

Chapter 2

Validation of Control Measures¹

2.1 Introduction

ICMSF previously discussed validation of control measures in the supply chain (Zwietering et al. 2010) and portions of that paper are included in this chapter. The flexibility offered by an outcome based risk management system must be supported by demonstration that the selected control measures actually are capable of achieving the intended level of control on a consistent basis. Validation is defined by the Codex Alimentarius Commission (2008) as:

“**Validation:** Obtaining evidence that a control measure or combination of control measures, if properly implemented, is capable of controlling the hazard to a specified outcome.”

The overall effectiveness of the control measures should be validated according to the prevalence of the hazards in the food of concern, taking into consideration the characteristics of the individual hazard(s) of concern, established Food Safety Objectives or Performance Objectives and level of risk to the consumer.

2.1.1 Relationship of Validation to Monitoring and Verification

In addition to the definition of validation cited above, the Codex Alimentarius Commission (2008) adopted the following definitions:

“**Monitoring:** The act of conducting a planned sequence of observations or measurements of control parameters to assess whether a control measure is under control.”

“**Verification:** The application of methods, procedures, tests and other evaluations, in addition to monitoring, to determine whether a control measure is or has been operating as intended.”

Validation focuses on the collection and evaluation of scientific, technical and observational information and is different from verification and monitoring. Monitoring is the on-going collection of information on a control measure at the time the control measure is applied and verification is used to determine that the control measures have been appropriately implemented. The successful implementation of HACCP requires validation, which includes the clear identification of hazards, control measures available, critical control points, critical limits and corrective actions. The outcomes of

¹Part of this chapter was published as: Zwietering MH, Stewart CM, Whiting RC, ICMSF (2010) Validation of control measures in a food chain using the FSO concept. Food Control 21:1716–1722.

monitoring and verification activities associated with a HACCP system assist in determining when re-evaluation may be necessary. To be effective, the scope of validation may go beyond the control measures used in the manufacturing facility and may include control areas such as primary processing and consumer handling.

The production of safe food requires the application of GHP and HACCP principles to develop and implement a total food safety management system that controls the significant hazards in the food being produced. Some risk management principles are best addressed through GHP measures (e.g., controlling the initial levels of a hazard through good hygiene) and others are clearly part of a defined CCP within HACCP (e.g., reducing the level of a hazard, through a decontamination step).

Food manufacturers design processes to meet Performance Objectives (PO) or Performance Criteria (PC), which can be set at specific points throughout the food chain to assure food safety. Regulatory authorities are concerned with whether a group of products or the consequences of a series of processing and handling steps prior to consumption can meet the Food Safety Objective (FSO) and ensure that those foods achieve levels that are consistent with the Appropriate Level of Protection (ALOP) (see Chap. 1, Utility of Microbiological Testing for Food Safety and Quality).

Various control measures include the control of ingredients at the initial stage of food processing or food chain, and intensive protocols to reduce or eliminate the contamination by washing, heating, disinfecting and other measures. Control measures are also designed to prevent an increase of hazards during transportation and storage, by cross-contamination during the processing or cooking, or even by re-contamination after those steps.

Control measures should be validated to determine whether the products meet with objectives; however, different segments of the food industry undertake these activities depending on the situation. Food processors may validate the control measures for the processes they use, and validation should focus on achievement of meeting the given PO or PC. In this case of validation, both within-lot and between-lot variability should be considered. On the other hand, control measures validated under the responsibility of regulatory authorities cover all control actions in the system for multiple products and processes, including consideration of between-lot variability. In this case validation is targeted at assessing the established PCs, POs and FSOs. For example, the effective risk management of a meat production system may include validation of:

- Farm practices aimed at ensuring animal health and minimizing the level of infection in the herd (zoonosis).
- Slaughter practices aimed at minimizing contamination.
- Chilling regimes and temperature control aimed at minimizing the potential for pathogen growth.
- Consumer instructions aimed at ensuring that the product is cooked to the minimum temperature required to inactivate pathogens.

In this chapter, the prevalence and levels of microorganisms from the initial contamination (H_0), reduction (ΣR), growth and re-contamination (ΣI), and factors that influence these are considered throughout food production until consumption. The influence of these factors on meeting the FSO is represented by the equation $H_0 - \Sigma R + \Sigma I \leq \text{FSO}$. Stochastic aspects of the parameters are taken into account as well as deterministic values. Potential key factors, data and data analysis methods are described. However, some of these factors may not be relevant for a particular processing line or processor. Examples of the use of data to validate one or a series of processes, including statistical insights, are provided.

2.2 Considerations for Validation

Processes can be validated through the use of a variety of approaches (Codex Alimentarius 2008) including predictive modeling, the literature, microbiological challenge studies and use of safe harbors (i.e., approaches that have been previously approved as delivering a safe product (see Chap. 1)).

Not all these need to be used, but often several approaches are combined to supply sufficient validation evidence. When a safe harbor approach is used, it may not be necessary to conduct validation studies for that process. For example, a safe harbor for milk pasteurization is to deliver a minimum heat process of 72°C for 15s. This process criterion has been validated and therefore can be implemented by manufacturers without re-validation of the process.

Numerous considerations for establishing the efficacy and equivalency of processes are discussed by NACMCF (2006), which proposed the following steps for the development of processes intended to reduce the pathogen(s) of concern:

- Conduct a hazard analysis to identify the microorganism(s) of public health concern for the food.
- Determine the most resistant pathogen of public health concern that is likely to survive the process.
- Assess the level of inactivation needed. Ideally this would involve determining the initial cell numbers and normal variation in concentration that occurs before processing.
- Consider the impact of the food matrix on pathogen survival and possible growth during storage.
- Validate the efficacy of the process.
- Define the critical limits that need to be met during processing so that the food will meet the performance objectives and performance criteria.
- Define the specific equipment and operating parameters for the proposed process.
- Implementation within GHP and/or HACCP.

Regardless of the methods used to determine and validate process criteria, similar microbiological considerations need to be taken into account (NACMCF 2010). These include:

- What is the most resistant microorganism of public health significance for each process? When determining the target microorganism, it is necessary to consider all pathogens that have an epidemiologically relevant association with a product, as the most resistant pathogen may not be present in the highest numbers. Conversely, pathogens controlled by other means may not be of public health significance in a product when growth is required in order to cause illness (i.e., *C. botulinum* controlled by pH).
- Choice of strains used to conduct validation studies
- The phase of growth in which the microorganisms are harvested
- The substrate upon which the culture is grown and the associated environmental conditions (e.g., pH, temperature, atmospheric conditions), including adaptation of culture when appropriate
- The suspending medium
- The food's intrinsic factors, such as pH, a_w , and preservative levels
- The sample size, preparation and handling (i.e., compositing, homogenizing, subsamples)
- Packaging conditions (packaging material and atmospheric conditions, including modified atmosphere gas mixtures)
- Cell enumeration methods following the process and selection of appropriate measurement systems
- Processing variability

Three commonly used strategies for process validation include concurrent, retrospective and prospective process validation. *Concurrent process validation* is based on simultaneous collection and evaluation of data from a process concurrently with its application. This is used when there is a change or modification to an established and previously validated process. *Retrospective process validation* is validation of product already in distribution based upon accumulated production, testing and control data. This technique is often used in analyzing process failures that result in product recalls. *Prospective process validation* is a deliberate, forward-looking, planned approach that determines if the process can be relied upon with a high degree of confidence to deliver safe food. Prospective validation is best suited for evaluating novel processes and must consider the equipment, the process and the product (Keener 2006).

A team of experts is required for system validation because of the many skills required such as engineering, microbiology, physical chemistry, etc. Involvement of external experts and regulatory officials in the development of both the master validation plan and the validation protocols is essential to ensure technical adequacy and acceptance by authorities. Process validation requires proper analysis of objective data.

2.3 Validation of Control Measures

Validation generally begins with microbiological studies on a laboratory scale, progresses to a pilot plant scale and ends with full validation on a commercial scale when possible or necessary. Microbiological challenge testing is useful to validate process lethality against a target microorganism(s) to determine the ability of a food to support microbial growth and to determine the potential shelf life of ambient or refrigerated foods. For example inactivation kinetic studies can be conducted over a small range of treatments such as a unique combination of factors and levels (e.g., pH 6.5 and 70°C). Conversely, studies can also be conducted over a broad range of treatments, and can illustrate where failure occurs and help assess the margin of safety in any process, as well as providing data that can be used in evaluation of deviations. Furthermore this facilitates development of predictive models for future public or private use. Several microbiological predictive models are available, including the USDA Pathogen Modeling Program (USDA 2006) and COMBASE (2010). Challenge studies can also be used to determine processing criteria, although they are of less generic use than models and often are used for particular products or as a way of validating the model predictions. On the other hand models are often generic, and therefore do not contain all factors that are of relevance for a specific food. Therefore models and challenge studies should be combined in an iterative way. This is further discussed by NACMCF (2010). Finally, on a commercial scale, challenge studies can be conducted using nonpathogenic surrogate microorganisms and shelf life studies with uninoculated product can also provide useful information for validating a process.

While microbiological challenge testing can be used to determine the stability of a product with regards to spoilage over the intended shelf life, the remainder of this discussion focuses on microbiological safety of food products. In the following sections, the initial contamination (H_0), reduction (ΣR), growth and re-contamination (ΣI), and factors influencing these are discussed sequentially, including data needs and experimental considerations.

It is important to note that in this text, diagnostic methods are assumed to be 100% sensitive and 100% specific, which is not the case. These characteristics of methods depend largely on the target microorganism, diagnostic method used and investigated food product. Especially for low level pathogens false negative results might be expected. These aspects need to be clearly considered in validation studies.

2.3.1 Initial Level (H_0), Standard Deviation and Distribution

The design of the food process influences the importance of incoming material for product safety. The main source of the pathogen of concern may be from a major or minor ingredient, one incorporated in the initial processing steps or one added later. It is important to understand which ingredient(s) may harbor the pathogen and if there is a seasonal effect on the level of the pathogen. For example, the number of *Escherichia coli* O157:H7-positive lots of ground beef sampled from 2001 to 2009 increased in the June-October period in the USA (USDA-FSIS 2009). The geographical source of the ingredient may also play a role in the likelihood of whether a certain pathogen is present in the raw ingredient. If contamination is not avoidable, the goal is to develop specifications and criteria for the incoming

material that will lead to achievement of the final PO and FSO, in conjunction with the performance criteria for the other steps in the food process. The specifications for accepting the incoming materials include the acceptable proportion above a limit or the mean log level and standard deviation.

Information for validating that incoming materials comply with required specifications can come from:

- Baseline data from government agencies.
- Documentation from suppliers that specifications are met (supplier provides validation and end product testing).
- Baseline data from the processor's experience or
- Test results for incoming lots.

Microbiological testing is one of the tools that can be used to evaluate whether a food safety system is providing the level of control it was designed to deliver. A number of different types of microbiological testing may be employed by industry and government. One of the most commonly used is within lot testing, which compares the level of a microbiological hazard detected in a food against a prespecified limit, i.e., a Microbiological Criterion (MC) (ICMSF 2002). MCs are designed to determine adherence to GHP and HACCP (i.e., verification) when more effective and efficient means are not available. In this context, FSOs and POs are limits to be met, and within-lot testing can provide a statistically-designed means of determining whether these limits are being met (van Schothorst et al. 2009). To assess compliance of a lot to a MC, a sampling plan based on the MC specified and the confidence level desired can be established. To do this, the recommendations for setting MCs as outlined in Appendix A should be followed. The MC should specify the concentration to be met (m in CFU/g), the proportion of defective samples (c) allowed above the m value, the number of samples to be tested (n) and an evaluation of the implications for a given sampling plan.

A sampling plan appropriate to assess compliance with a specified concentration can be developed using the ICMSF spreadsheet (Legan et al. 2002, <http://www.icmsf.org>). The calculations underlying the spreadsheet determine the probability that an analytical unit from a lot contains more than any specified number of cells/g. That probability can be estimated from the mean concentration of the cells in the lot, and its standard deviation. It is assumed that the distribution of concentrations of cells in a lot is log-normally distributed. A Performance Objective is determined, e.g., that 99% of units must contain less than a specified concentration of cells, and a corresponding mean log concentration determined from the assumed standard deviation. Then the number of samples required to be taken from the batch, to provide 95% confidence that an unacceptable batch will be rejected by sampling, can be calculated taking into account the size of the analytical unit. In an example on *Listeria monocytogenes* in cooked sausage (ICMSF 2002), the initial number in the raw materials prior to cooking is assured to be no more than 10^3 CFU/g (i.e., $H_0 = 3$). Often a PO for H_0 can also be regarded as the PO for the output of a previous stage of the food chain.

In any sampling process in microbiology, the actual number of organisms recovered in a sample taken from a lot will also be affected by the random distribution of cells within the region that is actually sampled. This randomness is described by the Poisson distribution. The relative effect of this randomness is relatively small when large number of cells are contained, and counted, from the sample (e.g., the standard deviation when the true mean is 100, is ± 10), but it is relatively large when the target concentration is one cell per sample, such as in presence absence testing. Including this consideration in design of a sampling plan is more important when the result of testing is presence or absence, and has also been incorporated into the spreadsheet calculation (van Schothorst et al. 2009). As for the evaluation of sampling plans based on testing against a specific number of cells, for evaluation of sampling plans based on presence/absence testing it is also assumed that the distribution of the concentration of cells in the batch is log-normally distributed, and is characterized by a mean log and standard deviation. The Poisson effect is also included in the calculations for the first alternative, but is relatively minor.

2.3.2 Inactivation Studies (ΣR)

2.3.2.1 Modeling Studies

A microbiological predictive model can describe or predict the growth, survival or death of microorganisms in foods. These models typically relate the microbial growth, survival or death responses to the levels of the controlling factors, such as temperature, pH, water activity etc. Models generally should not be used outside the range of the factors used to create them because there is no underlying principle on which to base extrapolation. Thus consideration of the range over which they will be used is required before beginning experimentation (Legan et al. 2002). Where extrapolation is necessary, tests should be conducted to confirm that the extrapolation is valid, e.g., confirm that the established process destroys a specific population of the target microorganism. However, models that can predict the rate of death of pathogens can be used to design safe and effective processes.

Several authors describe experimental design for modeling in food microbiology (Ratkowsky et al. 1983; Davies 1993, Ratkowsky 1993, McMeekin et al. 1993). Guidelines for data collection and storage are also available (Kilsby and Walker 1990, Walker and Jones 1993). A practical guide to modeling, supported by references to primary sources of modeling information is discussed by Legan et al. (2002). The reader should consult these references for details on development of a microbiological predictive model.

2.3.2.2 Microbiological Challenge Studies

Detailed information on the design and implementation of microbiological challenge studies has been described (IFT 2001, Scott et al. 2005, NACMCF 2010). Microbiological challenge testing is useful to validate process lethality against a target microorganism(s).

When designing and carrying out a microbiological challenge study, some factors to consider include the selection of appropriate pathogens or surrogates, the level of the challenge inoculum, the inoculum preparation and method of inoculation, the duration of the study, formulation factors and storage conditions, and sample analyses (Vestergaard 2001). Multiple replicates of such studies should be done to reflect variation in the production lots and other factors. The extent of replication and the impact on the results of the study must be considered.

2.3.2.3 Challenge Microorganism Selection

The ideal microorganisms for challenge testing are those previously isolated from similar formulations. If possible, pathogens from known foodborne outbreaks should be included. In contrast to kinetic studies, challenge studies frequently use a mixture of five or more strains of the target pathogen because a single strain may not be the most resistant to each of the multiple stress factors involved in the product/process combination. Additionally, strains with the shortest generation time may not have the shortest lag time under the test conditions. Likewise, strains may vary in response to changes in the inactivation treatment (Scott et al. 2005). The strains in the cocktail should be present in approximately equal numbers. It is also important to incubate and prepare the challenge suspension under standardized conditions and format.

When possible, it is desirable to use a pathogen rather than a surrogate microorganism for validation studies. However, surrogates are sometimes used in place of specific pathogens, for example, in challenge studies conducted in a processing facility. The characteristics of the surrogate in relation to those of the pathogen should be determined and the difference accounted for in the interpretation of the challenge studies (Scott et al. 2005). Detailed information on the desirable attributes for surrogates can be found in IFT (2001).

2.3.2.4 Inoculum Level

The inoculum level depends on the purpose of the study; whether the objective is to determine product stability or shelf life, or to validate a step in the process designed to reduce microbial numbers. When validating a process lethality step, it is usually necessary to use a high inoculum level, such as 10^6 – 10^7 CFU/g of product or higher, to demonstrate the log reduction of the challenge microorganisms. The actual concentration of the inoculum before and after inoculation should be confirmed. Also uninoculated samples should be analyzed to investigate intrinsic product contamination. Total inactivation of the inoculum may not be necessary, especially in situations where the H_0 is likely to be low (e.g., when the initial population is $<10^3$ CFU/g a 5D process is required and the inoculum level in the experiment is 10^7 CFU/g). This may be relevant when validating post lethality treatments, where the process is being designed to inactivate low levels of pathogens resulting from recontamination of product after an initial lethal treatment, such as might occur during slicing or packaging operations.

2.3.2.5 Inoculum Preparation and Inoculation Method

Preparation of the inoculum is an important component of the overall protocol. Typically, the challenge cultures should be grown in media and under conditions optimal for growth of the specific challenge culture. In some studies, specific challenge microorganisms may be pre-adapted to certain conditions.

The method of inoculation is another important consideration. It is essential to avoid changes in the critical parameters of the product formulation undergoing the challenge. For example, the use of a diluent adjusted to the approximate water activity of the product using the humectant present in the food minimizes the potential for erroneous results in intermediate moisture foods. Preliminary analyses should be done to ensure the water activity or moisture level of the formulation is not changed after inoculation. For guidelines for inoculation of low water activity products or for challenge studies with spores refer to IFT (2001).

2.3.2.6 Duration of Challenge Studies for Potential Growth

It is prudent to conduct the challenge study longer than the desired shelf life to determine what would happen if users stored and consumed the product beyond its intended shelf life. Additionally, when validating inactivation processes, it is possible that sublethal injury may occur in some products, leading to a long lag period (Busta 1978). If the product is not tested for at least its entire shelf life, it is possible to miss the recovery and subsequent growth of the challenge microorganism late in shelf life. Some regulatory agencies require data for 1.3 times the shelf life of the product when stored as intended. Shorter times may be considered for refrigerated products that are stored under abuse conditions.

The frequency of testing is governed by the duration of the challenge study. If the shelf life is measured in weeks, the test frequency is typically no less than once per week. It is desirable to have a minimum of 5–7 data points over the shelf life to have a good indication of inoculum behavior. All studies should start with “zero time” testing, i.e., analysis of the product right after inoculation and, for inactivation studies, right after processing. It may also be desirable to test more frequently early in the challenge study and then reduce the frequency of testing to longer intervals.

A sufficient quantity of product should be inoculated so that a minimum of three replicates per sampling time is available throughout the challenge study. In some cases, such as in certain revalidation studies and for uninoculated control samples, fewer replicates may be used.

2.3.2.7 Formulation Factors and Storage Conditions

When evaluating formulation, it is important to understand the range of key factors that control its microbiological stability such as pH, preservative level and water activity. These intrinsic properties should be documented. It is useful to collect data on the inherent manufacturing variability of the critical parameters and ensure that the challenge test conditions encompass this variability by a specified margin (e.g., with 95% confidence). These parameters should be adjusted to the worst case condition expected for the product with respect to microbial growth or inactivation (e.g., highest pH). One approach would be to use the 95% confidence interval for the parameter or the mean plus 2 standard deviations. If there is only one critical parameter, this 95% confidence would mean that one out of 20 times reality could be outside this range. However, if there are many critical parameters, setting all at their 95% confidence level might simulate an unrealistic condition. The level of confidence desired must be considered in evaluating these parameters.

It is important to test each key variable singly or in combination under worst case conditions. For example, if the target pH is 4.5 ± 0.2 (95% confidence interval) and the processing capability is within that range, the challenge product should be on the high side of that range (pH 4.7). This should be carefully assessed for different parameters. For example, decreasing the water activity of a product may delay or prevent growth of microorganisms; however, using a different humectant in the system is a change in the critical factor even if the same water activity (a_w) is achieved because growth rates may vary with different humectants. Further, decreasing the a_w of a system may reduce the lethality of a process (Mattick et al. 2001). Inclusion of the impact of variability in critical factors helps to ensure that the challenge study covers the process capability range for each critical factor in the formulation.

2.3.2.8 Sample Analysis

Typically, enumeration is conducted at each sampling time. It is desirable to have at least duplicate and preferably triplicate samples for analyses at each time point. The selection of enumeration media and method depends on the microorganisms used in the challenge study. In situations where toxin-producing microorganisms are used, test for appropriate toxins at each sampling time using the most current validated method. Growth may occur without the formation of toxin.

It is prudent to analyze inoculated product and uninoculated control samples at each selected sampling time to determine how the background microbiota behaves during shelf life. It is also important to track pertinent physical and chemical parameters over the shelf life as they may influence the behavior of the microorganism. Understanding how factors such as a_w , moisture content, salt level, pH, Modified Atmosphere Packaging (MAP) gas concentrations, preservative levels and other variables may change over product shelf life is important to understanding the microbiological stability of the product. Quality attributes should also be noted.

2.3.2.9 Data Interpretation

Once the challenge study is completed, the data should be analyzed to determine how the microorganisms behaved over time. For toxin-producing pathogens, no toxin should be detected over the designated challenge period. Combining quantitative inoculum data for each time point with data on the background microbiota and the relevant physical and chemical parameters provides a broad representation of the microbiological stability of the formulation under evaluation. A well-designed challenge study can provide critical information on the microbiological safety and stability of a food formulation. Such studies are also invaluable in validating the key lethality or microbiological control points in a process.

2.3.3 Growth Studies (ΣI)

An increase in the numbers of pathogen or spoilage microorganism can occur through growth or recontamination. This section addresses growth.

Growth may occur if the food, temperature and packaging atmosphere support growth, and sufficient time is provided under favorable conditions. Growth potential should be assessed for raw ingredients, intermediate points during the manufacturing and after manufacture during distribution, retail, food service and home storage and use. Generally, public health cannot be assured unless the potential for growth is minimized. If the pathogen is not completely inactivated and growth is possible, then an accurate estimation of the amount of growth that may occur is important in validating product safety and stability.

As previously described for validating inactivation, estimates for growth may be obtained from a variety of sources including the literature, models and challenge tests (Scott et al. 2005). Increasing reliance is given to studies with experimental conditions that more closely reflect the actual conditions of the food. Satisfactory validation of a pathogen's growth in a food includes challenge tests with the normal background microbiota. Models and broth studies can provide support for evaluating minor changes in formulation and strain differences and for interpolating to conditions not explicitly tested in the challenge tests. Applications of predictive models in food microbiology include models that predict the growth rate of bacterial pathogens in response to product or environmental factors such as a_w , temperature or pH. Growth models can be used to design safe product formulations, to set appropriate storage conditions, to explore the maximum interval between cleaning and sanitizing of process equipment, and can also be used to inform decisions about when a challenge study is needed and to design the test parameters.

Factors that should be considered when evaluating growth include the strain(s) used, surrogates, physiological state of the inoculum, inoculation method, simulation of the experimental or pilot plant conditions to the commercial process, inclusion of all environmental factors in the food (pH, a_w , acid anions) and external factors (temperature, packaging), and inclusion of the spoilage microorganisms. Many of these factors were described in the inactivation section; considerations particular to estimating growth are discussed below.

2.3.3.1 Inoculum Level

IFT (2001) provided a list of microorganisms that can be used in microbiological challenge studies and recommendations for selection and assessment of tolerable growth. When the objective is to determine product safety and the extent of growth over its shelf life (ΣI), an inoculum level of between 10^2 and 10^3 CFU/g of product is frequently used. Lower or multiple inoculum levels may be considered if microbial spoilage is a common mode of failure and low numbers are anticipated in the product. See Sects. 2.3.3.3 and 2.3.3.6, for additional considerations on inoculum level.

2.3.3.2 Formulation Factors and Storage Conditions

When similar products are under evaluation, testing formulations that are more favorable to growth can limit the need to conduct challenge studies on formulations less favorable to growth. For example, studying products with a pH near neutrality may represent a worst case when similar products have a lower pH.

Test samples should ideally be stored in the same packaging and under the same conditions (e.g., MAP) used for the commercial marketplace. The storage temperatures used in the challenge study should include the typical temperature range at which the product is to be held and distributed.

Refrigerated products should be challenged under representative abuse temperatures. Some challenge studies may incorporate temperature cycling into the protocol.

2.3.3.3 Lag Phase

A lag phase occurs when cells require time to adjust to a new environment. The lag phase is influenced by the magnitude of the change and the favorability of the new environment. In general, a lengthy lag phase occurs when cells experience a significant shift to a less favorable environment such as to a lower temperature or water activity.

The physiological state of the cell also plays a role in the length of the lag phase. Generally, cells in the exponential growth phase adapt more rapidly than cells in the stationary phase. Cells that are starved in nutrient poor environments such as water, frozen or desiccated on a food contact surface typically have an increased lag time compared to the other cells. Following an inactivation treatment or other severe stress, surviving cells may need time to repair, which can also appear as a lag phase before growth. Significant lag times are most likely when certain ingredients are added (e.g., salt, acidulant) or after a stressful process (heating, thawing, sudden temperature change). A lag phase as result of temperature changes is less likely in a finished product because the mass of the food, retail packaging and box/pallet moderate temperature changes. Validation should recognize that the temperature reduction during a cooling period may extend over one or more days, especially if the food is boxed and palletized. Validation of a process should strive to replicate the initial physiological state and environmental changes in order to accurately determine the length of the lag phase, if any.

The length of the lag phase can be affected by the initial number of cells because a log normal distribution exists for the lag times of individual cells. Validation studies with high cell numbers ($>10^2$ CFU/package or unit) will inevitably have some cells with the shortest lag times and daughter cells will almost entirely originate from these cells. When low levels of contamination occur, it is possible that none of these fastest cells are present in some of the packages and the apparent lag times will become longer and more varied in those packages.

2.3.3.4 Exponential Growth Rate

The exponential growth rate (EGR) increases with storage temperature up to the pathogen's optimum temperature (typically 35–45°C for pathogens). The EGR depends on other intrinsic characteristics of the food such as acidity, water activity and inhibitors in a complex manner that can be estimated by models. However, challenge studies are required to demonstrate that the model's prediction is accurate for a specific food. Once a model is validated, it can be used to estimate the impact of the environmental factor changes (T, pH, a_w etc.) on the EGR.

2.3.3.5 Maximum Growth Level

A pathogen has a maximum level of growth that it achieves in a microbial medium or food. In broth and in pure culture, this level is typically 10^8 – 10^9 CFU/mL; however, it is sometimes lower in a food. The maximum in a food is affected also by storage temperature. For *L. monocytogenes* in the FDA-FSIS risk assessment the maximum growth levels (CFU/g) selected were 10^5 for temperatures $<5^\circ\text{C}$, $10^{6.5}$ for 5 – 7°C and 10^8 for temperatures $>7^\circ\text{C}$ (FDA-FSIS 2003) based on various literature sources.

2.3.3.6 Competition and the Spoilage Flora

Competition between the pathogen and spoilage microorganism is difficult to predict. For many pathogen-spoilage microorganism pairs, growth of both groups is reasonably independent until the spoilage microorganisms have grown significantly. Spoilage microorganisms may decrease the pH or produce inhibitors such as bacteriocins. Pathogens are typically at low populations and do not interfere with the spoilage microorganisms. Typical microbiota found in commercial settings should be present in challenge studies. Pathogens should be inoculated in the appropriate physiological state, location in the food (e.g., surface, interior or interface of components as appropriate) and concentrations that will likely occur in the commercial setting.

Another important consideration in determining the safety of a food is the storage conditions that lead to spoilage, particularly spoilage before the pathogen reaches the PO. Evaluation of growth during storage requires knowledge of the typical times and temperatures characteristic of that stage. This may be easy for the relatively short growth periods during the commercial phases of the food chain. However, time and temperature are highly variable in the home or food service operation. A temperature of moderate abuse should be selected and the maximum length of the storage period before spoilage at that temperature ascertained for determination of the amount of growth. Foods should be tested for 1.25–1.5 times their intended shelf life unless spoilage occurs first.

2.3.3.7 Effect Variation on Growth

In addition to determining the average increase in cell population during each growth period, it is important to estimate the variation about that estimate (for example the 95% confidence interval). This variation is the consequence of the different characteristics of various strains, fluctuations in the environmental conditions within the food (pH, salt levels) and the ranges in times and temperatures of storage. The challenge test can provide an estimate of the mean log value; varying the parameters within a model can provide additional data to estimate the variation. This variation includes the differences in growth from the factors calculated above but may also be increased by the analyst to account for uncertainties because of a lack of high quality data.

2.3.4 Recontamination (ΣI)

If a food process includes a lethal step that eliminates the pathogen, then any pathogen present at consumption is the result of recontamination. Foods receiving 6–8-log reductions rarely have a contaminated package immediately after that step. For example, if a product initially has a homogeneous contamination of 10^2 CFU/g in every 100 g package, after a 7 log reduction only one in 1,000 packages will be contaminated and it will have ~ 1 CFU/package. When determining whether such a food meets an FSO or PO at a further step, calculation begins after the lethal step. The frequency and level of contamination represent the new H_0 .

Little literature exists on the frequencies and levels of recontamination and few applicable models have been developed to estimate the results of recontamination. Sufficient sampling of the specific process at this step or at a subsequent step with a back calculation is the only way to obtain valid data on recontamination. A food process without a lethal step and with several potential points of additional recontamination is difficult to predict, especially since quantitative information related to recontamination is usually not available. Sufficient sampling of the food after the last point of recontamination is a possible way to validate whether a PO or FSO is being

achieved. Another approach is environmental monitoring and monitoring of food contact surfaces. Other factors to consider are packaging integrity and proper training of employees on handling practices.

2.4 Effect of Process Variability on FSO Compliance Validation

One way to demonstrate compliance to an FSO is by using the equation:

$$H_0 - \Sigma R + \Sigma I \leq \text{FSO}$$

By combining information on the initial level (H_0), reductions (ΣR) and increases (ΣI) of the microbial hazard throughout the production and distribution chain, one can determine if the FSO or PO will be reliably met. The variability of the microbial levels at different steps in the process and food chain will influence the ability to meet the FSO.

The following examples illustrate the impact of including the effect of statistical distributions for H_0 , ΣR and ΣI on the hazard level and the percent of nonconformance (% product above the PO or FSO) is calculated. First, a point estimate, without considering variability is used; then the impact of variability in the initial levels, reductions delivered through processing, and increases due to growth during food distribution are included to evaluate the ability to meet the PO or FSO. Fresh cut, washed and packaged lettuce is used as an example, with *L. monocytogenes* as the pathogen of concern. For illustrative purposes, it is assumed that to reach an ALOP, a maximum exposure of *L. monocytogenes* of 10^2 CFU/g (i.e., an FSO = 2 log CFU/g or 10^2 CFU/g) for ready-to-eat foods is set.

2.4.1 Point Estimate Approach

Szabo et al. (2003) estimated the initial contamination level of *L. monocytogenes* on precut lettuce, reduction using sanitized washing, and the increases after packaging and during storage and distribution. For a given initial level of *L. monocytogenes* on lettuce and the expected level of growth (ΣI) during storage and distribution, the necessary reduction level to achieve a given FSO can be determined. From Szabo et al. (2003), the initial population was $H_0 = 0.1$ log CFU/g, the potential increase was $\Sigma I = 2.7$ log CFU/g during storage for 14 days at 8°C, a $\Sigma R \geq 0.8$ log CFU/g was deemed necessary to achieve the FSO of 2 log CFU/g:

$$H_0 - \Sigma R + \Sigma I = 2 \rightarrow 0.1 - 0.8 + 2.7 = 2.$$

In this example, the process can be considered to achieve the FSO exactly. However, this calculation does not consider the impact of process variation.

2.4.2 Including Variability in the Process

2.4.2.1 Variability for One Parameter

The next example illustrates the impact of variability on calculations using data from Szabo et al. (2003). Assume the standard deviation for ΣI is 0.59, and assume the log increase of *L. monocytogenes* is normally distributed. For ease of calculation and explanation, H_0 and ΣR levels do not include variation. Because of the distribution of ΣI , the producer must target a lower average level of

Table 2.1 Results of various levels of reduction (ΣR) on the proportion of defective units (P) with a standard deviation for the increase of 0.59, assuming the log increase is normally distributed

Reduction (ΣR)	$H_0 - \Sigma R + \Sigma I$	Probability that FSO=2 is exceeded $P(H_0 - \Sigma R + \Sigma I) > 2$ (sd=0.59)
0.8	$0.1 - 0.8 + 2.7 = 2$	0.5 (50%)
1.2	$0.1 - 1.2 + 2.7 = 1.6$	0.25 (25%)
1.77	$0.1 - 1.77 + 2.7 = 1.03$	0.05 (5%)
2.17	$0.1 - 2.17 + 2.7 = 0.63$	0.01 (1%)
2.62	$0.1 - 2.62 + 2.7 = 0.18$	0.001 (0.1%)

Note: The proportion above the FSO determined by the cumulative normal distribution $F(2; \mu, \sigma^2)$ calculated in Excel by 1-NORMDIST(2,x,s,1). For example, for the last line=1-NORMDIST(2,0.18,0.59,1)=0.001019

Table 2.2 Results on the proportion of products that do not meet the FSO (packages of fresh cut lettuce calculated to have greater than 2 log CFU/g *L. monocytogenes* present at the point of consumption), with various mean log and standard deviation values for H_0 , ΣI and ΣR

	H_0	ΣR	ΣI	Total ^a	
mean log	-2.5	1.4	2.7	-1.2	$H_0 - \Sigma R + \Sigma I$
sd	0.80	0.50	0.59	1.11	$sd = \sqrt{sd_1^2 + sd_2^2 + sd_3^2}$
				$P(>FSO)$	0.2%

^aThe level (log CFU/g) of *L. monocytogenes* present in a package of lettuce at the point of consumption

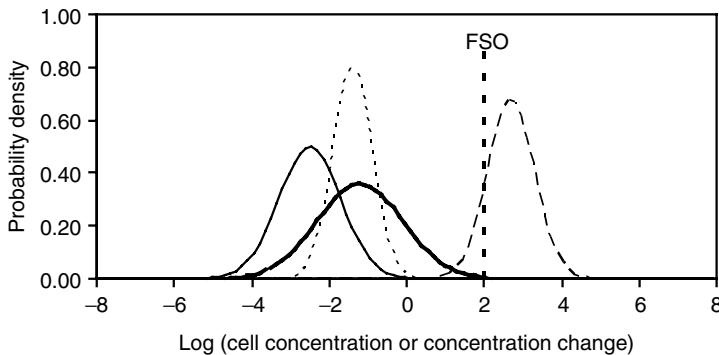


Fig. 2.1 Probability distribution of initial cell level (H_0 —), reduction in concentration ($-\Sigma R$ - -) and increase in concentration (ΣI - · -) of *L. monocytogenes* on fresh cut lettuce, and resulting cell concentration distribution (—) in packages of lettuce at the point of consumption using input values in Table 2.2

L. monocytogenes in the finished product to reliably meet the FSO. If the same average level was targeted (i.e., FSO=2 log CFU/g), 50% of the products would be above the FSO to some extent. The processor can consider other sanitizing wash methods to provide a greater reduction step to help to achieve the FSO through process control. The level of reduction needed to achieve different levels of conformity is presented in Table 2.1. For example, if the ΣR is 2.62, the proportion product above 2 logs, for a log normal distribution with mean 0.18 and standard deviation 0.59 is 0.1%.

2.4.2.2 Including Variability in the Process for all Process Stages

The example in 2.4.2.1 did not include estimates of variability for H_0 or ΣR , but variation does exist. This section assumes variation for H_0 , ΣI and ΣR (values in Table 2.2). The resulting total describes the distribution of levels of *L. monocytogenes* in packages of fresh cut lettuce at the point of consumption, and is equal to the sum of the log means for H_0 , ΣI and ΣR . The mean is not a correct indicator of the risk without considering the variance. The variance of the total distribution equals the sum of the variances, thus the standard deviation is the square root of the sum of the squares of the standard deviations. The distributions are illustrated in Fig. 2.1. Given this distribution of outcomes, the proportion of packages of lettuce not meeting an FSO=2 in this example is 0.2%.

2.4.2.3 Ineffective Washing Step

Assuming that the lettuce washing step (ΣR) is not effective in reducing the level of *L. monocytogenes* (Table 2.3, Fig. 2.2), the overall effectiveness of the process can be determined. The mean log level of *L. monocytogenes* in packages of fresh cut lettuce increases from -1.2 to 0.2 and the overall standard deviation of the level decreases from 1.11 to 0.99 . The proportion of packages that have *L. monocytogenes* levels above the FSO ($2 \log \text{CFU/g}$) at the point of consumption increases to 3.5% (Table 2.3). Note that the standard deviation does not differ much since the overall standard deviation

Table 2.3 Impact of a lettuce washing step (ΣR) that does not reduce *L. monocytogenes* levels on the proportion of packages of fresh cut lettuce that do not meet the Food Safety Objective

	H_0	ΣR	ΣI	Total ^a	
Mean log	-2.5	0	2.7	0.2	$H_0 - \Sigma R + \Sigma I$
sd	0.80	-	0.59	0.99	$\text{sd} = \sqrt{\text{sd}_1^2 + \text{sd}_2^2 + \text{sd}_3^2}$
			$P(>\text{FSO})$	3.5%	

^aThe level ($\log \text{CFU/g}$) of *L. monocytogenes* present in a package of lettuce at the point of consumption

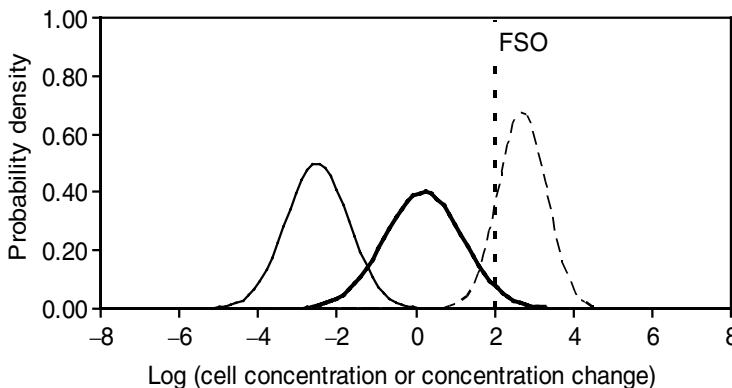


Fig. 2.2 Probability distribution of the initial cell level (H_0 —), increase in concentration (ΣI - - -) and resulting overall final distribution (—) of the levels of *L. monocytogenes* in packages of lettuce at the point of consumption for a process in which the washing step does not reduce the level of *L. monocytogenes* ($\Sigma R=0$), following the input values in Table 2.3

Table 2.4 The impact of shortening the shelf life of the product from 14 to 7 days, thus reducing the level of growth (ΣI) on the proportion of packages of fresh cut lettuce that do not meet the Food Safety Objective

	H_0	ΣR	ΣI	Total ¹	
mean log	-2.5	1.4	1.9	-2	$H_0 - \Sigma R + \Sigma I$
sd	0.80	0.50	0.56	1.10	$sd = \sqrt{sd_1^2 + sd_2^2 + sd_3^2}$
				P(>FSO)	0.01%

¹ The level (log CFU/g) of *L. monocytogenes* present in a package of lettuce at the point of consumption

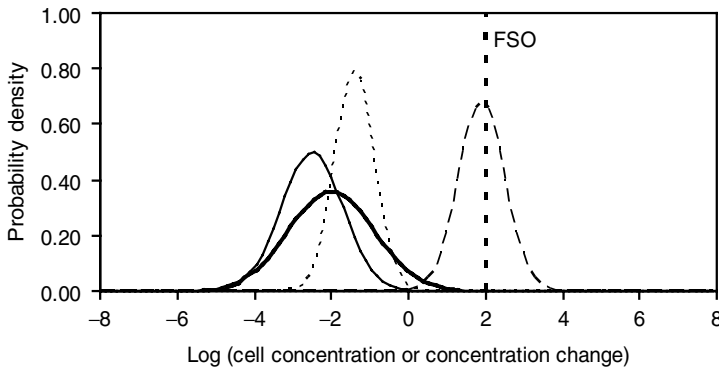


Fig. 2.3 Probability distribution of the initial level (H_0 —), reduction in concentration ($-\Sigma R$ - -), increase in concentration (ΣI - · -) and resulting final distribution of *L. monocytogenes* levels in packages of lettuce at the point of consumption (—) for a product with a shortened shelf life (see Table 2.4)

tion is influenced by the largest contributors, which is H_0 in this example. Due to the ineffectiveness of the washing procedure, a higher proportion (3.5%) of packages do not meet the FSO (2 log CFU/g).

2.4.2.4 Effect of Shortening the Shelf Life of the Packaged Lettuce

If the product contains pathogens and supports growth of the pathogen, the length of the shelf life can influence the impact on public health. In this example, the effect of a shorter shelf life on the proportion of packages of lettuce that do not meet the FSO is evaluated by reducing the predicted value for ΣI . If the product is stored for 7 days at 8°C, rather than 14 days, the increase in *L. monocytogenes* over 7 days is estimated to be 1.9 log CFU/g with a standard deviation of 0.56 (Szabo et al. 2003) (Table 2.4, Fig. 2.3). By decreasing the shelf life, which decreases the extent of growth of *L. monocytogenes*, the proportion of packages of lettuce that do not meet the FSO is decreased to 0.01% compared to 0.2%, over a 10-fold decrease in risk.

2.4.2.5 Meeting the FSO by Changing Levels or Variability

The same proportion of products can meet an FSO, by reducing the variability of one of the inputs. For example, if the variability of the initial levels of *L. monocytogenes* on the raw materials is reduced from 0.8 to 0.4, the level of *L. monocytogenes* reduction required during the lettuce washing step (ΣR)

Table 2.5 Effect of reducing variability of H_0 and lowering ΣR during washing on the proportion of packages of fresh cut lettuce that do not meet the FSO (compare to Table 2.2)

	H_0	ΣR	ΣI	Total ¹	
mean log	-2.5	0.7	2.7	-0.5	$H_0 - \Sigma R + \Sigma I$
sd	0.40	0.50	0.59	0.87	$sd = \sqrt{sd_1^2 + sd_2^2 + sd_3^2}$
			P(>FSO)	0.2%	

¹The level (log CFU/g) of *L. monocytogenes* present in a package of lettuce at the point of consumption

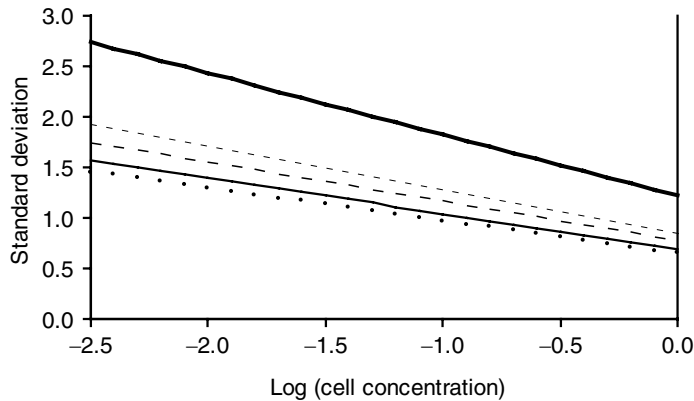


Fig. 2.4 Various combinations of mean log cell levels and standard deviation of the combined distributions for H_0 , ΣR and ΣI resulting in a particular proportion of product that does not meet the FSO=2 log CFU/g. Lines represent percent of products not meeting the FSO. Proportion not satisfying the criterion: 0.1% defective (· · ·), 0.2% defective (—), 0.5% defective (— —), 1.0% defective (- - -), 2.0% defective (— — —)

could be decreased from 1.4 to 0.7 with the same proportion of product meeting the FSO (Table 2.5). While the practicality of reducing the standard deviation for a raw agricultural commodity such as lettuce may be difficult to achieve given control measures available at this time, this strategy may be applicable for other product types.

2.4.3 Log Mean Value, Standard Deviation and Meeting the FSO

The proportion of products in which the level of the microorganism of concern is above the FSO or PO is determined by both the mean log levels and the standard deviation of the combined distributions for H_0 , ΣR and ΣI . Different combinations of the mean and standard deviation resulting in the same overall proportion of products not meeting the FSO can be calculated. The results are shown in Fig. 2.4.

The examples presented in this chapter illustrate the impact of both the mean log level and the variability of H_0 , ΣR and ΣI on the proportion of product meeting the FSO. With this deeper level of understanding of the influence of both the levels and variability of the initial microbiological load on the incoming materials, the steps in the process that reduce the level of the microorganism of concern and the increase of the pathogen of concern during storage and distribution, a food manufacturer can determine where they can have the biggest impact on ensuring that the appropriate proportion of product meets the FSO. Control strategies can focus on decreasing variability of the process, decreasing the initial level of the microorganism of concern on the raw materials, or other parameters based on

the levels or variability observed for a particular situation. Calculations used for Fig. 2.4 are presented in Appendix B.

The following assumptions are made with these calculations:

- All variables are assumed log normally distributed, therefore the log of the variables as used in the FSO equation is normally distributed. This also makes their sum in the FSO equation have a normal distribution. If values have other distributions, Monte-Carlo type calculations are needed to determine the statistical distribution of the sum. While a normal distribution for log initial level, log increase and log reduction is often described in the literature, in real life the distribution of pathogens may be highly heterogeneous and not possible to describe by a log normal distribution.
- These examples assume that calculations hold even for very low levels. This may have further implications in some situations. For example, if a 6D inactivation step is applied to containers with a 100-g unit size and an initial concentration of 2 log CFU/g, the calculated level in each unit after inactivation is -4 log CFU/g. If each CFU contains only one microorganism, then this process would actually yield one microorganism in one 100 g unit (i.e., -2 log CFU/g) for every 100 units produced (1% of the units). The other 99% of the units would be free of the microorganism. For some microorganisms, a CFU may contain more than one cell, thus a greater percentage of units could theoretically contain a contaminant. This illustrates the importance of using these calculations as general principles to compare the relative effect of changes to a food safety management strategy rather than as absolute figures.
- If no data on standard deviation are available but minimum and maximum data are known, representing the range where 95% of the data will lie, the standard deviation can be estimated by $sd = 0.5 \times (\text{maximum} - \text{minimum}) / 1.96^2$.

2.5 Validation of Cleaning and Other GHP Control Measures

Effective application of GHP provides the foundation upon which HACCP systems are developed and implemented. Failure to maintain and implement GHP can invalidate a HACCP system and result in the production of unsafe food.

Effective control of a hazard in a food necessitates consideration of the components of GHP likely to have significant impact in controlling the hazard. For example, incoming material requirements are very important to control the risks of certain hazards in seafood (e.g., paralytic shellfish poisoning, ciguatera toxin, scombroid poisoning). Incoming material requirements are of lesser importance for a food that will be cooked sufficiently to eliminate vegetative pathogens (e.g., salmonellae in raw meat or poultry) that may be present. Thus, the various components of GHP do not carry equal weight in all food operations. It is necessary to consider the hazards that are most likely to occur and then apply those GHP that will be effective for controlling the hazards. This does not mean that the other components of GHP, such as equipment maintenance or calibration, are ignored. Some are very important to ensure a food meets established safety and quality requirements.

In certain situations selected components of GHP may carry particular significance and should be incorporated into the HACCP plan. For example, equipment maintenance and calibration are important for large continuous ovens used in cooking meat products. In this example, the procedure and frequency (e. g., monthly, quarterly) for conducting checks on heat distribution during cooking could

²The minimum and maximum 95% limits are $\text{minimum} = \text{average} - 1.96sd$; $\text{maximum} = \text{average} + 1.96sd$. This results in $\text{maximum} - \text{minimum} = 2 \times 1.96sd$, so $sd = 0.5(\text{maximum} - \text{minimum}) / 1.96$.

be incorporated into the HACCP plan as a verification procedure. In addition, it is necessary to verify the accuracy of the thermometers used for monitoring oven temperatures during cooking.

Information on hygienic design of facilities and equipment, cleaning and disinfection, health and hygiene of personnel, and education and training was discussed previously (ICMSF 1988). Preventing contamination or recontamination of the product during processing is a critical component of a control program. Validation means that the facilities and equipment, choice of cleaners and sanitizers, and conduct of the operations are designed to achieve the necessary level of control. Initial considerations in designing the sanitation program include food characteristics, equipment construction and materials, and microorganisms of concern for safety and spoilage. Validation of the program ensures all parts of the system are properly treated to remove food soil and inactivate microorganisms. Residual food soil in wet environments not only provides a source of nutrients for subsequent microbial growth, but also can reduce the effectiveness of sanitation steps. Clean-in-place (CIP) systems require careful verification that all parts are treated and that the system operates as intended.

The effectiveness of many sanitizers is affected by the presence of organic residues from the food and processing environment. Scientific criteria needed to determine a sanitizer's immediate and residual effect include:

- Concentration of the sanitizer and conditions for efficacy (e.g., temperature).
- Immediate and long term antimicrobial effectiveness (stability of the sanitizer).
- Microorganism susceptibility to the sanitizer.
- Characteristics of the surfaces to be sanitized (temperature, organic load).
- Impact of processing steps (thermal treatments, packaging conditions).

As with validation of other components of the food process, validation of the sanitation program is the accumulation of knowledge from laboratory, pilot plant and commercial facility studies. Sufficient information of increasing specificity needs to be acquired to ensure the functioning of the process will be understood. In laboratory studies, pathogens can be inoculated into media or product. Specialized pilot plant studies might use pathogens if exposure to food and humans can be controlled; however, GMP plants must use surrogates. In commercial facilities, data is acquired using surrogates when pathogen presence is a rare event, or from monitoring when naturally-occurring pathogens are present in sufficient frequencies and numbers (e.g., in slaughter operations). Appropriate pathogen strains or surrogates must be used. Chemical agents must be tested according to directions using potable water of appropriate hardness, concentration, pH, temperature and contact time. Variations in the food and process must be considered, the critical factors that determine the margin of safety identified and the minimum lethal treatment specified to be assured that appropriate control will always be achieved. Periodic verification is necessary to ensure that efficacy is not lost over time (e.g., due to development of resistance).

2.6 Shelf Life Determination

One approach to management of the safety of the food is to have the food spoil and be rejected by the consumer for poor quality before pathogens that might be present grow to levels that become a public health threat. In the absence of spoilage, other means of limiting shelf life such as use-by labeling or time-temperature indicators could be employed. These issues are discussed below and in more detail in NACMCF (2005).

Distribution and storage conditions may include moderate time and temperature abuse. Process design and validation should include these conditions when validating that the products meet the FSO. Decisions about the temperature abuse can be based in part on retail and home storage temperatures survey databases from e.g., EcoSure (2008) where retail display temperatures varied by product type (5% of home refrigerators exceeded 7.2°C and 0.7% exceeded 10°C). For some products and

regions, a shelf life short enough to cope with the growth at abusive temperatures may result in times that do not permit normal commercial handling or meet consumer's expectations. Specifying the maximum storage temperatures is a public health risk management decision.

Shelf life validation would include determining the distribution of contamination at the end of processing and establishing a PO at that point. The allowable amount of growth that potentially could occur for the food to still meet the FSO can then be determined. With specification of the maximum abuse temperature, laboratory and challenge testing can determine the length of time for repair/lag and growth before exceeding the FSO as explained in previous examples.

For foods that are continually refrigerated from manufacture to consumption, the use-by date can be estimated by the manufacturer. Times for commercial and retail periods and home storage are included in the determination and a calendar date can be applied by the manufacturer. If a food is frozen and then thawed at retail, the growth time is the remaining retail and home storage time. For this product, a label indicating the number of days after purchase is appropriate.

Time temperature integrators (TTI) for retail packages produce a noticeable color change at the end of the allowable storage based on a biological, physical or chemical reaction. The kinetics of the reaction varies among devices and end points may be set for specific time/temperature standards, for quality concerns or theoretically for growth in a specific food-pathogen combination. TTIs are not widely used on consumer packages in 2010 because high cost, complexity of reaction kinetics for different food/microorganism combinations, and lack of consumer awareness and understanding have limited their use. TTIs have a potential benefit of indicating the end of the permissible shelf life because the ongoing reaction rate is continuously affected by the temperature. If the temperature is below the designated optimum, the rate is correspondingly slowed and the time before the indicating color change is lengthened. If the temperature exceeds the designated optimum, the TTI reaction rate appropriately shortens the storage time. Future developments may make it possible to choose a TTI that continuously monitors the temperature during the entire storage period and provides an end point specific to the conditions that a specific individual package experiences.

2.7 When to Revalidate

Validation data should be periodically reviewed to determine whether new scientific data or changes in operating conditions would alter the previous validation conclusions. Emergence of a new pathogen requires re-evaluation of the process based on the characteristics of the pathogen. A change in the initial contamination of the ingredients, the formulation of the product, processing parameters or the storage conditions of a food may require the process be revalidated. The impact of specific changes on the concentration, homogeneity or frequency of contamination for the affected step should be estimated. This information may be obtained from the literature, models, and laboratory or pilot plant experiments. The magnitude of the change can be compared to the corresponding mean log and standard deviation of the validated process. If the change is within the values of the original validation, there may be no need for further validation. The final impact of the change at the point of consumption can be estimated and compared to the FSO. For example, a 0.2 log increase in the contamination of an ingredient may increase the contamination by 0.2 log for all subsequent steps to consumption. If this increase does not result in exceeding the FSO, further validation is not needed. However, if the change in the process was an increase in pH that permitted a 1 log increase in pathogen concentration at consumption, this process would likely require revalidation. It would perhaps require redesign of the process to compensate elsewhere for the increased growth and revalidation of the new process.

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