Ministry for Primary Industries
Manatū Ahu Matua

Guidance Document

Further Processing

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Title

Guidance Document: Further Processing

About this document

This Section deals with high pressure processing. It provides guidance to assist operators (you) to comply with the Animal Products Act 1999 (APA), as you develop and validate your RMPs for high pressure processing, and for routine processing.

Related Requirements

- Animal Products Regulations 2000 [AP regs]
- Animal Products (Risk Management Programme Registration—Required Part) Regulations 2020
- Animal Products (Risk Management Programme Specifications) Notice 2008 [RMP Spec]
- Animal Products Notice: Risk Management Programme Specifications Amendment and Requirements for Risk Management Programme Outlines Revocation 2020
- Animal Products Notice: Specifications for Products Intended for Human Consumption [HC Spec]
- Australia New Zealand Food Standards Code (FSC)

Document history

Version	Version Date	Section Changed	Change(s) Description
1	August 2011		
2	May 2020	Entire Part	Content updated, reformatted and rebranded.

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1 Purpose

1.1 Purpose

High pressure processing (HPP), also known as high hydrostatic pressure (HHP) or ultra-high pressure processing (UHP), is the process of applying pressures, usually between 100 and 600 MPa to packaged or unpackaged liquid or solid foods, to achieve a particular technological effect, make them safe and/or to extend shelf life. It is a non-thermal technique with a typical pressurisation time of a few seconds to over 20 minutes.

Increasingly, processors are looking to use HPP as an alternative to thermal pasteurisation. However in many cases, there is limited or no published information that describes validated parameters for HPP, such as is available for thermal processing. As a result processors often need to carry out their own product specific validation to demonstrate that it will be safe both immediately after processing and for its shelf life.

As bacterial spores are resistant to HPP applied at ambient temperatures, products produced using HPP are often high acid and are stored chilled.

Although there is evidence to support the ability of HPP applied together with high heat (known as Pressure Assisted Thermal Sterilisation (PATS) or HPP with heat), to achieve significant reductions in spore formers, this requires the HPP unit to be equipped to control the temperature during processing. Units capable of achieving this have limited commercially availability, and so this technique is not addressed in this guidance.

This Section focuses on HPP where it is applied to eliminate or reduce micro-organisms to acceptable levels to:

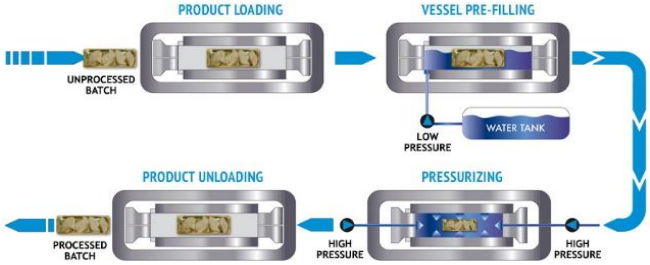
- extend shelf life; and/or
- to replace traditional pasteurisation processes to make foods safe.

When developing and validating a HPP, you should also consider other risk factors such as chemical and physical hazards.

Additional Information - HPP Units

Generally, products are HPP treated in their final commercial package. HPP system consist of a pressure vessel, pressure-generating device (high pressure pump) and loading and unloading conveyors. The process initiates with loading product into HPP carrier baskets. The baskets are then inserted into the pressure vessel. The vessel is sealed and filled with pressure transmitting fluid (usually water or oil) at low pressure. A high pressure pump compresses the fluid inside the vessel until the target pressure is reached. This pressure is held for a period of time. Finally, depressurisation occurs and the product is unloaded.

Figure 1: Diagram of operation of a HPP unit (http://www.hiperbaric.com/en/high-pressure)



1.2 Layout of section

This Section has been written to align with the activities carried out as you develop, validate and operate a HP process. Some or all of this Section may be relevant, depending on your product, process and equipment.

1.3 Definitions

In this Guidance Document, unless the context otherwise requires:

critical control point (CCP) means a step at which control can be applied and is essential to prevent or eliminate a food safety hazard or reduce it to an acceptable level

headspace means the air or empty space left above the food contents in a sealed package

operator-defined limit means a measurable limit established by the operator to manage the fitness for intended purpose of a product and is not defined in legislation

HPP or HP process means high pressure process

pasteurisation means any process, treatment or combination thereof, applied to product to reduce the most resistant microorganism(s) of public health significance to a level that is not likely to present a public health risk under normal conditions of distribution and storage (NACMCF, 2006)

pathogen means an organism such as bacteria (e.g. *Salmonella*), viruses (e.g. norovirus, hepatitis A virus), or parasites (e.g. Giardia, Cryptosporidium) that may causes disease in people

regulatory limit means a measurable regulatory requirement that is critical to the fitness for intended purpose of a particular product

shelf life means the period of time, established under intended conditions of distribution, storage, retail and use, that the product would remain fit for its intended purpose

spoilage organisms means microorganisms which cause deterioration of a product and limit their shelf life by producing objectionable flavours, odours, and slime

s**ub-lethally injured cells** means microorganism that are stressed or injured by HPP, which given favourable environmental conditions and time can become functionally normal again

suitably skilled person means a person, who in the opinion of the operator, is skilled in a particular activity or task through training, experience, or qualifications

surrogate organisms are non-pathogenic species and strains responding to HPP treatment in a manner equivalent to a pathogenic species and strain

validation means a process by which evidence is obtained to demonstrate the process operating at defined parameters, is consistently capable of producing animal material or products that meet the requirements to be fit for purpose

water activity (a_w) means a measure of the water available for microbial growth. It is the ratio of the water vapour pressure of the food (p) to that of pure water (p_o) at the same temperature: $a_w = p/p_o$

1.4 High Pressure Processing Procedures

1.4.1 General requirements

(1) When operating a HPP, you must:

a) document and meet any regulatory limits applicable to the product [RMP Spec 7 & 11];
b) establish, document and meet operator-defined limits that are appropriate for the product and have evidence to justify their selection [RMP Spec 7 & 11]; and
c) demonstrate that the process is capable of consistently achieving the regulatory and/or operator-defined limits [RMP Spec 18].

Additional Information – Operator-defined limits

Operator-defined limits are measurable limits that are established by you and are not defined in legislation. They may be sourced from reputable codes of practice or guidance documents, peer-reviewed scientific information, predictive models, scientific information from people or organisations known to be competent, other regulatory authorities or developed from trials and experiments. Examples of operator-defined limits include:

- microbiological limits for products not currently addressed in the FSC;
- a specified $\log_{10}$ reduction of a target pathogen; or
- HP processing parameters.

In setting operator-defined limits, consider the product's intended use, intended consumer, and the handling it is likely to receive during its shelf life.

(2) Processes should be developed and validated[1] by suitably skilled persons.

Additional Information- Suitably skilled persons

Suitably skilled people who develop and validate HP processes should have a good working knowledge of factors critical to the process. It is recommended that they have knowledge of:

a) the capabilities and limitations of HP processing and the unit;
b) product and packaging characteristics;
c) pathogens of concern and their response to pressure;
d) validation techniques for HPP (e.g. challenge trials and shelf life studies);
e) analysis of validation data; and
f) dealing with process deviations.

You are responsible for ensuring that people with the appropriate knowledge and skills are used to develop and validate your RMP.

If you are looking for consultants to assist with this work, a good starting point is the NZ Food Innovation Network.

(3) People carrying out key tasks must be identified, and any required competencies specified, in the RMP [RMP Spec 15];

(4) Training records must be kept [HC Spec 9.3 and RMP Spec 15].

[1] For more general information about how to validate a process, refer to the Risk Management Programme Manual.

Additional information

If a processing step is a CCP, training expectations are heightened. You need to ensure that adequate training is provided. The training should cover the operation, control and monitoring of that step.

(5) A report of the validation work must be documented by the suitably skilled person [RMP Spec 18].

(6) The validation report must be kept:

a) while the product is in production; and
b) for an additional four years or the shelf life of the last product produced, whichever is longer, if its production ceases.

(7) If the validation report is replaced, the obsolete report must be kept for 4 years [RMP Spec 18].

Additional information

The validation report should include the documentation recommended in section 5.2.5 of the Risk Management Programme Manual.

(8) A suitably skilled person should review the process or product whenever there is a change that could impact on food safety and revalidate where necessary.

(9) Calibrated equipment with sufficient accuracy should be used during any validation work and routine processing, and calibration records kept [HC Spec Part 6].

(10) All equipment used for critical measurements must meet HC Spec 6.2.

1.5 Equipment

(1) HPP units must be fitted with a control system that is capable of generating permanent process records that enable you to identify process deviations.

Additional Information
The following table is an example of the transducers recommended by the Food and Drug Administration (2000), their precision, accuracy, and appropriate location in the vessel.

Table 1: Recommended transducers with accuracy/precision and appropriate location in pressure vessel

Measuring Transducer	Accuracy/Precision	Location in Pressure Vessel
Pressure Gauge (Electronic)	+/- 1/2% / 3.4 MPa	Anywhere in HPP system
Pressure Gauge (Dial Display)	+/- 1% / 6.8 MPa	Anywhere in HPP system
Time (Recorder)	+/- 1% / one second	(not applicable)

(2) Equipment should be installed and commissioned by suitably skilled people.

1.6 Raw Materials

(1) Raw materials must comply with the requirements of the FSC.

(2) Raw materials must be protected from contamination, handled hygienically and stored in a manner that will minimise deterioration [AP Reg 9].

(3) When developing new products or modifying existing formulations, consider the microbiological loading of the raw material.

(4) You should have procedures to ensure that raw materials are not changed in a formulation (including supplier, type, or addition rate) without input from a suitably skilled person.

Additional Information

Information about the microbiological quality of the raw materials may be obtained from:

- certificates of analysis from suppliers:
- the National Microbiological Database (NMD) for some meat types;
- testing the materials; or
- knowledge about any previous processing steps applied to the material.

The capability of the process, intended use of the product, any further processing and/or preparation prior to consumption should be considered when determining the appropriate microbiological criteria for raw materials.

1.7 Packaging and Labelling

(1) Packaging must be:

a) used in accordance with the manufacturer's specifications;
b) appropriate to the intended use, and filling and sealing equipment used [HC Spec 7.2(1)]; and
c) clean and sound before filling [HC Spec 7.2(2)].

(2) Packaging must be stored in a way that prevents contamination. It should be wrapped and stored off the floor until used in production [AP Reg 16 and HC Spec 7.2(2)].

(3) The barrier properties of the packaging such as the permeability (water, oxygen, or carbon dioxide) should not change due to HPP.

(4) Where necessary to ensure the safety of the product, procedures must be implemented to check the packaging seal or closure integrity.

(5) Materials used for sealing product must be controlled to ensure that they are not a source of physical contamination to the product [AP Reg 6].

(6) Product must be labelled in accordance with the requirements of the FSC, including any “Directions for Storage and Use”.

Additional Information

As the volume of the product reduces during HPP, the packaging needs to have flexibility. The compression and re-expansion means that the packaging should be capable of withstanding up to a 15% reduction in volume without losing seal integrity or barrier properties (USFDA, 2000).

The head space should be kept to a minimum to ensure that pressure is well transferred to the product, to help minimise deformation of packaging and to make the most of the space inside the pressure vessel.

Packaging should be obtained from reputable suppliers who can provide technical support, including specifications for package sealing parameters. The most common packaging materials are polypropylene (PP), polyester tubes, polyethylene (PE) pouches, and nylon cast polypropylene pouches.

Packaging seal or closure integrity checks may include visual or physical testing such checking that complete seals are being formed, no cracking, wrinkling, delamination, or blisters. Seal quality check should be carried out after HPP on randomly selected production packs (not on empty packs).

1.8 Outcome of High Pressure Processing

Additional Information

Example validation templates are in Appendix 1.

(1) HPP applied for the purpose of food safety must ensure the elimination or reduction to acceptable levels of the pathogens of concern, identified by applying the principles of HACCP. The pathogens may include:

a) bacteria (e.g. *Salmonella* spp., *Escherichia coli* O157:H7, *Campylobacter* spp., *Listeria monocytogenes*, *Staphylococcus aureus*, *Vibrio parahaemolyticus*, *Clostridium* spp, *Bacillus cereus*, and *Cronobacter* spp.);
b) viruses (e.g. Norovirus, Hepatitis A); and
c) parasites (e.g. *Toxoplasma gondii*, *Trichinella spiralis, Anisakis simplex)*.

(2) The process should be sufficient (either alone or in combination with other control measures) to ensure the product is fit for its intended purpose, method of storage, and shelf life.

Additional Information
The application of HACCP principles will identify those pathogens that are reasonably likely to occur and that need to be controlled by your HPP. The MPI Hazard database and model HACCP plans can be used to assist with hazard identification and analysis.

As an example, Table 2 identifies the pathogens of concern and the level of inactivation that is expected when developing and validating a HPP that is to be applied to raw milk, instead of using thermal pasteurisation.

Table 2: Target pathogens and level of inactivation when applying HPP to raw milk

Pathogen	Log_{10} reduction for product to be sold in NZ	Log_{10} reduction to achieve a standard similar to thermal pasteurisation (export product must meet at least this standard)
Campylobacter spp.	5	>7
Listeria monocytogenes	5	>7
STEC	5	>7
Salmonella spp.	5	>7
Staphylococcus aureus	5	>7
Mycobacterium avium sub spp *paratuberculosis (MAP)*[a]	6[b]	Estimate >7, may need data on *M. bovis*
Bacillus cereus	No growth	No data available[c]

[a]The inclusion of MAP is as a surrogate for *M.bovis*. The requirement to include the elimination of *M. bovis* is based on the current NZ TB status.
[b]An additional 1 log_{10} reduction has been applied to allow for the possibility that *M. bovis* inactivation under HPP may differ to MAP.
[c]Comparison cannot be made as pasteurisation does not inactivate spores.

1.9 Development and Validation of the High Pressure Process

(1) When determining appropriate HHP parameters to apply to your product (such as holding times and target pressure), you should consider the:

a) purpose of applying the HPP process (e.g. shelf life extension, pasteurisation);
b) pathogens of concern as identified during the application of HACCP principles and their initial concentration in the raw materials;
c) presence of pressure resistant strains of any pathogens of concern;
d) spoilage organisms and their initial concentrations;
e) food matrix;
f) other control measures (pH, a_w, antimicrobial agents etc);
g) packaging size;
h) packaging material and its mechanical and thermal properties (strength and barrier properties);
i) potential microbiological growth before HPP, including during any product hold steps;
j) regulatory and/or operator-defined limits;
k) level of pathogen or spoilage organism reduction to be achieved by the process; and
l) intended purpose and consumer of the product, storage conditions and shelf life.

Additional Information - Source of HPP parameters

HPP parameters maybe derived from:

- scientific publications or other reference material, where the conditions directly match those being applied to the product; and
- trials and experimentation.

(2) During the validation work, the suitably skilled person should address all product, process and validation parameters (Refer Appendix 1 for more details).

Additional Information Validation Considerations

To assist with understanding the factors that are critical to HPP, and to be considered when developing and validating a HP process, New Zealand Food Safety commissioned the report "Review of the High Pressure Processes applied as an alternative to thermal process pasteurisation". The following information is taken from that report.

HPP for shelf life extension
Shelf life extension trials aim to demonstrate that HPP is able to eliminate or reduce any spoilage microorganisms present in the food to acceptable levels and thereby extend the shelf life of the food. HP treated product is compared with untreated product to identify if it delays the growth of spoilage microorganisms during the proposed shelf life.

HPP for food safety
HPP is capable of eliminating or reducing foodborne pathogens to acceptable levels for the shelf life of the product. However, the effectiveness of a HP process depends on a number of intrinsic factors (growth phase, cell membrane, food composition) and extrinsic factors (pH, a_w).

The HP process should be designed and validated to ensure the reduction to acceptable levels of the most pressure resistant strains of the pathogen(s) likely to occur in the food. If spore forming microorganisms are likely to occur in the product, appropriate control measures will need to be in place.

Inactivation of pathogens
Different bacterial strains of the same species can be reduced by significantly different amounts when subject to the same HP process. Also, often those pathogens that are susceptible to heat are not necessarily more readily inactivated by pressure. In most cases, trials, and experimentation will be needed.

Use of high pressure (>550MPa) with elevated pre-pressure product temperature (>50^{0}C) for greater than 5minutes has achieved 5 $\log_{10}$ reduction of most non-spore forming foodborne bacterial pathogens in NZ.

Product composition/formulation/
The composition of the food matrix can affect the level of microbial inactivation by HPP. Parameters such as pH, a_w, and fat content need to be considered. In general, HPP shows efficient microbial inactivation when a_w of the product is more than 0.87. Microorganisms in product with lower pH value (acidic) are more sensitive to HPP. Due to the significant influence that composition and formulation has on the inactivation that can be achieved by HPP, it is important that the validation work is carried out using the product as formulated for commercial production.

Product packaging and size
As packaging may affect the temperature and pressure of the product during HPP, wherever possible, the packaging should be the same material as used in the commercial production. If this is not possible, consider whether this impacts on the inactivation achieved the process. For heterogeneous food products, complete packaged food that will be produced in commercial HPP need to be tested.

If the formulation or packaging changes, trials would most likely need to be repeated, so it is important that the validation work is carried out on product that is as close to commercial production as possible.

Challenge trials
This may include microbial challenge trials, where a cocktail of strains of the target pathogen(s) is inoculated into the product and then processed to determine whether the required pathogen reduction is achieved. Cells in the stationary phase are more pressure resistant than actively growing cells. However, it is not possible for processors to know the physiological state of pathogens before a HPP. Therefore when carrying out trials, cells should be in a stationary growth phase.

Surrogate organisms
Challenge trials involving microbial pathogens should not be carried out in the commercial processing environment. Surrogates can be useful as they allow validation of a HP treatment without introducing pathogens into a processing area. The choice of surrogate used for HPP validation needs to be justified. Surrogate microorganisms need to have comparable pressure resistance to the pathogens they are representing, in an equivalent food matrix. Validating a surrogate is not a simple task and currently there are limited validated surrogates that are recommended for use for HPP validation. The ESR report includes a review of HPP surrogates.

Sub-lethally injured cells
HPP can result in sub-lethally injured cells that are able to recover and grow during storage. Because of this, it is important that trial samples are stored for longer than the intended shelf life to confirm (or otherwise) that the process has fully inactivated the target micro-organisms. When validating a HP process, the microbiological method should be able to detect stressed or injured (but repairable) cells.

Holding time and pressure
Length of holding time is not necessarily proportional to microbial inactivation. Therefore, inactivation data from validation studies should not be extrapolated between or beyond the holding times tested.
Cyclic pressurisation or pulsed HPP can also achieve greater inactivation of pathogens. This treatment involves series of pressure cycles with full decompression between cycles.

Initial temperature
The temperatures used during the validation work should cover the range likely to be encountered during commercial processing. Unlike thermal processing, the least inactivation is not necessarily delivered using the lowest initial temperature. The initial product temperature should homogeneous with no cold spots.

(3) The validation needs to be comprehensive and include a number of repetitions to ensure confidence in the results.

(4) Trials carried out to validate a HPP should be repeated if a change is made to the ingredients supply, formulation, pre-HPP steps, packaging or HP process that could impact on food safety.

(5) Process and product parameters that are critical to food safety and shelf life should be identified in the RMP and managed, including identifying those that would require revalidation if changed.

1.10 Pressure transmitting fluid

(1) You must ensure that water (pressure transmitting fluid) that comes into direct contact or indirect contact with the product is suitable water [HC Spec 2.5].

Additional Information - Pressure-transmitting fluid
Pressure-transmitting fluid is used to transmit pressure to pre-packaged product. Water is the most commonly used pressure-transmitting fluid in commercial equipment.

As the products do not come into direct contact with the pressure transmitting fluid (water), it can be recycled. Most of spoilage and pathogenic microorganisms (if any) in the fluid are eliminated when it is recycled at high pressure (600MPa). However, pressure resistant microorganisms can potentially remain and build up in water, which may then contaminate the product or attach to the product packaging and contaminate the product when opened. To avoid this, water is changed frequently (minimum once week is recommended for most of HP processes).[2]

1.11 Routine processing

(1) The process must be operated in accordance with the established parameters documented in the RMP [RMP Spec 11].

Additional Information
All validated critical factors and process parameters should be transferred into the RMP and be readily available to staff.

(2) HPP should commence promptly after product preparation. Any delays or holding between product preparation and treatment must be in accordance with the RMP [RMP Spec 11].

Additional Information
The maximum time from product preparation to HPP should be specified in the RMP, taking into account conditions that may permit microbial growth and toxin production. Ideally, the time should not exceed 2 hours.

(3) Handle raw materials and products to avoid any contamination that the process is not designed to eliminate [AP Reg 9].

[2] Arranz I., Hiperbaric (2020), personal communication

(4) If pre-programmed controls are used to operate the HPP unit, unauthorised access to the programmed parameters must be prevented [HC Spec 22.3].

(5) If product could mistakenly bypass the HP process, procedures and/or systems must be put in place to prevent this.

(6) The process must be monitored and results recorded to demonstrate that the validated parameters are met for every batch [HC Spec Part 9, RMP Spec 20].

(7) Permanent records must be kept for each product batch for 4 years or the shelf life of the product - whichever is longer [HC Spec 9.3 (2)(b), RMP Spec 20].

(8) Records must allow traceability of raw materials and final products from the supplier to the next person in the supply chain.

Additional Information

Process records may include:

- processing date and time;
- product name, code and/or other identification;
- formulation;
- pack size;
- product parameters (pH, a_w, product composition etc.);
- approximate number of containers in the load;
- initial product temperature;
- pressure transmitting fluid (water) initial temperature;
- compression time;
- target pressure;
- holding time;
- decompression time;
- results of seal checks;
- results of final product testing; and
- water suitability checks.

(9) Any parameters that had been applied when validating the process but are not routinely monitored during processing should be periodically verified to ensure that the process continues to operate within those parameters [RMP Spec 16].

(10) Implement operator verification procedures, including ensuring that regulatory and operator-defined limits are met [RMP Spec 16].

(11) Verification of compliance with the microbiological limits in FSC Part 1.6 Standard 1.6.1 is required.

Additional Information

It is good practice to verify the records from each batch prior to product release. Routine microbiological testing of all product batches is not required, but it is recommended that batches are tested periodically as part of operator verification.

1.12 Post-process Handling

(1) Product must be handled in a manner that prevents recontamination and deterioration [AP Reg 9].

1.13 Deviation from the Validated Process

(1) Take immediate action if there is a process deviation that could impact on food safety or suitability, including if any regulatory or operator-defined limit is not met [RMP Spec 8].

(2) Any product lot(s) affected by a deviation that could impact on food safety should be identified, segregated, and detained pending the outcome of an assessment by a suitably skilled person.

(3) A record of the assessment and corrective actions taken must be prepared by the suitably skilled person. The record should be appropriate to the nature of the deviation and include:

a) date and time of deviation;
b) description of the deviation, including the processing records;
c) description of affected product, including batch identifiers and quantity;
d) corrective action taken, including restoration of control, product disposition and prevention of recurrence;
e) records of any tests undertaken; and
f) the name and signature of the suitably skilled person who carried out the assessment [RMP Spec 20].

Additional Information

If a corrective action such as extending the pressurised holding time is applied, you would need to have evidence that the new process is capable of delivering a safe product.

1.14 Shelf life

(1) You must have evidence to support the shelf life of your products[3].

Additional Information – Chilled storage temperatures

When selecting storage temperatures at which to carry out shelf life trials, the maximum chilled storage temperature at retail required under the Food Act 2015 is 5°C.

[3] Refer to the MPI Guidance document "How to Determine the Shelf Life of Food" for further guidance.

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Appendix 1: Microbiological Validation Templates:

1A: Shelf Life Extension

1B: Food Safety

1C: Guidance for laboratories carrying out validation trials

1 Background

The following templates have been developed to assist you to validate a HP processes. The templates are generic and can be applied to all product types. They will be amended over time as more information is made available. Also provided is a basic guide for laboratories carrying out the microbiological aspects of the validation work.

1.1 What is validation?

Validation is the work carried out to demonstrate that your HP process will produce safe and suitable product. RMP Spec 18 provides the legal underpinning for validation. Validation should be carried out to confirm that HPP parameters such as the target pressure and holding time are effective at reducing the microorganisms of concern to an acceptable level when applied to your product. Validation may be carried out to demonstrate that the following parameters, will produce safe and suitable product:

- parameters that you have previously validated on very similar products;
- parameters from reference material, reputable scientific literature, codes of practice, or international standards and that are directly applicable to your product;
- parameters from equipment suppliers and/or others with expertise in HPP that are directly applicable to your product; and
- parameters that you are developing for products or applications that have not previously been validated.

Challenge trials are likely to be needed if the HPP step is being applied to inactivate pathogens, for example to replace a thermal pasteurisation process, and the parameters have not previously been validated as effective for your particular product and process.

1.2 Who should use this template?

This template should be used if you are validating a HP process that is being applied for the purpose of:

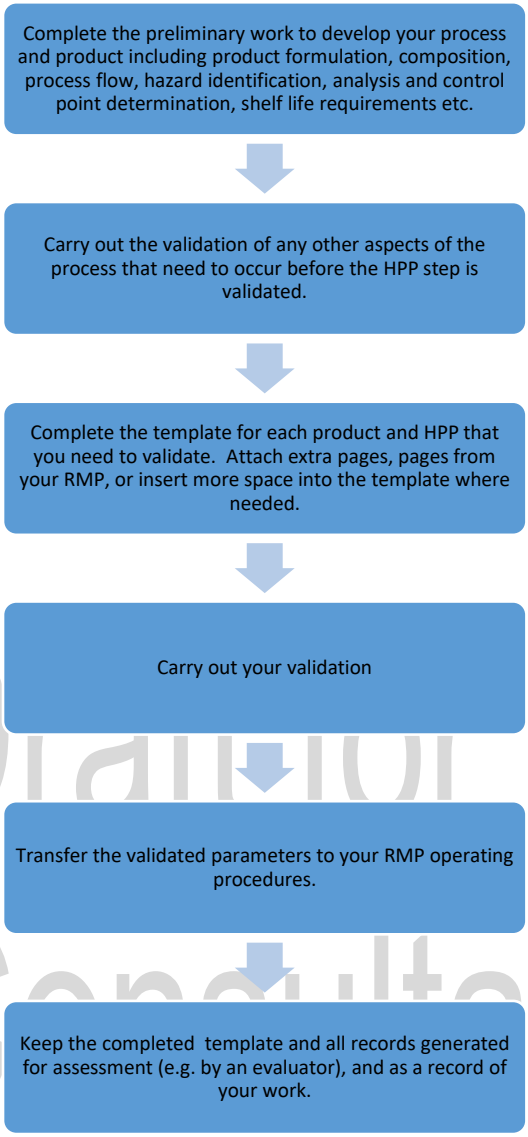
- **shelf life extension**, i.e. ensuring that the product remains microbiologically suitable for the duration of its shelf; and/or
- **food safety,** i.e. reducing the vegetative pathogenic microorganisms of concern by a known amount and maintaining its safety for the duration of its shelf life.

This template does not need to be used if the HPP step is:

- not intended to preserve the product; or
- being applied for a technological or physical effect only and the HPP is, such as the shucking of shellfish or the removal of meat from crustaceans.

1.3 How to use the template

Figure 1 describes the steps to be carried out when validating your process using this template:



Often your product will be subject to processing steps (hurdles) prior to the HPP and you will need to have evidence that these other steps are effective. However, validation of these steps is not covered by this template. For example, if applying a HPP to retail ready sliced ham or to a pasta salad, validation of the ham or pasta manufacture process is not covered. You can expand the template to include all aspects of the validation so that you have all the information in one place.

Your process flow diagram, and HACCP plan should include all process steps used to process the product, including steps prior to the HPP. Control points and limits for the pre-HPP steps should be provided. This will then inform about the hazards that have been identified and where and how they will be controlled. It will clarify the hazards that remain to be addressed by the HPP and for which validation information will be needed.

Remember:

- Plan thoroughly and get technical help if needed.
- Keep good records so that the process can be reproduced by someone with no prior knowledge

- If data is not recorded, the impact of changing a parameter on food safety or shelf life may not be known and the value of the work will be lost.

1.4 Records

(1) During the validation work, the records to be kept should include:

a) name of the person who developed the process;
b) date of development;
c) product name, formulation, composition and product parameters (pH, a_w, brix, composition);
d) product preparation, packaging and closure;
e) microbiological hazards and spoilage organisms associated with the raw materials and their loadings;
f) log reduction of target pathogen(s) and/or spoilage organism ;
g) product intended use, storage conditions and shelf life.
h) trial conditions used when validating the process, including the data acquisition system; and
i) critical process parameters such as:

i) initial product temperature;
ii) initial temperature of pressure transmitting fluid;
iii) compression profile;
iv) target pressure ;
v) time at target pressure (holding time);
vi) in the case of pulsed processes: number of pulses, pause time-interval between pulses, pause pressure, product temperature and holding time for each pulse;
vii) decompression profile;
viii) final product temperature; and
ix) final temperature of pressure transmitting fluid.

Template 1A: Shelf life Extension

This template should be used if validation is to demonstrate that the HPP will extend the shelf life of a product beyond that which would be achieved if the product was not HPP treated. These trials will provide justification for setting the product shelf life in relation to microbiological spoilage of the product. This template does not address loss of shelf life due to other quality issues or deterioration due to chemical factors.

Shelf life studies should not be undertaken without a review of potential pathogens. A process to extend shelf life may give rise to hazards as a consequence of pathogens that survive the process. See the MPI Guide: How to Determine the Shelf life of Food, for further detail.

The most common way to perform a trial to demonstrate shelf life extension using HPP is to compare microbiological results from products that have been HPP treated against untreated controls. Untreated samples are tested at the start of the validation. Treated samples are tested immediately after the HPP step and the remaining product is held under controlled storage conditions and tested at various times up to and beyond the proposed shelf life.

During trials:

- product should be stored and microbiological testing should be continued beyond expected shelf life;
- the variability between ingredient batches, product batches, processing days, staff, storage and transport conditions should be taken into account by testing a number of batches; and
- consider the product's intended use, intended consumer, and whether the food will be consumed in one sitting or over a period of time when deciding on the storage conditions to be tested.

The areas in white in the template need to be filled in.

A: Product information

Product details	Name or other identifier:	Weight/volume:
Microbiological regulatory or operator-defined limits to be met in the product.	Microorganism	Limit
Any microbiological limits to be met prior to the HPP step *This may be in individual ingredients or the formulated product just prior to the HPP step.*	Microorganism	Limit
Product formulation *List ingredients, amounts added and form (e.g. dried, fresh etc).*	*Write the complete recipe here or attach from your RMP:*	
Product characteristics *If these characteristics don't apply to your product, delete and replace with more appropriate characteristics. You can also add more rows.*	pH:	a_w:
	Salt/sugar content:	Protein content:
	Fat content:	Other *(list)*:
	Preservative(s):	
Product packaging to be used	Type and form of packaging:	Is the packaging appropriate for use in the HPP and capable of forming a hermetic seal?

Remember, you will need evidence of compliance with HC spec Part 7.	Confirmed compliance with HC Spec? Yes [ ]	Yes [ ] No [ ] Can the material withstand the loading and unloading into/from the unit? Yes [ ] No [ ]
	Is the same packaging to be used during the trials? Yes [ ] No [ ] Is same pack size to be used during the trial? Yes [ ] No [ ] If no, you will need to provide evidence that the results will be applicable when using the commercial packaging.	What is the gas composition inside the product packaging/headspace e.g. vacuum, composition of modified atmosphere: Is the same gas composition to be applied to the trial samples: Yes [ ] No [ ] If no, you will need to provide evidence that the results will be applicable when using the commercial packaging.
Intended shelf life and would product be used in one sitting or consumed over time?	Months/Weeks/Days: Single use/multiple use:	Storage conditions:

B: Process information

Full process details *Include preparation, filling, closing, holding, HPP, cooling, storage etc.*	Attach details, including all processing parameters and limits. This may be provided as a process flow.	
HACCP plan or equivalent RMP documentation	Attach for the entire process.	
Proposed HPP test parameters *There may be a number of process variations to be tested.* *If these parameters don't apply to your product, delete and replace with more parameters. Add more rows if needed.*	Initial product temperature before the HPP step:	Holding time(s) at target pressure (state mins/sec etc, also if pulses applied):
	Target pressure (give units e.g. MPa, bar):	Compression and decompression profile (e.g. pressure increase/decrease vs time):
	Pressure transmitting fluid (e.g. water) initial temperature:	Other (e.g. number of cycles):
Source of proposed HPP parameters *This may be peer reviewed research, a person with expertise in HPP, the equipment supplier etc. If possible, provide an electronic link to the source.*	State how the proposed HPP parameters have been determined, or the source from which they have been taken:	

C: Trial Information

Date(s) of validation work		
Suitably skilled person(s) involved in the validation trials *You may have one or more.*	Name: Evidence of competency:	Name: Evidence of competency:
How is trial product to be disposed of?	Product disposition:	Confirmation that product disposed of as stated:

<table>
<tr><td>Name and contact details of laboratory carrying out testing.

Some testing must be carried out by a laboratory with accreditation to ISO 17025, for example if testing for Listeria monocytogenes in animal products. Check if this is a requirement for your product. Check that the laboratory has the competency and capability to conduct your validation trials – this can be a complex area.</td><td colspan="2"></td></tr>
<tr><td>Compatibility of food matrix and test methods.</td><td colspan="2">Are the laboratory test methods appropriate for food matrix and pathogen?
Yes [ ] No [ ]</td></tr>
<tr><td rowspan="4">Enumeration method(s) for target microorganisms
Ensure these methods are capable of detecting sub-lethally injured cells This information should be provided by your laboratory. It is recommended that you obtain this information from the laboratory and attached it to this template.</td><td>Microorganism</td><td>Enumeration method</td></tr>
<tr><td></td><td></td></tr>
<tr><td></td><td></td></tr>
<tr><td></td><td></td></tr>
<tr><td>Details of the HPP equipment used for the trials</td><td>Premises where HPP is located and equipment details (e.g. make, model, size):</td><td>Evidence viewed that instrumentation calibrated:
Yes [ ]</td></tr>
<tr><td>Is this the same HPP unit that will be used for commercial production?</td><td colspan="2">Yes [ ] No [ ]

If no, you will need to provide evidence that the results will be applicable when used on the commercial unit. If the results cannot be applied to the commercial unit, these trials will need to be repeated).</td></tr>
<tr><td rowspan="5">Spoilage microorganisms of concern and limits to be met
Confirming that these limits will be met is the purpose of this validation.</td><td>Microorganism</td><td>Limit</td></tr>
<tr><td></td><td></td></tr>
<tr><td></td><td></td></tr>
<tr><td></td><td></td></tr>
<tr><td></td><td></td></tr>
<tr><td>Processing conditions.</td><td colspan="2">As provided in Section B. Product should be processed under worst case conditions.</td></tr>
<tr><td>Sample storage conditions during the trial.
Examples:
Optimum e.g. storage as on the label for entire shelf life;
Realistic e.g. repeated short periods of elevated temperatures;
Worst case e.g. poor temperature control in a domestic refrigerator or variable temperature control.</td><td>Set of storage conditions (1):</td><td>Set of storage conditions 2 (add more rows if more sets of conditions are used):</td></tr>
<tr><td>Number of batches to be tested (N_B).
It is recommended that more than one batch is tested so that batch to batch variability can be detected.</td><td colspan="2">Number:</td></tr>
</table>

Total number of samples needed for the shelf life trials from each test batch. *Sufficient samples should be stored so that testing can be carried out beyond the stated shelf life, to ensure that any surviving cells that may grow during the shelf life are detected.* *A full set of samples will be needed for each storage condition tested. Determine the number needed from your sampling programme plus a few spares.*	If your sampling programme requires you to test non-HPP treated controls throughout the shelf life, you will also need to store a sufficient number of these as identified in your sampling programme. Number of samples: Batch 1: Controls (non-HPP treated) [ ] HPP treated [ ] Batch 2: Controls (non-HPP treated) [ ] HPP treated [ ] Batch 3: Controls (non-HPP treated) [ ] HPP treated [ ]

Sampling programme for shelf life trial:

An example sampling programme is provided here. This should be deleted and replaced with your own programme or attach your sampling programme to this template.

The information includes the dates of testing and number of samples tested at each sampling period.

Studies should be repeated on at least three batches to identify variability within and between batches. Ideally these will be on different dates with different ingredients batches etc. A sampling programme should be prepared for each batch tested.

Further trials will be needed if the shelf life is not met, or the shelf life must be reduced.

At each testing period it is recommended that 3-5 replicate samples are tested.

Batch ID: X Batch number: 1 Date of Manufacture: 2.2.20

Sample description	*Sample date (N_p)*	*Sampling points*	*Number of samples tested (N_R)*	*Testing required (e.g. yeasts, moulds, bacteria, pH etc):*
*Green juice, **untreated control***	*2.2.20*	*Take sample immediately before HPP step*	*5*	*Y, M, APC, pH, a_w, temp*
*Green juice, **HPP treated***	*2.2.20*	*Take sample immediately after HPP step*	*5*	Y, M, APC, pH, a_w, temp
*Green juice, HPP, **untreated control***	*9.2.20*	*Condition **1**: 4°C/After week 1*	*5*	Y, M, APC, pH, a_w, temp
*Green juice, **HPP treated***	*9.2.20*	*Condition **1**: 4°C/After week 1*	*5*	Y, M, APC, pH, a_w, temp
*Green juice, HPP **untreated control***	*9.2.20*	*Condition **2**: 8°C/After week 1*	*5*	Y, M, APC, pH, a_w, temp
*Green juice, **HPP treated***	*9.2.20*	*Condition **2**: 8°C/After week 1*	*5*	Y, M, APC, pH, a_w, temp
Etc				

Data collected when processing the samples for shelf life testing

Data collected on **product entering the HPP:** *It is recommended that at least 5 samples are tested for each parameter (n=5). If product is not homogeneous more samples will need to be tested.*	pH:	a_w:
	Salt/sugar:	Fat content:
	Initial temperature of product *(hold samples of the product and measure the temperature just before the HPP step starts)*:	Pressure transmitting fluid (e.g. water) initial temperature:

These parameters are suggestions only. Generally pH, a_w (unless liquids with an a_w close to 1), and IT is expected. *Make sure these parameters are the same as will occur during commercial operations.*	Target Pressure (give units e.g. MPa, bar):	Holding time at pressure (state mins/sec etc):
	Compression and decompression profile (e.g. pressure increase/decrease vs time):	Pressure transmitting fluid (e.g. water) final temperature:
	Final product temperature immediately after the HPP step:	Other important parameters *(list)*:
Raw data and analysis		
Data collected and its analysis: *Attach the tables of raw data and its analysis for all batches to this template and tick the boxes to indicate that this has been done (add more rows if needed).*	Product temperature during storage and times Yes [ ]	Microbiological results tabulated and analysed Yes [ ]
	Product characteristic test results tabulated and analysed Yes [ ]	Other (list):
Results		
Were the results as expected?	Yes [ ] Explain:	No [ ] See next row.
Were there any unusual or unexpected results? *Unexpected result should be investigated and explained.* *Examples include high growth levels in some samples when most are not detected, results that are much higher or lower than expected, large variations.*	Explain unexpected results	
Shelf life achieved. *This should be the earliest time at which spoilage or other limiting factor occurs as determined from these trials.*	Shelf life: months/weeks/days	
Validated parameters		
Validated HPP parameters transferred to the RMP operating procedures, including procedures to ensure correct shelf life is applied.	Yes [ ]	No [ ]

Template 1B: Challenge trials; Evidence to demonstrate inactivation of vegetative pathogenic microorganisms

This template should be used if validation is to demonstrate that the HPP will inactivate pathogenic microorganisms. Inoculation of products with known quantities of target pathogenic microorganisms can be used for two purposes:

- to assess the growth potential of the pathogen in product under specific circumstances; and
- to assess the efficiency of an inactivation process.

Depending of the HPP parameters used (e.g. pressure, time and temperature) combined with the characteristics of the product (e.g. a_w, pH, salt/sugar/fat content), the effect on inactivation can vary. Some pathogenic cells may not be completely killed, but only injured. These cells may not be detected/enumerated through the analyses performed just after the HPP treatment, especially when selective media are used, but the conditions of storage during shelf-life may allow the recovery of these injured cells and therefore their detection at some stage during the shelf-life.

It is important to consider - do the product characteristics and storage conditions allow the recovery and growth of the target microorganism during the shelf-life? If the answer is no, assessment of the $\log_{10}$ reduction in the concentration of the pathogen just after the HPP treatment may be sufficient. If the answer is yes, the $\log_{10}$ reduction needs to be assessed at the end of the product's shelf-life.

Given this, it is likely that you will need to demonstrate that the pathogens remain at an acceptable level for the shelf life of the product and so sufficient samples would need to be produced and stored for at least the product shelf life.

Additional Information

The following factors are critical for HPP validation studies:

- Inoculum for each pathogen of concern should be a cocktail of strains including human related strains and pressure resistant strains;
- If spore forming bacteria are pathogens of concern, these should be included as one of the microorganisms in challenge study;
- The inoculum should be in a stationary growth phase;
- The enumeration method should be appropriate to detect stressed or injured cells;
- The same product formulation needs to be used as for the commercial product;
- Packaging should be the same as used in the commercial production;
- For heterogeneous products, the same pack sizes as for commercial production should be tested;
- Equipment used for challenge trail should provide comparable pressure, temperature against time profile as commercial equipment that will be used;
- Post HPP storage conditions should be comparable to commercial storage conditions;and
- A change in ingredients, product formulation, pre-HPP steps, packaging or the HPP process may require further validation studies to be completed.

The areas in white in the template need to be filled in.

A: Product information

Product details	Name or other identifier:	Weight/volume:
Microbiological regulatory or operator-defined limits to be met in the product.	Microorganism	Limit
Any microbiological limits to be met prior to the HPP step. *This may be in individual ingredients or the formulated product just prior to the HPP step.*	Microorganism	Limit
Product formulation *List ingredients, amounts added, and form (e.g. dried, fresh etc).*	*Write the complete recipe here or attach from your RMP:*	
Product characteristics *If these characteristics don't apply to your product, delete and replace with more appropriate characteristics.*	pH:	a_w:
	Salt/sugar:	Protein content:
	Fat content:	Other (list):
	Preservative(s):	
Product packaging to be used *Remember, you will need evidence of compliance with HC spec Part 7.*	Type and form of packaging: Confirmed compliance with HC Spec? Yes [ ]	Is the packaging appropriate for use in the HPP and capable of forming a hermetic seal? Yes [ ] No [ ] Can the material withstand the loading and unloading into the unit? Yes [ ] No [ ]
	Is the same packaging to be used during the trials? Yes [ ] No [ ] Is same pack size to be used during the trial? Yes [ ] No [ ] If no, you will need to provide evidence that the results will be applicable when used on the commercial unit.	What is the gas composition inside the product packaging/headspace e.g. vacuum, composition of modified atmosphere: Is the same gas composition to be applied to the trial samples: Yes [ ] No [ ] If no, you will need to provide evidence that the results will be applicable when used on the commercial unit.
Intended shelf life and would product be used in one sitting or consumed over time?	Months/Weeks/Days: Single use/multiple use:	Storage conditions:

B: Process information

Full process details *Include preparation, filling, closing, holding, HPP, cooling, storage etc.*	Attach details, including all processing parameters and limits. This may be provided as a process flow.
HACCP plan or equivalent RMP documentation	Attach for the entire process.

Proposed HPP test parameters *There may be a number of process variations to be tested.* *If these parameters don't apply to your product, delete and replace with more parameters. Add more rows if needed.*	Initial product temperature immediately before the HPP step:	Holding time at target pressure (state mins/sec etc, also if pulses applied):
	Target Pressure (give units e.g. MPa, bar):	Compression and decompression profile (e.g. pressure increase/decreased vs time):
	Pressure transmitting fluid (e.g. water) initial temperature:	Other (e.g. number of cycles):
List of technical references used to support the HPP parameters selected e.g. literature, international standards. *If possible, provide an electronic link to the source.* *This may be peer reviewed research, a person with expertise in HPP, the equipment supplier etc.*	State how the proposed HPP parameters have been determined, or the source from which they have been taken:	

C: Trial Information

Date(s) of validation work		
Suitably skilled person(s) involved in the validation trials *You may have one or more.*	Name: Evidence of competency:	Name: Evidence of competency:
How trial product is to be disposed of.	Product disposition:	Confirmation that product disposed of as stated:
Name and contact details of laboratory carrying out testing. *Some testing **must be** carried out by a laboratory with accreditation to ISO 17025, for example if testing for Listeria monocytogenes in animal products. Check if this is a requirement for your product.* *Check that the laboratory has the competency and capability to conduct your validation trials – this can be a complex area.*		
Compatibility of food matrix and test methods.	Are the laboratory test methods appropriate for food matrix and pathogen? Yes [ ] No [ ]	
Enumeration method(s) for target microorganisms. *Ensure these methods are capable of detecting sub-lethally injured cells. This information should be provided by your laboratory. It is recommended that you obtain this information from the laboratory and attached it to this template.*	Microorganism	Enumeration method
Details of the HPP equipment used for the trials. *Note it is generally unacceptable to introduce pathogens into your processing site. A laboratory or pilot*	Premises where HPP is located and equipment details (e.g. make, model, size):	Evidence viewed that instrumentation calibrated: Yes [ ]

plant with the appropriate facilities is likely to be needed for challenge trials unless an approved procedure is in place or validated surrogate(s) are used.		
Is this the same HPP that will be used for commercial production?	Yes [ ] No [ ] If no, you will need to provide evidence that the results will be applicable when used on the commercial unit. If the results cannot be applied to the commercial unit, these trials will need to be repeated).	
Microbial pathogens to be inactivated by the HPP and the reduction to be achieved and/or limits to be met. *These are the biological hazards identified in your hazard identification and analysis that are to be inactivated by the HPP step. Confirming that these limits will be met is the purpose of this validation.*	Target pathogenic microorganism	Limit and/or target log_{10} reduction to be achieved by the HPP step
Processing conditions.	*As provided in **Section B.** Product should be processed under worst case conditions.*	
Cocktail of microbiological strains used for each pathogen and justification for their selection. *The strains should be relevant to the product (those isolated from similar products), include a reference strain, human related strains and also pressure-resistant strains.*	Cocktail of strains and justification for each target pathogen: *For details of what is needed, see Template 1C: MPI guidance for laboratories carrying out validation trials. This information should be provided by your laboratory. It is recommended that you obtain this information from the laboratory and attached it to this template.* *Organism/Cocktail 1:* *Organism/Cocktail 2:* *Organism/Cocktail 3:* *Etc*	
Sample inoculation. *Inoculum preparation etc should be managed by the laboratory. Information needed here should be provided by the laboratory.*	Level of inoculation into product samples for each cocktail: Are all inocula are in stationary growth phase: Yes [ ]	
Sampling programme for the duration of the trial. *This information includes the dates and number of samples tested at each sampling period. Details may be written here or attach the sampling programme to this template.* *Studies should be repeated on more than one batch to identify variability within and between batches. Ideally these will be on different dates with different ingredient batches etc. Further trials will needed if the required reduction is not achieved or the stated shelf life is not met.* *A sampling programme should be prepared for each batch tested.* *At each testing period it is recommended that at least 3-5 replicate samples are tested.*	*See example of sampling programme provided in template 1A.*	

Sample storage conditions during the trial. *Examples:* *Optimum e.g. storage as on the label for entire shelf life;* *Realistic e.g. repeated short periods of elevated temperatures;* *Worst case e.g. poor temperature control in a domestic refrigerator or variable temperature control.*	Set of storage conditions (1):	Set of storage conditions (2) *(add more rows if more conditions are used)*:
Number of batches to be tested (N_B): *It is recommended that more than one batch is tested so that batch to batch variability can be detected*	Number:	
Total number of samples needed for the trials from each test batch. *Sufficient samples should be produced and stored so that testing can be carried out beyond the stated shelf life e.g. to ensure that any surviving cells that may grow during the shelf life are detected.* *A full set of samples will be needed for each pathogen and storage condition tested. Determine the number needed from your sampling programme plus a few spares.*	Total number of inoculated controls needed: [ ] Total number of inoculated but non-HPP treated control samples for each pathogen tested *(these are tested at the start and end of the shelf life)*. Total number of inoculated HPP treated samples to be produced for each pathogen tested *(these are tested straight after the HPP step and then at various times until after the end of the shelf life)*. Number of samples: Batch 1: Controls (non-HPP treated) [ ] HPP treated per pathogen [ ] Batch 2: Controls (non-HPP treated) [ ] HPP treated per pathogen [ ] Batch 3: Controls (non-HPP treated) [ ] HPP treated per pathogen [ ]	
Data collected when processing the samples for Challenge trial		
Data collected by you on **product entering the HPP:** *It is recommended that at least 5 samples are tested for each variable (n=5). If product is not homogeneous more samples will need to be tested.* *These parameters are suggestions only. Generally pH, a_w (unless liquids with an a_w close to 1), and initial temperature is expected.* *Make sure these parameters are the same as will occur during commercial operations.* *Note: testing of intrinsic product characteristics by you does not need to be carried out on inoculated product.*	pH:	a_w:
	Salt/sugar content:	Protein content
	Fat content:	Other (list):
	Preservative/s:	
	Initial temperature of product *(hold samples of the product and measure the temperature just before the HPP step starts):*	Pressure transmitting fluid (e.g. water) initial temperature:
	Target pressure (give units e.g. MPa, bar):	Holding time at target pressure (state mins/sec etc):
	Compression and decompression profile (e.g. pressure increase/decrease vs time):	Pressure transmitting fluid (e.g. water) final temperature:
	Final product temperature immediately after the HPP step:	Other important parameters *(list)*:

Raw data and its analysis		
Data collected and its analysis: *Attach the tables of raw data and its analysis for all batches to this template and tick the boxes to indicate that this has been done (add more rows if needed).*	Storage temperatures and times	Microbiological test results tabulated and analysed Yes [ ]
	Intrinsic product characteristic results tabulated and analysed Yes [ ]	Other (list):
Results		
Were the results as expected?	Yes [ ] Explain:	No [ ] See next row
Were there any unusual or unexpected results? a_w *Unexpected result should be investigated and explained.* *Examples include high growth levels in some samples when most are not detected, results that are much higher or lower than expected, large variations.*	Explained unexpected results.	
Log count reduction (LCR) of the target organisms achieved by the process: *Attach the data and calculations used to determine the LCR.* *See the guide for laboratories for an example of how this should be calculated.*	LCR=$\log_{10}$ cfu/ml non-HPP treated control (average) – $\log_{10}$ cfu/ml HPP treated (average) Log count reduction achieved:	
Shelf life achieved.	Shelf life:	
Validated parameters		
Validated HPP parameters transferred to the RMP operating procedures.	Yes [ ]	No [ ]

Add extra template pages, if needed

Template 1C: Guidance for laboratories carrying out validation trials

This template should be completed by the laboratory carries out microbiological validation study. More space may be inserted where needed.

<table>
<tr><th>Step</th><th colspan="3">Information</th><th>Comments</th></tr>
<tr><td rowspan="2">Target organisms</td><td>Cocktail 1 /Organism:</td><td>Strains:
-
-
-
-</td><td>Origin:
-
-
-
-</td><td rowspan="2">• Rationale for the choice of target microorganism(s)
• A cocktail of about 3-5 strains for each microorganism genus is needed (although the exact number is under the laboratory responsibility, provided they give a rationale to their choice)
• Different species should not be mixed in the same cocktail (for example. Salmonella and Listeria)
• For each strain: provide the origin of the strain: reference strain from a collection (ex ATCC), environmental strain obtained from environment/ water/food, clinical strain obtained from human cases.
• When possible, include strains with known high pressure resistance
• Surrogates can be used but they should be validated and their choice should be justified (references needed).</td></tr>
<tr><td>Cocktail 2 /Organism:</td><td>-
-
-
-</td><td>-
-
-
-</td></tr>
<tr><td rowspan="5">Inoculum preparation</td><td colspan="3">Media used:</td><td rowspan="5">• Describe the complete protocol for the growth and preparation of the inoculation cocktail
• Indicate if there is a pre-adaptation step: for example adaptation to cold.
• Growth phase: the target microorganisms should be in stationary phase because they are more pressure resistant than bacteria in exponential phase</td></tr>
<tr><td rowspan="2">Incubation</td><td>Temperature:</td><td></td></tr>
<tr><td>Duration:</td><td></td></tr>
<tr><td colspan="3">Number sub-cultivation steps:</td></tr>
<tr><td colspan="3">Pre-adaptation:</td></tr>
<tr><td colspan="3">Growth phase:</td></tr>
</table>

Inoculation level	Number CFU/g or CFU/ml in the cocktail and /or in the food sample:		• The inoculation level should be high enough (10^6-10^7 cfu/g) to demonstrate the level of inactivation if HPP is intended to replace a heat treatment (demonstration of a minimum of 5 $\log_{10}$ reductions). • If the HPP treatment is intended to be used as a post lethality treatment (for example for cooked RTE products after slicing), this level can be reduced, as it is expected that the main pathogen reduction is performed by other inactivation treatments. Therefore the inoculum level can be lower than 10^6 cfu/g. • Indicate how you control the final inoculation level in the product
	Volume of inoculation:		
Inoculation methodology			• Indicate how the microorganism cocktail is inoculated to the food sample: surface, in deep, how long is the waiting time after inoculation, if there is mixing of the food sample
Duration of the study	Intended shelf-life:	…… days / weeks / months	• Frequency of testing: no less than once a week if shelf-life in weeks. Minimum: just after HPP treatment, half the shelf-life, end of shelf life, and eventually beyond shelf-life. However, if the main purpose of the challenge study is the process inactivation assessment, the number of sampling points may be reduced to 2 (just after HPP treatment and at the end of shelf life).
	Number of sampling points (N_P)	N_P =	
	Sampling points	HPP = T0	
		T1	HPP + …….
		T2	HPP +……
		T3	HPP + …..
		T4	HPP + ….
Number of samples	Number of replicates per sampling point (N_R):	N_R =	• Number of replicates per sampling point for each batch: minimum of 3 replicates • Number of batches: batches should be processed at different times with different ingredients. The number of batches tested can be reduced if the low inter-batch variability is demonstrated (stable intrinsic characteristics such as pH, a_w, etc). • NS = NR x NP • Number of controls and nature of controls
	Number of batches (N_B):	N_B =	
	Total number of inoculated samples (N_S):	N_S =	
	Number and nature of controls:		

			• Un-inoculated control samples should be analysed, it is prudent to perform these analyses for each sampling point to determine the behaviour of background flora
Product storage conditions	Storage temperature:		• Indicate if temperature abuse is considered and how.
	Temperature abuse?: Yes [ ] No [ ]		
Analyses	Parameter	Method	• Parameters to be analysed: microbiological and non- microbiological, with the corresponding methods. • It is better to use normalised methods, accredited by IANZ • If no normalized methods used, justify this choice and describe extensively the method: media, incubation time and temperature. • Indicate if recovery of injured cells will be assessed and how: medium and protocol use
	Recovery: injured cells?: Yes [ ] No [ ]		
	Method		
Data treatment	Control validation: Yes [ ] No [ ]		• All the raw data and calculated results should be provided in the separate table. • Colony counts and identification should comply with the corresponding normalized methods • Controls: assessment of controls => do they validate the test? (no pathogen detection in un-inoculated samples). • For technical replicates within a batch, it is necessary to calculate the average or median with the confidence interval or SD.
	Results:	Results template to be completed	

	Log reduction for this challenge test:		• log reduction calculation ➔ log initial inoculum level (non HPP treated)– log final count (HPP treated at the end of shelf life) • Calculation of separate Log reductions for each batch. • Log reduction between each batch will be compared and discussed: the final log reduction to be taken should be the worst case scenario (the least log reduction).