the food's safety under expected storage conditions.

### **Q3 What is a *Clostridium botulinum* challenge test?**

A *C. botulinum* challenge test involves the addition of relevant organisms (or their spores – in this case non-proteolytic *C. botulinum*) to a food, to determine whether neurotoxin production occurs under expected storage conditions for that food's formulation, production and packaging method, assessing the safety and stability of the food. Guidance produced in the USA/Canada emphasises the importance of demonstrating that neurotoxin formation is prevented, and this recommendation is based on neurotoxin being the hazard, and the observation that neurotoxin can be formed without an increase in viable count (Doyle 1991<sup>5</sup>, NACMCF 2010<sup>6</sup>, Health Canada Food Directorate 2010<sup>7</sup>, NACMCF 1992<sup>8</sup>, Brown & Gaze 1990<sup>2</sup>, Brown *et al* 1991<sup>3</sup>, Hyttiä *et al* 1999<sup>4</sup>, Bell & Kyriakides 2000<sup>1</sup>). If viable counts are carried out, then a significant increase in viable count should be taken to indicate a hazardous scenario even when neurotoxin formation is not detected. Importantly, however, a failure to measure an increase in viable count does not prove that neurotoxin has not been formed.

### **Q4 In what circumstances would a challenge test be appropriate?**

It should be noted that there is no legal requirement in the EU or UK to carry out challenge testing for any microorganism. The primary responsibility for food safety rests with the FBO. General implementation of procedures based on the HACCP principles, together with the application of good hygiene practice, should reinforce FBOs' responsibility.

A challenge test may be advisable:

- If risk assessment (see below) identifies areas where further data are needed, or
- If changes are made to a food's formulation, thermal process, packaging system used, distribution or storage regime, or shelf life, and risk assessment identifies reduction in the level of control, or

- If a primary controlling factor is reduced so that it may cease to be effective (e.g. salt reduced), or
- If food has been produced in a standard way over a period of time but is found not to comply with guidance

#### **Q5 How might the results of a challenge test help set shelf life for a new/existing product?**

In the case of *C. botulinum*, absence of neurotoxin production provides an indication of the absolute limit for safety under the inoculum level, storage conditions, product formulation, production process and packaging technology assessed. However and very importantly, given the toxicity of botulinum neurotoxin, allowable shelf life will always be significantly shorter than the time when neurotoxin production occurs.

Note that if viable counts are carried out, then a significant increase in viable count should be taken to indicate a hazardous scenario even when neurotoxin formation is not detected. Importantly, however, a failure to measure an increase in viable count does not prove that neurotoxin has not been formed.

Examples of this include setting shelf life:

- By taking time (days) off the last negative challenge test result
- As a proportion of the time before the last negative challenge test result
- A combination of the above

Section 4 provides organising laboratories with guidance on how to present information to FBOs so they can decide the maximum allowable shelf life.

#### **4. Factors for an organising laboratory to consider when designing a challenge test of chilled foods with non-proteolytic *Clostridium botulinum***

The laboratory protocol needs to be agreed with the FBO in advance of any testing being carried out. This should include the provision of valid samples by the FBO, conditions for the transportation of foods to the organising laboratory, at what point during shelf life sampling will take place, in addition to satisfactorily addressing the points below. This document reflects guidance produced in the USA/Canada to consider when designing a challenge test with *C. botulinum* and the current ISO standard for challenge testing (Doyle 1991<sup>5</sup>, NACMCF 2010<sup>6</sup>, Health Canada Food Directorate 2010<sup>7</sup>, NACMCF 1992<sup>8</sup>, ISO/DIS 20976-1:2016<sup>17</sup>).

##### **4.1 Background**

###### 4.1.1 Purpose of challenge test and scope

- Ensure that the customer understands why a challenge test is needed, and that other options (e.g. modelling, risk assessment, review of literature data) are not more appropriate
- Ensure that the customer understands how generated data will answer the question asked and that these data can be used in risk management decisions (e.g. converted to shelf life)
- Ensure that customer understands how the project maximises value for money in terms of the number of samples to be tested and the wider applicability of results to products

###### 4.1.2 General information

- A *C. botulinum* challenge test should only be carried out by an organising laboratory with specialised containment facilities, and with suitable safety precautions
- A production flow diagram setting out CCPs including any thermal processes should be provided by the customer to enable the challenge test protocol to be designed appropriately

##### **4.2. *C. botulinum* strains to be used**

###### 4.2.1 Selection of strains of non-proteolytic *C. botulinum* to be used in spore cocktail

- Number of strains (e.g. 5-10)
- Origin of strains/traceability – where possible, source should be appropriate to tested product matrix. Use of reference strains (e.g. Eklund 17B and Beluga)
- Toxin types - strains forming type B or E neurotoxin should always be included, with strains forming type F neurotoxin sometimes also included. It should be checked at regular intervals (e.g. annually) that strains form good quantities of neurotoxin (e.g. >1000 MLD<sub>50</sub>/ml)
- Suitability of strains – proven neurotoxin formation at specific temperature, pH, salt concentration, etc.

###### 4.2.2 Production, enumeration and storage of spores

- Suitable spore production method (e.g. broth/plate, duration, incubation temperature)
- Washing of spores so that they are free of neurotoxin, vegetative cells and sporulation medium
- Any prior adaptation of strains to growth/germination at specific temperature/pH/salt/other
- Spore enumeration by viable count determination
- Storage of spore crops for valid periods of time, temperature, at optimal levels of spores
- Preparation of spore cocktail containing equal number of spores of each strain, and confirmation of viable count

##### **4.3. Food product and its inoculation**

###### 4.3.1 Optimising experimental design

- Number of replicates – for example 3 or 5
- Negative control samples – un-inoculated, or inoculated with inoculum carrier
- Positive control broths (or other suitable test samples) – to confirm neurotoxin formation at test temperature(s) or other control conditions

- Storage temperature of samples to reflect real situation, e.g. one temperature or change in temperature to reflect manufacture, retail, consumer storage (could include abuse step to simulate purchase)
- Duration of test
- Frequency of test points (minimum of 4-6 time points, including T=0)

#### 4.3.2 Selection and preparation of realistic worst-case food products

- Test portion, pack size of product to be tested (e.g. 10g – 100g)
- Consider variability of pH/A<sub>w</sub>/other e.g. preservative levels across batches/samples, particularly for multicomponent products, over their shelf lives
- If consistency between batches can be proved then test a single batch. If inconsistent batches, or multi-phasic or composite product then identify worst case scenario or test multiple batches (consider effect of age of individual batches / stage in shelf life)

#### 4.3.3 Selection of suitable spore concentration for inoculation of food product

- Representative spore concentration, but recovery must be repeatable/reproducible
- Suggest 10-100 spores/g product or 100-1000 spores/pack on the basis of known food spore loadings<sup>21</sup> and laboratory practicalities

#### 4.3.4 Selection of method of inoculation

- Spore heat activation or spore heat damage to mimic food production process prior to inoculation, or heat in product. It is recommended that spores are used immediately after heating
- Volume of inoculum (recommended volume-to-sample ratio ≤1:100, e.g. 100µl in 10g test portion)
- It is common practice to inoculate the product at a number of sites
- Distribution of inoculum in the product (e.g. surface inoculation, specific components within product)
- Inoculation likely to be product/packaging dependent. Such as: (i) initial packaging with septum and needle, or (ii) open pack inoculate (homogenise if appropriate) and repack under same VP/MAP using same packaging materials (or alternative with same technical properties), or (iii) open, aliquot, inoculate, pack under same conditions (e.g. for large volumes/masses)
- Consideration of effect of inoculation on pH / A<sub>w</sub>/VP/MAP conditions – confirm effect through analysis of pH/A<sub>w</sub>/gas

#### 4.3.5 Processing of food post inoculation

- Does a process(es) need to be applied post inoculation to represent manufacturing process?
- If a heat treatment is applied, then it must reflect the manufacturing process
- Should the product be VP/MAP to reflect the manufacturing process? (need to consider worst-case situation – low or no oxygen)

#### 4.3.6 Incubation of the food product (time and temperature)

- Note optimisation of experimental design (section 4.4.1)
- Storage temperatures to reflect practice. Monitoring and reporting of temperature profiling. Temperature tolerances
- Packing of product within storage equipment

### **4.4 Sampling the inoculated food product**

#### 4.4.1 Optimising sampling design

- See sections 4.1.1 and 4.3.1 above
- Also consider frequency of sampling, relationship of sampling to desired shelf life (e.g. demonstrate safety by subtracting time (days) off last negative challenge test result, or as a proportion of the time before the last negative challenge test result, or as combination of the two). It is common practice to test more frequently towards the end of shelf life

#### 4.4.2 Testing for botulinum neurotoxin

- Extract whole test portion for botulinum neurotoxin

- Test for botulinum neurotoxin using an ELISA, and compare with standard calibration curves to take account of food matrix effects. Using the mouse bioassay (according to the BAM manual (Solomon & Lilly<sup>23</sup>)) the detection limit for botulinum neurotoxin is *ca.* 20-40pg neurotoxin/g food. This has previously been accepted as the detection limit in challenge tests. The detection limit of the ELISA for botulinum neurotoxin should be within an order of magnitude of the mouse bioassay, i.e. 0.2-0.4 ng neurotoxin/g food (Solomon & Lilly<sup>23</sup>, Ferreira *et al* 2001<sup>24</sup>). This detection limit is *ca.* one hundredth of a human lethal dose (approximately 30ng) of botulinum neurotoxin
- Alternative methods to the ELISA that have a similar sensitivity (e.g. endo Mass Spectrometry) may also be suitable to detect botulinum neurotoxin

#### 4.4.3 Additional tests to support or in addition to measurement of botulinum neurotoxin

- Anaerobic plate counts to be correlated with neurotoxin results – consider media, incubation time/temp., replicates, etc.
- Detection of genomic botulinum neurotoxin <sup>§</sup>genes using PCR (Solomon & Lilly 2001<sup>23</sup>, ISO 2017<sup>25</sup>)
- Intrinsic analysis – pH/A<sub>w</sub> etc., particularly where these are controlling factors
- Analysis of other key controlling parameters e.g. salt, preservatives

<sup>§</sup> The spore inoculum used in the challenge test is unlikely to give a positive result, as (i) most methods will not extract genomic DNA from spores, (ii) the low inoculum level will mean that the number of copies of genomic DNA will be below the detection limit.

### **4.5 Presenting the experimental findings**

#### 4.5.1 Need for customer to understand findings

- The customer needs to understand how results can be converted to risk management decisions, and the limitations of findings (must be clear before project starts, see section 4.1). Two examples of how this might be applied are: set shelf life by either taking time (days) off the last negative challenge test result, and/or as a proportion of the time before the last negative challenge test result. In addition to considering the result of the challenge test, the FBO may wish to take account of other information (e.g. safety record of existing similar products) in setting shelf life.
- Expert advice must be sought from the organising laboratory, taking into account the agreed *a priori* criteria for the challenge test

#### 4.5.2 Final report

- Should clearly detail the results and their limitations, and provide a basis for risk management decisions
- Suitable sections for the report include: aim and type of test, experimental protocol, sample analysis, results and conclusions

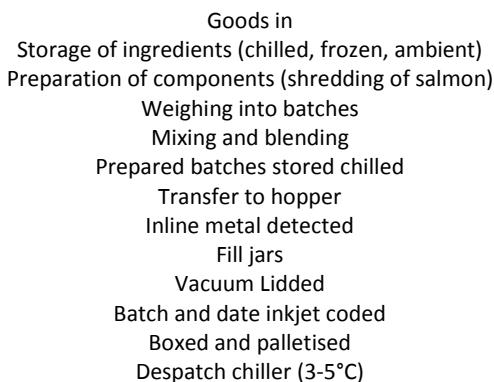
## Appendix 1

### Worked Example: Smoked Salmon Pâté Jars

The Smokin' Seafood Co. are developing a new smoked salmon pâté. The proposal is a “cold mixed” product, made up from the following ingredients:

**Smoked salmon** (Aq. salt 3.5% frozen), **poached salmon** (cooked to 70°C/2 minutes, stored at <5°C, 10 days shelf life), **soft cheese** (cooked and hot filled >70°C, pH 4.5, stored at <5°C, 60 days shelf life), **cream** (pasteurised 80°C/15s stored at <5°C 10 days shelf life), **mayonnaise** pH 4.2 ambient stable (containing oil, egg yolk, cornflour, spirit vinegar, sugar, salt).

The process is summarised as:

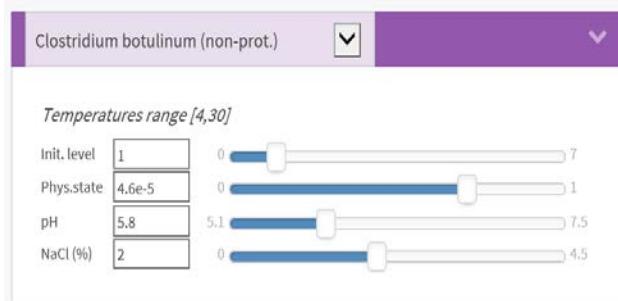


The shelf life starts when the product is  $\geq 3.0^{\circ}\text{C}$ . The desired shelf life is Production+12 days at 5-8°C (storage will be 2 days at up to 5°C on site and in retail depots, after which it could be purchased by a consumer and stored in a fridge at up to 8°C).

The product's parameters are given below against the ACMSF's requirements to prevent *C. botulinum* growth and neurotoxin formation in a chilled food:

ACMSF requirement	Process/product parameters	Requirement met?
A heat treatment of 90°C for 10 minutes or equivalent	Cold mixed	✗
A pH of 5.0 or less throughout the food	5.6 - 5.8	✗
A water activity ( $A_w$ ) of 0.97 or less throughout the food	0.98	✗
An aqueous salt concentration of 3.5% throughout the food	2.0 - 2.2	✗
A combination of heat and preservative factors which can be shown consistently to prevent growth and toxin production by non-proteolytic <i>C. botulinum</i>	?	?

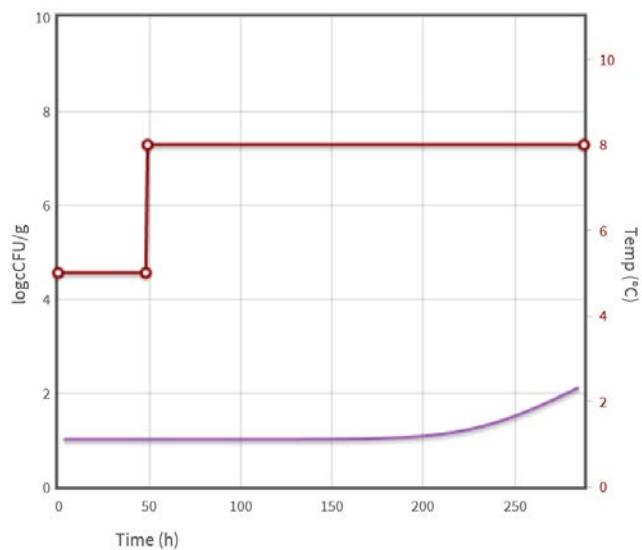
The effect of the combination of preservative factors is unknown, therefore the company seeks expert advice, and obtains a prediction of growth of *C. botulinum* in the product, using the product's “worst case” parameters (lowest salt, highest pH, assume that a customer buys the product straight away and stores it at 8°C):



The prediction shows that growth starts to occur around 200 hours (8 days). The product does not prevent growth of *C. botulinum*. Therefore, unless a challenge test were performed to demonstrate that neurotoxin is not produced, could only be given a shelf life of up to 10 days in accordance with the FSA guidance.

As the product is cold mixed however, the shelf life of the ingredients should also be considered. All of the ingredients inhibit growth/toxin formation, apart from the poached salmon and the cream.

Therefore the shelf life of these ingredients before and after addition to the product must not exceed 10 days (e.g. poached salmon must be used at 2 days old if the final product has 8 days shelf life).



### New Product Development options

The following options are trialled:

- Heating the product in its final packaging to a temperature of 90°C/10 minutes
- Increasing the salt to 3.5%
- Reducing the pH to 5.0 using lemon juice

The first two options result in poor organoleptic quality, but reducing the pH still gives a good quality product. To ensure that the pH never exceeds 5.0, a target of 4.8 is set.

### Providing due diligence

pH becomes a CCP for control of *C. botulinum*. The company routinely monitors and records this on every batch on a number of pots to confirm correct ingredient control and thorough mixing. pH is also checked before launch on several batches, throughout shelf life and periodically thereafter at the end of shelf life, to ensure it does not change with time. pH is monitored using a calibrated probe. Because neurotoxin formation by *C. botulinum* is arrested in the product, theoretically the poached salmon and cream ingredients could be used up to 10 days after preparation, this being extended in the final product owing to appropriate acidification and reduction of pH to become a controlling factor for non-proteolytic *C. botulinum*\*.

\*In this example *Listeria monocytogenes* (and potentially other pathogens, e.g. *Bacillus cereus*) is another potential hazard, and growth in the finished product would be predicted, therefore would need to be considered for ingredient and finished product shelf life.

**Appendix 2**

**Drafting Group Membership**

Mr Kim Matthews	Agriculture & Horticulture Development Board
Dr Andrew Millman	ASDA Stores Ltd
Mr Mark Young	Centre of Innovation Excellence in Livestock
Ms Kaarin Goodburn MBE	Chilled Food Association
Miss Louise Grinyer	Leatherhead Food Research
Dr Ian Jenson	Meat & Livestock Australia
Prof Mike Peck	Quadram Institute Bioscience
Mr Steve Batchford	Sainsbury's Supermarkets Ltd
Dr Sam Kirk	Tesco Stores Ltd
Mrs Karen Sims	Waitrose Ltd
Dr Nicola Wilson	Westward Laboratories (Samworth Brothers)

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