Relating microbiological criteria to food safety objectives and performance objectives

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ABSTRACT

Microbiological criteria, food safety objectives and performance objectives, and the relationship between them are discussed and described in the context of risk-based food safety management. A modified method to quantify the sensitivity of attributes sampling plans is presented to show how sampling plans can be designed to assess a microbiological criterion. Examples presented show that testing of processed foods for confirmation of safety is often not a practical option, because too many samples would need to be analysed. Nonetheless, in such cases the classical “ICMSF cases” and sampling schemes still offer a risk-based approach for examining food lots for regulatory or trade purposes.

1. Introduction

The Risk Analysis framework described by Codex Alimentarius (CAC, 2007a) provides a structured approach to the management of the safety of food. In the Codex document on Microbiological Risk Management (CAC, 2007a) and in ICMSF’s “Microorganisms in Foods 7: Microbiological Testing in Food Safety Management” (ICMSF, 2002), the establishment of a food safety objective (FSO) is described as a tool to meet a public health goal such as an appropriate level of protection (ALOP). More recently, an FAO/WHO expert consultation re-emphasised the original definition for ALOP that was part of the Sanitary and Phytosanitary (SPS) Measures Agreement (WTO, 1994), namely that it is the “expression of the level of protection in relation to food safety that is currently achieved. Hence, it is not an expression of a future or desirable level of protection” (FAO/WHO, 2006). An FSO specifies the maximum permissible level of a microbiological hazard in a food at the moment of consumption. Maximum hazard levels at other points along the food chain are called performance objectives (POs). The current definitions for FSO and PO (CAC, 2007b) are that an FSO is “the maximum frequency and/or concentration of a hazard at a specified step in the food chain before consumption that provides or contributes to an FSO or ALOP, as applicable”. Safe food is produced by adhering to good hygienic practices (GHP), good manufacturing practices (GMP), good agricultural practices (GAP), etc. and implementation of food safety risk management systems such as hazard analysis critical control points (HACCP), but the level of safety that these food safety systems are expected to deliver has seldom been defined in quantitative terms. Establishment of FSOs and POs provides the industry with quantitative targets to be met. When necessary, industry may have to validate that their food safety system is capable of controlling the hazard of concern, i.e., to provide evidence that control measures can meet the targets. In addition, industry must periodically verify that their measures are functioning as intended. To assess compliance with FSOs and POs, control authorities rely on inspection procedures (e.g., physical examination of manufacturing facilities, review of HACCP monitoring and verification records, analysis of samples) to verify the adequacy of control measures adopted by industry. In the context of the SPS Agreement (WTO, 1994), national governments may also need to quantitatively demonstrate the equivalence of their inspection procedures to ensure that food safety concerns do not result in an inappropriate barrier to trade. Similarly, a control authority may require individual manufacturers to provide evidence of equivalence of control measures, particularly when non-traditional technologies are being used to control a hazard.
Although FSOs and POs are expressed in quantitative terms, they are not microbiological criteria which are defined as the acceptability of a product or a food lot, based on the absence/presence or number of microorganisms including parasites, and/or quantity of their toxins/metabolites, per unit(s) of mass, volume, area or lot (CAC, 1997; ICMSF, 2002). A more detailed description of the elements and uses of microbiological criteria are presented in Section 2, below.

Microbiological testing is one of the potential tools that can be used to evaluate whether a food safety risk management system is providing the level of control it was designed to deliver. It is one of a number of tools that, when used correctly, can provide industry and regulatory authorities with tangible evidence of control.

A number of different types of microbiological testing may be used by industry and government (e.g., within lot, process control, investigational). One of the forms of testing most commonly used in relation to microbiological criteria is within-lot testing, which compares the level of a microbiological hazard detected in a food against a pre-specified limit, i.e., a microbiological criterion (MC; ICMSF, 2002). Microbiological criteria are designed to determine adherence to GHPs and HACCP (i.e., verification) when more effective and efficient means are not available. FSOs and POs are targets to be met. In this context, microbiological criteria based on within-lot testing are meant to provide a statistically-designed means for determining whether these targets are being achieved. Such sampling plans need to consider either:

(i) the ‘consumer’s risk’, i.e., the chance that a lot will be accepted that exceeds a level that has been determined, usually by government, to pose an unacceptable risk to public health and which, for convenience here, we will call ‘Acceptable Level for Safety’ (ALS, see Appendix 1), or
(ii) the ‘producer’s risk’, i.e., the possibility that an acceptable lot will be rejected by the sampling scheme (see also Section 5, below), recognising that both ‘risks’ are interdependent.

The current paper provides information on the data that are necessary, and the types of decisions that have to be made, to develop meaningful sampling plans and ensure that microbiological criteria based on within-lot microbiological testing are being used appropriately. For the purposes of this paper, a lot is considered a grouping of a product manufactured during a certain period of time or under the same conditions, or a consignment of a food arriving at a border. A sample is taken from that lot to assess the concentration of the hazard in that sample. A sample may comprise the entire analytical unit, or the analytical unit may be an aliquot derived from the sample. It is assumed that the concentration of the hazard in an aliquot of the sample is representative of the concentration in the whole sample, but that different samples can have different concentrations.

2. Nature and use of microbiological criteria

Developing meaningful within-lot microbiological criteria for a food or ingredient is a complex process that requires considerable effort. Furthermore, their application demands considerable resources. Therefore, microbiological criteria should be established only when there is a need and when it can be shown to be effective and practical. The criterion must be capable of accomplishing one or more clearly defined objectives, such as to assess:

– the safety of a food;
– adherence, on a lot-by-lot basis, to GHP and/or HACCP requirements;
– the acceptability of a food or ingredient from another country or region for which the history of the product is unknown or uncer-

tain, i.e., evidence of adherence to GHP or HACCP-based control systems is not available;
– compliance of a food with an FSO and/or a PO.

An MC consists of:

– a statement of the microorganism(s) of concern and/or their toxins/metabolites and the reason for that concern;
– the food to which the criterion applies;
– the specific point(s) in the food chain where the MC should be applied;
– microbiological limits considered appropriate to the food at that specified point(s) of the food chain, and
– a sampling plan defining the number and size of samples to be taken, and the method of sampling and handling,
– the number and size of the analytical units to be tested. For the purposes of this manuscript a sample refers to the portion of a batch that is collected and sent to a laboratory for testing. Part, or all, of the sample is analyzed. The actual amount of the sample that is analysed is the “analytical unit”. For example, if a product was sold in 100 g packages, and one package of a lot was sent to the laboratory for analysis, this would be the sample. If 50 g was removed from the package and then divided into two 25-g aliquots that were then tested separately, then one would have two 25-g analytical units (n = 2).
– the analytical methods to be used to detect and/or quantify the microorganism(s) or their toxins/metabolites;
– the number of analytical units that should conform to these limits; and
– any actions to be taken when the criterion is not met.

An MC can be used to define the microbiological quality of raw materials, food ingredients, and end-products at any stage in the food chain, or can be used to evaluate or compare the stringency of alternative food control systems and product and process requirements. Three classes of MC are distinguished based on regulatory consequences (ICMSF, 2002):

– ‘Standards’ are microbiological criteria that are written into law or government regulations, e.g., an MC specified by government to protect public health.
– ‘Specifications’ are microbiological criteria established between buyers and producers that define product quality and safety attributes required by the buyer; failure to meet the MC could result in rejection of the product or a reduction in price.
– ‘Guidelines’ are microbiological criteria that provide advice to industry about acceptable or expected microbial levels when the food production process is under control. They are used by producers, to assess their own processes and by government inspectors when conducting audits.

To develop an MC, the following information is needed:

– the distribution of the microorganism within the lot,
– the sensitivity and specificity of the test method,
– the randomness and efficacy of the sampling scheme (i.e., number and size of samples, that samples are randomly drawn from the batch),

and several decisions have to be made, e.g.,

– the quality/safety level as expressed in an FSO or PO, that is required, e.g., absence of E. coli O157 in 99% of 100 ml packages of apple juice,
the expected standard deviation of counts in samples taken from
the lot. (From these first two decisions, the microbiological sta-
tus of a lot that is just acceptable can be inferred),
– the statistical confidence required for the acceptance or rejec-
tion of a non-conforming lot (see Appendix 1),
– the required level of benefit derived from the application of an
MC compared to cost of testing or the potential consequences
of not applying and enforcing an MC.

It should be emphasised that statistical interpretation of test re-
sults can be misleading if the representativeness of the samples ta-
taken from the lot as a whole, or homogeneity of contamination
within a lot, cannot be assumed. Historical data relating to that
product and/or process are often relied upon when knowledge
about the distribution and variability of microorganisms in a spe-
cific lot of food is unknown. Several of the points mentioned above
will be further elaborated in the following sections.

3. Distribution of the pathogen of concern

The distribution of pathogens within the lot must be under-
stood if informed decisions are to be made concerning the applica-
ability of within-lot microbiological testing to verify compliance
with GHP/HACCP or to determine whether a food lot meets an
FSO or PO. Often, however, this is not known and, to enable com-
parison of the relative stringency of sampling plans, an assumed
distribution is used. Furthermore, the level and standard deviation
associated with a microbial population is often dynamic as a food
proceeds along the food chain. A pathogen may be present in the
raw material, but it may be partly or totally eliminated during pro-
cessing or preparation. It may be reintroduced as a result of subse-
quent contamination, or increase its concentration over time in
products that support its growth. This can influence the prevalence
and/or concentration in any specific lot. In an “ideal” situation,
microorganisms would be homogeneously distributed throughout
the lot, so that whatever sample is taken, it would have the same
level of contamination. Apart from liquid foods or after mixing pro-
cesses, this is usually not the case and, instead, the pathogens are
heterogeneously distributed. In many situations the frequency dis-
bution of the contamination levels across samples can be de-
scribed as log-normal (Jarvis, 1989), i.e., having a normal
distribution with expressed as log CPU values, and characterised
by a mean log concentration and a standard deviation. Ideally, to
apply statistical interpretations of non-stratified sampling plans
(i.e. when there is no reason to assume systematic differences be-
tween different samples), samples should be taken at random if the
hazard is heterogeneously distributed in the lot. Random sampling
cannot always be assured, nor the distribution assumed always to
be log-normal. However, experience has indicated that in most in-
stances these assumptions are appropriate for certain microorgan-
isms or groups of microorganisms. For illustration purposes in this
paper a log-normal distribution of the pathogen of concern in a
food is assumed because it provides the basis for establishing a
mathematical relationship between FSOs, POs and microbiological
criteria.

4. Performance of microbiological criteria

The ‘operating characteristic’ (OC) curve is a graph that relates
the probability of accepting a lot, based on the number of units
tested, to the proportion of units, or aliquots in the lot that do ex-
ceed some specified acceptable level, i.e., the maximum tolerated
defect rate. The OC-curve depends on both the number of samples
tested, ‘n’, and the maximum number, ‘c’, of those samples that
may exceed the specified level.

While not the usual situation, if the distribution of a pathogen
in a lot of food is known, an OC-curve can be generated to charac-
terise the performance of an MC (see Appendix 1) and to translate
information about the proportion of units that are defective into an
estimate of the concentration of the contaminant in the lot. OC-
curves can be used to evaluate the influence that parameters of
the MC, i.e. number of samples (n), microbiological limit (m), num-
ber of samples in excess of ‘m’ that would lead to rejection of the
batch or lot, (c), and the mean and standard deviation of the under-
lying lot distribution, have on the efficacy of the microbiological
testing program. This information quantifies the confidence that
we can have that a ‘defective’ lot will be rejected. If one were able
to test every unit of food within the lot, the OC-curve would
change from 100% probability of acceptance to a 100% probability
of rejection exactly at the proportion of defective units that distin-
guishes an acceptable from a defective lot. At the other extreme,
taking a single sample, particularly if negative, has virtually no
ability to discriminate between conforming and non-confirming
lots. Increasing the number of samples (n) examined is one of the
primary means for increasing the ability of a sampling plan to dis-
criminate ‘acceptable’ from ‘defective’ lots.

Evaluation of the OC-curves for the proposed MC is a critical
step in ensuring that the MC is able to assess whether food lots sat-
sify an FSO or PO. Thus, when an MC has to be set, a number of
decisions have to be made. These will be illustrated below.

5. Probabilities of accepting or rejecting lots

In the design of sampling plans it is necessary to define the
probability that a “defective” lot will be rejected.

The choice of this value has public health implications and is,
thus, a risk manager’s task. In the examples selected for illustra-
tion purposes in Section 8 we have chosen a value of 95% probability
of rejection of defective lots. In the following text, the consumer’s ALS
is the mean log concentration level or the proportion defective that
would result in lots contaminated at this level being rejected 95%
of the time. This implies, however, that 5% of the non-conforming
batches contaminated at this level would be accepted. This is called
type II error (i.e., a lot was accepted when it should have been re-
jected), and is referred to as “the consumer’s risk”.

Of concern to food producers is the possibility that, under the
sampling plan, acceptable lots are rejected. If a producer operated
at the level of control required to just meet the consumer ALS,
there would be a substantial number of lots that would fail the
microbiological criterion despite the lot actually meeting the FSO
or PO. This is sometimes called “type I error”, and describes the
producer’s risk. Thus, the producers are interested in determining
the lot quality that would need to be achieved so that there is a
high probability (e.g., 95%) that lots would be accepted and adjust
their production processes accordingly. In this manuscript, it is as-
sumed that the producer is operating with a degree of control that
is greater than that needed to achieve the consumer’s ALS. Thus,
the producer’s ALS is the mean log concentration level or that pro-
portion defective that ensures that lots are accepted 95% of the
time. This percentage could be set at other levels depending on
the willingness of the producer to accept rejection of conforming
lots. Setting either the consumer’s ALS or the producer’s ALS, im-
plies the other. On the other hand, it is not possible to elaborate
statistically-based microbiological criteria unless either the con-
sumer’s, or producer’s, ALS is specified.

6. Nature of an FSO or PO in statistical terms

FSOs are maximum frequencies or levels of pathogens that are
considered tolerable at the moment of consumption; POs specify
Salmonella the home (e.g., <15% of fresh poultry carcasses are contaminated) transfer of microorganisms to ready-to-eat foods. In this instance the PO would be the frequency of contaminated carcasses entering the home (e.g., <15% of fresh poultry carcasses are contaminated with Salmonella), and the FSO would be the frequency/levels of Salmonella–contaminated meals served in the home. This could then be used to reflect the potential control points, i.e., reduce the frequency of contaminated carcasses entering the home and reduce the incidence of cross-contamination.

In principle, an FSO communicates the level of a hazard that is unacceptable in any serving of a food at the moment of consumption while a PO communicates a (related) limit at some other defined point in the food chain. However, depending on the FSO or PO value chosen, some servings will exceed the specified limits because of the expected distribution of contamination levels in the food. It does not necessarily indicate, however, that the system is out of control as long as the proportion of such units is within the limits expected for the distribution (characterised by the standard deviation) around the mean contamination level required to achieve the FSO/PO. Thus, to establish a sampling plan that allows an MC to be specified to verify an FSO (or PO) the proportion of the lot that may be above the nominally ‘acceptable’ level must be specified. In examples given below this value is set at 1%, but other values could have been chosen. This value means that provided that no more than 1% of the lot exceeds the FSO (or PO) then the food safety risk management system is operating as intended. The FSO (or PO) is then understood as being the 99th percentile of a cumulative frequency distribution of log concentrations. Choosing this tolerance is a risk management decision, because it clearly has an influence on the levels of the hazard that are considered acceptable. It also establishes the level of Type II error that risk managers consider acceptable if the manufacturer produces product that just achieves the consumer ALS. It is not possible to elaborate an MC for FSO or PO confirmation purposes without specifying this tolerance.

7. Relating the performance of attributes plans to concentration

Previous evaluation of the performance of ICMSF sampling plans (Legan, Vandeven, Dahms, & Cole, 2001) did not consider the possibility that, even if the concentration of the microorganism of concern in the sample is homogenous, the sampling and enumeration method may over- or under-estimate the actual concentration of microorganisms, particularly if involving enrichment. As the concentration of microorganisms in the food sample increases, there is less likelihood that the sample will produce a false negative. Conversely, as the true concentration decreases there is less likelihood of obtaining a false-positive through sampling ‘chance’, i.e., detecting a target cell even though the overall concentration is below the level considered just acceptable. Therefore, in situations in which microbiological testing of samples involves enrichment and presence/absence testing rather than enumeration, a modification to the approach described by Legan et al. (2001) is appropriate. In the modified method described in Appendix 2, the overall probability of detecting a positive sample in a lot, characterised by a log-normal distribution, is estimated as the total probability of detecting a cell in any of the samples taken from that batch, i.e., by integrating over all possible concentrations in the batch. The probability of sampling any particular concentration in the batch is given by the log-normal distribution and is combined with the Poisson sampling process (and size of the analytical unit) to calculate the probability that a cell will be present in the sample taken and lead to a “positive” result after enrichment. The choice of this Poisson-log-normal is based on the assumption that at both lower and higher contamination levels the concentration of cells is log-normally distributed. This model is only one of several models that could be used, and might not be generally applicable.

8. Establishment of microbiological criteria intended to confirm an FSO/PO

As explained above, to establish an MC to assess compliance with the FSO/PO for a pathogenic microorganism, a series of assumptions/decisions must be made.

1. An assumption must firstly be made regarding the distribution of the pathogens in the lot of food. In the examples provided, we assume that the pathogens of concern are log-normally distributed and that the standard deviation (s.d.) is known. In the absence of available data, a log-normal distribution is often assumed and a default value for the standard deviation applied. For the purposes of the current examples, a standard deviation = 0.2 log10 CFU g⁻¹ is used to describe a food in which microbes would be expected to be rather homogeneously distributed within a batch (e.g., for liquid food with a high degree of mixing). A standard deviation of 0.4 log10 CFU g⁻¹ is assumed for a food of intermediate homogeneity (e.g., ground beef) and a standard deviation = 0.80 log10 CFU g⁻¹ for an inhomogeneous food (e.g., solid food). It could be that in certain cases even larger inhomogeneity could occur, e.g., if clumping occurs (Wilson et al., 1935) or if the contamination is restricted to surface contamination of a food.

2. The second requirement is to define the “maximum frequency and/or concentration” of the hazard that will be used to specify the FSO/PO, including what proportion (e.g., 95%, 99%, 99.9%, etc.) of the distribution of possible concentrations must satisfy the test limit so that the FSO/PO is met.

3. The third decision is to specify the level of confidence needed that a non-conforming lot is detected and rejected (i.e., the consumer’s ALS; examples below consider 95% or 99% confidence). Alternatively, the probability of rejecting a conforming lot (i.e., the producer’s risk) may be considered.

4. The fourth decision is the analytical methodology that should be employed.

The following examples illustrate the consequences of such decisions on the numbers of samples and/or sample sizes and analytical methodology required to assess compliance with the FSO/PO.

8.1. Salmonella in ice cream

Ice cream is a product that, when properly handled, does not support microbial growth. Thus, the PO for ice cream can be the same as the FSO. In the examples, three FSOS/POs are considered (one Salmonella per 100 g, one per kg and one per 10 kg; see
Table A2.1) and two possible standard deviations (0.4 and 0.8) are selected to illustrate the calculations. The maximum mean concentrations that can be tolerated, corresponding to each assumption about the standard deviation, are determined by subtracting a certain number of standard deviations from the hazard concentration nominated as the FSO so that the required percentage of the lot will have concentrations below the FSO/PO. The required number of standard deviations is called the z score. For example, to deduce a mean concentration in the lot such that 99% of the units are at or below the target FSO requires that 2.33 standard deviations are subtracted from the FSO. Determination of the number of samples to be examined to assess compliance is illustrated for three analytical sample sizes (25 g, 100 g and 250 g). The number of analytical units that need to be tested to have 95% confidence of rejection of non-conforming batches (with non-conforming batches defined as a lot with more than 1% of the units above the FSO) is shown in Table A2.1.

Table A2.1 illustrates that with increasing stringency of the FSO/PO, i.e., from 1 Salmonella/100 g to 1/10 kg, the number of samples that need to be analysed for confirmation becomes unacceptable for practical reasons. Table A2.1 also shows the changes in the number of samples that must be tested to assess compliance when the standard deviation (of the distribution of concentrations of Salmonella) increases from 0.4 to 0.8 log10 CFU g\(^{-1}\). A higher standard deviation also means that the mean log concentration that must be achieved so as not to exceed the FSO/PO must be decreased.

The effect of the size of the analytical unit on the number of samples that must be analysed, using the modified method described in Appendix 2, is demonstrable when one of the examples from Table A2.1 is considered in more detail. For the example, let us assume that the FSO/PO is set at one Salmonella per 100 g and the concentration of Salmonella is described as having a mean log10 concentration of \(-2.93\) with a standard deviation of log10 0.4. If the analytical unit is 25 g, 69 samples need to be analysed to determine compliance with the FSO/PO. If units of 100 g are taken, 19 samples need to be analysed and, in the case of 250 g analytical units, this number is reduced to nine. Clearly an increase in the size of the analytical units a reduction in number of samples can be achieved, although the validity of enrichment methods involving increased samples sizes (and potentially lower concentrations of the hazard) should also be considered (Jarvis, 2007). In general, however, if the PO is set at a level lower than one Salmonella per 100 g, testing may not be a practical option for assessing compliance.

8.2. Listeria monocytogenes in cold-smoked salmon

If a product permits the growth of the pathogen of concern during its shelf life, the PO will be lower than the FSO to take into account the growth that may be expected to occur between the point to which the PO relates and the point of consumption. Using as an example management of the risk from L. monocytogenes in cold-smoked salmon, we illustrate here how one might derive a PO at point of manufacture from a specified FSO. For the sake of the illustration a number of (untested) simplifying assumptions have been made. Thus, it is assumed that:

(i) the product has a shelf life of two weeks from point of manufacture to point of consumption when stored at or below 4 °C,
(ii) the specified FSO is 3.3 log10 CFU g\(^{-1}\).

The required PO would be 2.7 (3.3 – 0.6, the maximum increase of L. monocytogenes during shelf life) log\(_{10}\) CFU g\(^{-1}\). To ensure that the PO would be met by 99% of the food in the lot, the maximum permitted level should be 2.33 × SD below the calculated PO value of 2.7. Accordingly, the corresponding log-normally distributed population with an s.d. of 0.4 log10 CFU g\(^{-1}\) should have a mean concentration of 1.77 log10 CFU g\(^{-1}\) or less (2.7 – 2.33 × 0.4). Using statistical calculations it can be established that in order to reject a non-conforming lot with a 95% probability, requires that 10 samples (n) be tested: if any sample has \(\geq 100 \text{ L. monocytogenes } g^{-1}\) the lot does not conform.

To illustrate the effect of detection levels, three methods of analysis for L. monocytogenes are considered:

- a plate count technique able to determine \(\geq 100 \text{ L. monocytogenes } g^{-1}\);
- an 1/0.1/0.01 g three-tube MPN procedure with a lower limit of detection of 0.3 L. monocytogenes g\(^{-1}\), and
- an enrichment technique that examines a single analytical unit of 25 g.

In the example above, if the test used was presence/absence in 25 g, only one sample needs to be analysed to assure rejection (with >95% probability) of the lot. This is because the sample size is large relative to the required mean concentration that is commensurate with an acceptable batch, i.e., 1.77 log10 CFU g\(^{-1}\). Thus, a 25 g sample from a batch with acceptable mean concentration would almost certainly contain L. monocytogenes and return a positive result. However, using this presence/absence test or using the lowest level of detection with an MPN method has a substantial type I error; i.e., the risk of unnecessarily rejecting lots, as well as sometimes incorrectly accepting lots because sampling plans using only a single sample have limited discriminatory ability unless the sampling involves the compositing of randomly selected subsamples, e.g., a 25 g analytical unit consisting of the compositing of 25 1-g samples.

In Table A2.2 the key figures for the consumer’s ALS and the producer’s ALS (number of samples required and mean concentrations) for three distributions (s.d.’s of 0.2, 0.4 and 0.8) are presented, calculated to meet three FSOs. These figures show, for instance, that as the s.d. increases, the mean concentration needs to be reduced so as not to exceed the FSO/PO. The figures for the producer’s ALS demonstrate that the mean concentration of the pathogen in the lot should be lower than that calculated to be required to satisfy the consumer’s ALS. The numbers of samples that are required to be analysed show the same trends as discussed above. The figures also show that at the lowest values of the FSO/PO the m value can no longer be (realistically) set at 100 CFU g\(^{-1}\).

8.3. Salmonella in frozen poultry

In this example we illustrate the establishment of microbiological criteria designed to satisfy POs. Frozen poultry will be cooked before consumption, thus the PO will differ from the FSO (and may be higher than it). In Table A2.3 three POs were chosen to illustrate the effect these levels have on the number of samples that need to be analysed. The analytical unit in all three cases is the same, e.g., 5 g of neck-skin (Notermans, Kampelmacher, & Van Schothorst, 1975). If the PO is formulated as: “not more than 15% of chicken carcasses in a lot may test positive for Salmonella” and the consumer’s ALS is set at 95% probability, the analysis of 19 samples is sufficient to assess compliance of the lot. If a 10% contamination level is chosen, 29
samples are needed to assess compliance; if 5% is specified as the PO then 59 samples must be tested. Thus, as illustrated in Table A2.3, to produce lots that have a 95% probability of complying with these consumer ALS requirements, i.e., that no more than 15%, no more than 10% or no more than 5% of carcasses are contaminated with Salmonella, the producer needs to ensure that not more than 0.27%, 0.18% and 0.09%, respectively, of the carcasses are contaminated.

9. Developing microbiological criteria for pathogens when no FSOS/POs have been established and when no historical data are available

Ideally, verifying whether an FSO/PO is met is done at the site where the food is produced. However, in practice this is not always possible, or other circumstances require that control authorities have to assess the safety of lots of food and have to undertake testing themselves in the absence of historical data about contamination levels, and variation in contamination levels, in lots of that product. For this purpose ICMSF (1986, 2002) developed a series of "cases", and proposed sampling plans. Although, these sampling plans were not designed to assess compliance with an FSO/PO, using the analytical approach presented here it is possible to explore the numerical limits that correspond to the 'cases', i.e., FSOS/POs that are implicit in the sampling schemes corresponding to the 'cases'. Appendix 3 illustrates how one can derive an FSO/PO from a particular sampling plan.

Following the approach as set out in Appendix 2, the recommended sampling plan for Salmonella in ice cream can be analysed. In this example it is assumed that random sampling can be applied and that the standard deviation (s.d.) is 0.8 log10 CFU g⁻¹. The product/hazard combination is best described by case 11 for which no Salmonella should be detected in 10 samples of 25 g (i.e., c = 0, n = 10, m = 0/25 g). When the probability of rejection (consumer's ALS) is set at 95%, lots with a mean log concentration of ≥ -2.25, which corresponds to > 6 Salmonella per kg (or one per 179 g), will be rejected with at least 95% probability. With this sampling plan it would be possible to ensure, with 95% confidence, that a lot of food in which > 1% of servings have a concentration of Salmonella ≥ -0.39 log10 CFU g⁻¹ (ca. 0.4 Salmonella g⁻¹) would be rejected. For a producer who wants to ensure that that this food meets the MC with 95% probability (producer's ALS: mean log count accepted with 95% probability), the mean log concentration would need to be < -4.4 log10 CFU g⁻¹ of Salmonella (< 4 CFU 100 kg⁻¹).

It is important to note that the s.d. of 0.8 log units was based on data from national surveillance programmes for Salmonella, E. coli O157:H7 and some other pathogens in mainly raw products (Legan et al., 2001). The mean and s.d. associated with these data reflect the variability among a large number of different processors and practices. Even a few lots with higher concentrations can have a large effect on the s.d. value. Establishing an MC based on the standard deviation for all products of a certain category at a specified point in the food chain may be unnecessarily stringent for "good" producers, i.e. those that produce at a more consistent standard. Lots from a single producer will typically have a smaller s.d. A producer, who knows the s.d. of his/her products, could recalculate the likelihood that their product would be rejected. This is an advantage of using a PO based MC where the outcome (e.g., 95% confidence that 99% of the servings do not contain ≥ X log10 CFU g⁻¹) can be used by an individual manufacturer, in conjunction with their within-lot s.d., to develop the appropriate MC for their products. Alternatively, national surveillance programmes should measure both the between-lot and within-lot variability for the food category and use the s.d. associated with within-lot variability for developing MC and that associated with between-lot variability for conducting risk assessments and establishing the FSO/PO.

10. Practical aspects of these considerations

In this publication we have not discussed how to establish an FSO because this was considered in ICMSF (2002). Instead, we have demonstrated how, in certain cases, a PO can be derived from an FSO and, in turn, be used to develop an MC based on the PO. We have also demonstrated how articulating an MC could lead to an implied PO. In Section 8 the examples presented showed how the distribution of the numbers of the pathogen in a lot influences the number of samples that must be examined to verify that a food lot meets a prescribed PO. Moreover, it was shown that the level of the PO greatly influences the practicality of using microbiological testing for this purpose. The size of the analytical unit, the standard deviation, the probability of acceptance and the statistical definition of the PO are other factors that determine the practicality of testing lots for the presence of specified levels of pathogens for confirmation purposes. Whether testing for pathogens across lots may present better possibilities needs to be further explored. In certain situations testing for indicator microorganisms may offer an alternative (ICMSF, 2002).

When the practicality of testing and the interpretations of the results are considered, a few observations can be made. Firstly, the situation regarding fresh or raw foods may be different from foods processed for safety. In the case of such processed foods pathogens should, in principle, not be present or their presence (due to survival or unavoidable recontamination and growth) should be at levels that present a negligible risk to public health. Unacceptable levels of recontamination should not occur, or should be detected, and such incriminated batches should not be put on the market. Microbiological testing should detect such lots when no other means are available. However, while the distribution of the pathogens in the lot is not known it is most likely that they are not homogeneously distributed throughout a consignment. Moreover, random sampling is often not possible for reasons of accessibility of units in consignments on trucks, ships, etc. Consequently, in these cases, the calculations and interpretations of pathogen testing data have only limited validity: in simple terms it can be argued that a positive finding (i.e., presence of a pathogen) means something, while a negative one means very little. Even when the necessary data are available to allow statistical interpretation of the test results, the number of samples needed to obtain a meaningful result may be too large to be practical, as was shown in the Salmonella in ice cream example.

The situation may be different for foods that are not processed for safety, that are raw or that may originate from polluted environments. In these situations, testing may be useful because contamination levels and/or frequencies would be expected to be higher, and it is recommended to design sampling plans in a manner, such as demonstrated in this paper, to determine whether the POs set for such products are met.

11. Summary

FSOs and POs are targets to be met for pathogen/food product combinations. To assess whether consignments of foods conform to such targets, background information is needed. The best information is obtained during inspection/audits of the site of processing of the food. If information is available concerning the distribution of the pathogen of concern in the food batch (i.e., a log-normal distribution with a certain standard deviation) and if samples can be randomly taken, statistical methods can be used to specify microbiological criteria intended to verify achievement of an FSO or PO. However, this requires that a number of risk management decisions be made as described in this paper. The examples presented also demonstrate that when foods have received
treatments that greatly reduce microbiological levels, or that have very low prevalence due to other reasons, testing may not be the most effective means to verify microbiological status, and other approaches should be considered. When information to develop FSO/PO derived microbiological criteria is not available, the classical “ICMSF cases” and sampling schemes still offer a risk-based approach for examining lots for acceptance or rejection.

Acknowledgements

This paper is dedicated to Susanne Dahms who sadly lost her battle with cancer on July 23rd 2007.

Susanne was educated in Germany where she earned a university degree in economics and doctorate at the University of Bielefeld, and qualification for full professorship at the Free University of Berlin. Her research interests included biometrical and epidemiological approaches to evaluate dependencies between animal health management and food safety, statistical aspects of microbiological sampling plans and their relation with food safety objectives, and biometrical methods for validation of microbiological analytical techniques. Susanne was elected to serve as a member of the International Commission on Microbiological Specifications for Foods (ICMSF) in 1998, the advisory board of the German Region of the International Biometrical Society in 2002 and the Council of the International Biometrical Society in 2006.

She was a brilliant and talented mathematician who also had a unique ability to get difficult concepts across in simple ways. This was especially important to the ICMSF in order to carry out a number of important projects such as the various papers (including this one) and books produced by the Commission. Through this work Susanne has made a significant contribution to public health and food safety.

Susanne contributed much more than technical expertise to our group, she brought friendship, warmth and a great sense of humor. One of our enduring memories of Susanne will be the many lectures she used to give us, where she would take great delight and fun in teasing the mathematically challenged microbiologists among us, yet she would do this with great mutual respect and in a way that endeared her to the rest of the group. We feel privileged that Susanne was a member of our group and grateful for the time she spent with us. We will miss her as a colleague but especially as a very dear and special friend.

Appendix 1

1.1. Relating an OC-curve to concentrations of pathogens

If bacteria are log-normally distributed in a product and if the standard deviation of the distribution is known, an “operating characteristic” curve (OC-curve) can be established. For example, for L. monocytogenes the acceptable concentration in a lot could be set at less than 100 CFU g\(^{-1}\) according to the ICMSF sampling scheme (ICMSF, 1994); \(m = 100\) CFU g\(^{-1}\) \((= 2.0 \log_{10}\text{CFU g}^{-1})\). If the average concentration in the lot is 10 CFU g\(^{-1}\) \((\log N = 1 \log_{10}\text{CFU g}^{-1})\) and the standard deviation is 0.2 \(\log_{10}\text{CFU g}^{-1}\), the expected distribution of concentrations of L. monocytogenes in samples taken from the lot is as presented in Fig. A1.1. If one sample is taken, for example a 1 g sample, and the total number of L. monocytogenes in the sample enumerated by spreading the 1 g sample over multiple selective agar plates, the probability of accepting the lot will be virtually 100%. The probability of acceptance can be calculated in Microsoft\textsuperscript{\textregistered} Excel using the “Normdist” function, as shown:

\[
P(\text{acceptance for 1 sample}) = \text{Normdist}(x, \mu, \sigma, \text{cumulative})
\]

where \(x\) is the value for which the probability of occurrence is wanted (in this case \(x = m \log_{10}\text{CFU g}^{-1}\)), \(\mu\) is the mean \(\log_{10}\) concentration of the distribution, either a theoretical value (\(\mu\)) or an estimated value (\(m\)), \(\sigma\) is the standard deviation of the log-normal distribution, either a theoretical value (\(\sigma\)) or an estimated value (\(\hat{\sigma}\)), ‘cumulative’ is a logical value. If this is set to 1, ‘Normdist’ will return the cumulative distribution. If this is set to 0, it returns the probability mass function (frequency distribution).

If one sample is taken from the lot, the probability of acceptance for a distribution with mean concentration 1 \(\log_{10}\) CFU g\(^{-1}\) and standard deviation 0.2 \(\log_{10}\text{CFU g}^{-1}\) with a limit at 2 \(\log_{10}\text{CFU g}^{-1}\) \((m = 2)\) is then: \(P(\text{acceptance}) = \text{Normdist}(2,1,0.2,1) = 0.99999713\%\).

The same procedure \((m = 2 \log_{10}\text{CFU g}^{-1})\) can be used for other mean \(\log_{10}\) concentrations \((e.g., 1.0,1.5,2.0,2.5)\) with the same standard deviation of 0.2 \(\log_{10}\text{CFU g}^{-1}\) to illustrate the effect of mean microbial levels on the probabilities of acceptance (Fig. A1.2).

Once the probabilities of acceptance are determined for various mean log concentrations, they can be plotted in a graph to obtain an OC-curve as indicated in Fig. A1.3. The probability of acceptance for more then one sample can be calculated as follows:

\[
P(\text{acceptance for n samples}) = P(\text{acceptance for 1 sample})^n
\]

For \(n = 10\), the probability of acceptance for a mean log concentration of 1.5 \(\log_{10}\text{CFU g}^{-1}\) and standard deviation 0.2 \(\log_{10}\text{CFU g}^{-1}\) is then:

\[
P(\text{acceptance for 10 samples}) = \text{Normdist}(2.1,1.5,0.2,1)^{10}
\]

\[= (0.994)^{10} = 94\%
\]

In the same way as indicated in Fig. A1.3, the probability of acceptance can be determined for \(n = 10\) for various mean log concentrations resulting in an OC-curve for \(n = 10\), \(c = 0\) and \(m = 100\) CFU g\(^{-1}\). From this OC-curve, the concentration at which the lot will be accepted with 95% probability (producer’s ALS) and the concentration at which a lot will be rejected with 95% probability (consumer’s ALS) can be determined. These concentrations are shown in Fig. A1.1.
Fig. A1.4 for \( \sigma = 0.2 \). It can be seen by comparing Fig. A1.4 with Fig. A1.3 that the more samples are taken, the steeper the OC-curve becomes.

The OC-curve can be obtained for other standard deviations as well. Two examples are given in Fig. A1.5 for \( \sigma = 0.4 \) and Fig. A1.6 for \( \sigma = 0.8 \). It can be seen that the larger the standard deviation, the flatter the OC-curve becomes.

Appendix 2

2.1. A modified approach for estimation of the number of ‘analytical units’ that need to be tested to have 95% confidence of rejection of non-conforming batches (for enrichment tests)

In previous publications (Legan et al., 2001; ICMSF, 2002) an approach (described in Appendix 1) was developed to determine the number of analytical units and their size that, for a given distribution of microbial counts, must be tested to provide 95% confidence that a batch with unacceptable levels will be rejected by attributes sampling. In the following, a refinement of that approach is described for 2-class attribute testing where a specified number of analytical units are cultured via enrichment and then assessed for presence/absence of the microorganism.

In either approach, a lot has the usual meaning, i.e. a grouping of a product manufactured during a certain period of time or under the same conditions, or a consignment of a food arriving at a border. A sample is taken from that lot to assess the concentration of cells in that sample. A sample may comprise the entire analytical unit, or the analytical unit may be an aliquot derived from the sample. In certain cases a sample is made up of a composite of various mixed subsamples, in order to increase homogeneity in the sample, but this does not of course change the underlying distribution in the lot. It is assumed that the concentration in an aliquot of the sample is representative of the concentration in the whole sample, but that different samples can have different concentrations (it should be realised that in very heterogeneous samples this might not be correct). Also, in both approaches it is assumed that a log-normal distribution characterises the microbiological status of the lot but in this modified approach a further consideration, concerning the likelihood of sampling a contaminant in an analytical unit, is implemented.

2.2. Modified approach: Poisson-log-normal distribution

Microorganisms are discrete particles that are very small relative to the size of analytical units typically employed. Thus, even if the microorganisms were completely evenly distributed in the sample and were present at the level of one cell per sample unit, one would not expect every sample to be positive for growth; some samples selected at random would contain one or more cells and
produce a positive result, while others would not. Moreover, if the concentration of target cells was just less than the PO, even if the cells were perfectly homogenously distributed in the sample, some samples would produce a positive result leading to the inappropriate rejection of the batch (in a sampling scheme that specifies \( c = 0 \)). Thus, it is necessary to consider the consequences of sampling ‘coincidences’ (i.e. the detection of a cell in a set of samples even when such detection is highly improbable based on the mean concentration of the organism in the batch) on the interpretation of the results of analytical methods, particularly when inferring the concentration of cells in a sample. The probability of detecting cells, by randomly sampling from a well-mixed system can be described by a Poisson distribution:

\[
P_{\text{detection}} = 1 - \exp^{-\left(\frac{\text{concentration of cells}}{\text{sample size}}\right)}
\]

For the Poisson distribution the variance is the same as the mean and, consequently, the standard deviation is approximated by the square root of the mean count. Thus, for a concentration of 100 cells per analytical unit, we would expect 95% of test results to fall in the range from 80 to 120 cells, i.e. within \( \pm 2 \) standard deviations of the mean.

In other words, we need to recognise that even if the concentration of target cells in the sample is perfectly homogenous, our sampling and enumeration method will sometimes over-estimate the concentration and sometimes under-estimate it. We could calculate the total effect of the probabilities of over- and under-estimation of the concentration due to sampling coincidences, i.e. samples that have a concentration greater than the PO but that generate an estimate less than the target (i.e., a false-negative), and those samples that have a concentration less than the target that produce an estimate greater than the PO due to sampling coincidences (i.e., a false-positive). Clearly, as the true concentration in the sample increases, there is less likelihood that the sample will produce a false negative and, conversely, as the true concentration in the sample decreases there is less likelihood of obtaining a false-positive through sampling coincidences.

As the target concentration of cells declines, our confidence in the result of our test method also declines. From the above equations, it can be seen that when the target concentration is on average one cell per analytical unit, our chance of detecting a cell in a single sample is \( \approx 63\% \) only, while a sample that contains half that concentration of cells has \( \approx 40\% \) probability of yielding a (false) positive result. To explain this further, if the concentration of organisms is on average one cell per kilogram and we take samples of 100 g there will be, on average, one cell in every tenth sample. Thus, there is a probability that we will detect a positive even though, on average, the amount of cells is smaller than one per sample unit. This arises because, as noted earlier, cells are discrete units. Thus, calculating a
mean concentration of 0.1 cells per 100 g is somewhat misleading — it is more helpful to think of any ‘fractional’ cell concentration as being equivalent to one cell in a suitably large analytical unit, e.g. when we say “0.1 cells per 100 g” we mean “one cell per kg”. We can also express this as a proportion of positive analytical units e.g. “one in 10 units of 100 g are positive”.

Our aim, however, is to determine by sampling whether the mean log concentration in the lot is such that less than 1% of the lot exceed the PO. While one approach is to test a sufficient number of samples to determine whether 1% of the lot exceed this limit, equally we could base our sampling strategy on assessing the proportion of samples that exceeded any other concentration on the required distribution curve because we can determine the probability of detecting a cell in a sample of any concentration. Thus, as long as the distribution of concentrations is known, it is possible to calculate the overall probability of detecting a cell from any sample drawn from a lot. This is because the overall probability of obtaining a positive sample is the product of the probability of that concentration occurring in the lot, and the probability of detecting a cell in the sample based on the size of the analytical unit and the concentration of cells in the sample. For example, if on average the amount of cells in a sample is exactly one cell, one would intuitively expect that the value in a sample would be one. However, the probability for any single sample to be positive is not equal to one, since there is only, on average, one cell in the sample. Sometimes there will be really one organism in the sample, sometimes zero, and sometimes two, or even occasionally more than two. The probability that there is no organism in a sample (although on average the expected number would be one organism in a sample) is \( \exp(-1) = 0.368 \). Therefore the probability

---

### Table A2.1

Sampling plans derived from the modified approach for *Salmonella* in ice cream with different distributions intended to test compliance with different FSOs/POs.

<table>
<thead>
<tr>
<th>m Absence in (g)</th>
<th>FSO/PO (g)</th>
<th>Log mean (cfu g(^{-1})) ± s.d. (^a)</th>
<th>( n (c = 0) )</th>
<th>Mean log (cfu g(^{-1})) ± s.d. (^a) accept</th>
</tr>
</thead>
<tbody>
<tr>
<td>25</td>
<td>1/100</td>
<td>-2.93 ± 0.4</td>
<td>69</td>
<td>4.6 × 10(^4)</td>
</tr>
<tr>
<td>100</td>
<td>1/100</td>
<td>-</td>
<td>19</td>
<td>1</td>
</tr>
<tr>
<td>250</td>
<td>1/100</td>
<td>-</td>
<td>9</td>
<td>-0.8 ± 0.2</td>
</tr>
<tr>
<td>25</td>
<td>1/1000</td>
<td>-2.93 ± 0.4</td>
<td>671</td>
<td>2.9 × 10(^2)</td>
</tr>
<tr>
<td>100</td>
<td>1/1000</td>
<td>-</td>
<td>170</td>
<td>1</td>
</tr>
<tr>
<td>250</td>
<td>1/1000</td>
<td>-</td>
<td>69</td>
<td>-1.7 ± 0.2</td>
</tr>
<tr>
<td>25</td>
<td>1/10,000</td>
<td>-2.93 ± 0.4</td>
<td>6684</td>
<td>867</td>
</tr>
<tr>
<td>100</td>
<td>1/10,000</td>
<td>-</td>
<td>1673</td>
<td>3</td>
</tr>
<tr>
<td>250</td>
<td>1/10,000</td>
<td>-</td>
<td>671</td>
<td>-2.2 ± 0.8</td>
</tr>
<tr>
<td>25</td>
<td>1/100,000</td>
<td>-2.93 ± 0.4</td>
<td>15,994</td>
<td>15</td>
</tr>
<tr>
<td>100</td>
<td>1/100,000</td>
<td>-</td>
<td>4027</td>
<td>-3.0 ± 0.8</td>
</tr>
<tr>
<td>250</td>
<td>1/100,000</td>
<td>-</td>
<td>1631</td>
<td>-</td>
</tr>
</tbody>
</table>

These figures are based on a “99%-point definition” of the PO and 95% probability of rejection.

\(^a\) The maximum mean log concentration of a lot with the stated standard deviation that would permit the lot to meet the FSO. \( n (c = 0) \) is the number of samples that would need to be tested to ensure 95% confidence that the lot would meet the FSO. Log (FSO) = log (0.05); P \(_n\) = Poisson-log-normal (log(1/m), log(mean, s.d.); P \(_n\) = Poisson-log-normal (log(1/m), log(mean, s.d.)); P = Poisson-log-normal (log(1/m), log(mean, s.d.)); P = Poisson-log-normal (log(1/m), log(mean, s.d.)).

### Table A2.2

Sampling plans derived from the approach of Legan et al. (2001) for *L. monocytogenes* in smoked salmon with different distributions intended to test compliance with different FSOs and POs.

<table>
<thead>
<tr>
<th>The lower limit of detection for three analytical techniques used to examine the samples (see text)</th>
<th>FSO Log(cfu g(^{-1}))</th>
<th>PO Log(cfu g(^{-1}))</th>
<th>Mean log(cfu g(^{-1})) ± s.d. (^a)</th>
<th>( n (c = 0) ) (^b)</th>
<th>Mean log (cfu g(^{-1})) ± s.d. (^a) accept</th>
</tr>
</thead>
<tbody>
<tr>
<td>100/g</td>
<td>2.3</td>
<td>1.7</td>
<td>1.2 ± 0.2</td>
<td>4.6 × 10(^4)</td>
<td>1.1 ± 0.2</td>
</tr>
<tr>
<td>0.3/g</td>
<td></td>
<td></td>
<td>1</td>
<td>( n (c = 0) )</td>
<td>-0.8 ± 0.2</td>
</tr>
<tr>
<td>Abs. in 25 g</td>
<td></td>
<td></td>
<td>1</td>
<td></td>
<td>-1.7 ± 0.2</td>
</tr>
<tr>
<td>100/g</td>
<td></td>
<td></td>
<td>0.8 ± 0.4</td>
<td>2.9 × 10(^2)</td>
<td>0.35 ± 0.4</td>
</tr>
<tr>
<td>0.3/g</td>
<td></td>
<td></td>
<td>1</td>
<td>( n (c = 0) )</td>
<td>-1.1 ± 0.4</td>
</tr>
<tr>
<td>Abs. in 25 g</td>
<td></td>
<td></td>
<td>1</td>
<td></td>
<td>-2.1 ± 0.4</td>
</tr>
<tr>
<td>100/g</td>
<td></td>
<td></td>
<td>-0.2 ± 0.8</td>
<td>867</td>
<td>-1.1 ± 0.8</td>
</tr>
<tr>
<td>0.3/g</td>
<td></td>
<td></td>
<td>3</td>
<td>( n (c = 0) )</td>
<td>-2.2 ± 0.8</td>
</tr>
<tr>
<td>Abs. in 25 g</td>
<td></td>
<td></td>
<td>2</td>
<td></td>
<td>-3.0 ± 0.8</td>
</tr>
<tr>
<td>100/g</td>
<td>3.3</td>
<td>2.7</td>
<td>2.2 ± 0.2</td>
<td>2.0</td>
<td>1.6 ± 0.2</td>
</tr>
<tr>
<td>0.3/g</td>
<td></td>
<td></td>
<td>1</td>
<td>( n (c = 0) )</td>
<td>-0.8 ± 0.2</td>
</tr>
<tr>
<td>Abs. in 25 g</td>
<td></td>
<td></td>
<td>1</td>
<td></td>
<td>-1.7 ± 0.2</td>
</tr>
<tr>
<td>100/g</td>
<td></td>
<td></td>
<td>1.8 ± 0.4</td>
<td>10</td>
<td>1.0 ± 0.4</td>
</tr>
<tr>
<td>0.3/g</td>
<td></td>
<td></td>
<td>1</td>
<td>( n (c = 0) )</td>
<td>-1.1 ± 0.4</td>
</tr>
<tr>
<td>Abs. in 25 g</td>
<td></td>
<td></td>
<td>1</td>
<td></td>
<td>-2.1 ± 0.4</td>
</tr>
<tr>
<td>100/g</td>
<td></td>
<td></td>
<td>0.8 ± 0.8</td>
<td>40</td>
<td>0.4 ± 0.8</td>
</tr>
<tr>
<td>0.3/g</td>
<td></td>
<td></td>
<td>1</td>
<td>( n (c = 0) )</td>
<td>-1.8 ± 0.8</td>
</tr>
<tr>
<td>Abs. in 25 g</td>
<td></td>
<td></td>
<td>1</td>
<td></td>
<td>-2.7 ± 0.8</td>
</tr>
<tr>
<td>100/g</td>
<td>4.3</td>
<td>3.7</td>
<td>3.2 ± 0.2</td>
<td>1</td>
<td>1.7 ± 0.2</td>
</tr>
<tr>
<td>0.3/g</td>
<td></td>
<td></td>
<td>1</td>
<td>( n (c = 0) )</td>
<td>-0.8 ± 0.2</td>
</tr>
<tr>
<td>Abs. in 25 g</td>
<td></td>
<td></td>
<td>1</td>
<td></td>
<td>-1.7 ± 0.2</td>
</tr>
<tr>
<td>100/g</td>
<td></td>
<td></td>
<td>2.8 ± 0.4</td>
<td>1</td>
<td>1.3 ± 0.4</td>
</tr>
<tr>
<td>0.3/g</td>
<td></td>
<td></td>
<td>1</td>
<td>( n (c = 0) )</td>
<td>-1.1 ± 0.4</td>
</tr>
<tr>
<td>Abs. in 25 g</td>
<td></td>
<td></td>
<td>1</td>
<td></td>
<td>-2.0 ± 0.4</td>
</tr>
<tr>
<td>100/g</td>
<td></td>
<td></td>
<td>1.8 ± 0.8</td>
<td>6</td>
<td>0.1 ± 0.8</td>
</tr>
<tr>
<td>0.3/g</td>
<td></td>
<td></td>
<td>1</td>
<td>( n (c = 0) )</td>
<td>-1.8 ± 0.8</td>
</tr>
<tr>
<td>Abs. in 25 g</td>
<td></td>
<td></td>
<td>1</td>
<td></td>
<td>-2.7 ± 0.8</td>
</tr>
</tbody>
</table>

\(^a\) The maximum mean log concentration of a lot with the stated standard deviation that would permit the lot to meet the FSO.

\(^b\) Number of samples that would need to be tested to ensure 95% confidence that a lot was not exceeding the stated FSO.

\(^c\) The maximum mean log concentration of a lot that would be accepted with 95% probability given this number of samples.
that a sample will be positive (one or more organisms in the sample) is one minus the probability of no organism in the sample:

\[ \frac{1}{C_0} = 0.368 \]  

Alternatively the probability of a positive sample can be calculated as the sum of the individual probabilities, i.e., the Poisson probabilities for 1, 2, 3, 4, etc. cells per sample are 0.368, 0.184, 0.0613, 0.0153, etc. respectively, so the probability of obtaining 1 or more cells/sample is given by

\[ 0.368 + 0.184 + 0.0613 + 0.0153 + \cdots = 0.632. \]

The above concept can be used to estimate the overall probability of detecting a positive sample in a lot characterised by a known, or assumed, distribution, i.e. by integrating, over all possible concentrations in the batch, the overall probability of detecting a cell in any of the samples taken. The probability of sampling any particular concentration in the batch is given by the log-normal distribution and is combined with the Poisson sampling process (and sampling size) to calculate the probability that a cell is present in the sample taken and leads to detection of a ‘positive’. This can be expressed mathematically as

\[
p(+) = \int_{-\infty}^{\infty} \text{Normal}(\log C, \mu, \sigma) \cdot (1 - \text{Poisson}(0, C \cdot \text{samplesize})) \, d \log C
\]

Note that the concentration $C$ for the Poisson distribution is $10^{\log C}$.

Since $\text{Poisson}(0, x) = \exp(-x)$, this results in:

\[
p(+) = \int_{-\infty}^{\infty} \text{Normal}(\log C, \mu, \sigma) \cdot (1 - \exp(-C \cdot \text{samplesize})) \, d \log C
\]

Returning to the example of line 3 from Table A2.1 the above leads to:

<table>
<thead>
<tr>
<th>Proportion of contaminated carcasses tolerated (PO) (%)</th>
<th>Number of samples (n) required to reject defective lots with 95% probability (c = 0)</th>
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</thead>
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<td>0.27</td>
</tr>
<tr>
<td>10</td>
<td>29</td>
<td>0.18</td>
</tr>
<tr>
<td>5</td>
<td>59</td>
<td>0.09</td>
</tr>
<tr>
<td>1</td>
<td>298</td>
<td>0.02</td>
</tr>
</tbody>
</table>

This could also be calculated with the negative binomial distribution:

NEGBINOMIAL(0; 19; 1 – 0.15 = 0.05); NEGBINOMIAL(0; 19; 1 – 0.0027 = 0.95).

\[ a \quad (1 - P)^n = 0.05, \quad n \log (1 - P) = \log (0.05), \quad n = \log (0.05)/\log (1 - P). \]

\[ b \quad (1 - P)^n = 0.95, \quad n \log (1 - P) = \log (0.95)/n, \quad 1 - P = 0.95^{1/n}, \quad P = 1 - 0.95^{1/n}. \]

**Table A2.3**

Sampling plans derived for *Salmonella* in poultry carcasses intended to test compliance with different POs.

<table>
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<tr>
<th>Proportion of contaminated carcasses tolerated (PO) (%)</th>
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**Fig. A3.1.** (a) Probability of acceptance as a function of mean log$_{10}$ CFU g$^{-1}$ (assuming s.d. = 0.4), for sampling plan of $n = 10$, $c = 0$, $m = 100$ CFU g$^{-1}$. (b) Distribution of counts in a lot rejected with 95% probability (assuming s.d. = 0.4), for sampling plan of $n = 10$, $c = 0$, $m = 100$ CFU g$^{-1}$. (c) Distribution of counts in a lot accepted with 95% probability (assuming s.d. = 0.4), for sampling plan of $n = 10$, $c = 0$, $m = 100$ CFU g$^{-1}$. 

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\[ p(+) = \int_{-\infty}^{\infty} \text{normal}(C, -2.931, 0.4) \cdot (1 - \exp(-C \cdot 250)) \, d\log C \]

(7)

Calculating this integral results in \( p = 0.3068 \), i.e., the probability of acceptance of the batch based on a single sample is: \( \frac{1}{p} = 0.6932 \). This is well below the required 95% confidence, so that more (negative) samples are required to achieve 95% confidence that the lot meets the PO.

If we take eight samples, the probability that all eight are acceptable (i.e., that no sample contains a cell) is \((0.6932)^8 = 0.0533\), and with nine samples that probability is \((0.6932)^9 = 0.0369\). Thus, to reject with 95% certainty a batch that has greater than the desired log mean concentration (and with the specified standard deviation), requires nine negative samples, \((n = 9, c = 0)\) given this calculation scheme.

This approach differs from earlier analyses (e.g., Legan et al., 2001) because it specifically considers the likelihood of any concentration of cells within the limiting distribution generating a ‘positive’ result, rather than basing the sampling scheme on a specific concentration only. In other words, in this approach if a sample contains on average 0.1 cells, it can result in a positive outcome (i.e., with a probability of 10%). Therefore, samples are more often predicted to be positive and, consequently, fewer samples are needed to assure rejection at a specified level of confidence.

Appendix 3

3.1. FSOs/POs based on sampling plans using the original ICMSF approach

For the purposes of this Appendix, it is assumed that an FSO/PO is interpreted as the 99% or 99.5%-point of the exposure distribution that is accepted, i.e., that up to 1% or 0.5%, respectively, of units with concentrations exceeding this limit can be tolerated. As an example, a sampling plan for \( L. \) monocytogenes with \( n = 10, c = 0, m = 100 \text{ CFU g}^{-1} \) (or \( m = 2 \) in log_{10}-units) is used.

If log-normal distributions can be used to describe the frequencies of concentrations and experience shows that assumption of a standard deviation = 0.4 log_{10}-units can be justified, the situation as depicted in Fig. A3.1a applies. The Figure shows the OC-curve for this sampling plan and shows which mean log-concentrations
are rejected and those that are accepted with 95% probability (consumer’s and producer’s ALS). Lots with mean log_{10}-concentrations of 1.74 log_{10} CFU g^{-1} will be rejected with a 95% probability (consumers ALS) and lots with mean log_{10}-concentrations of 0.97 log_{10} CFU g^{-1} (producers ALS) will be accepted with a 95% probability. This is shown in more detail in Fig. A3.1.

Using the log_{10}-concentration distribution in Fig. A3.1b, which has a mean value corresponding to the consumer’s ALS (1.74), the 99% or 99.5%-point (i.e. the FSO/PO value) can be determined. Application of this sampling scheme for this distribution of L. monocytogenes in a lot would mean that the FSO/PO would need to be set at 2.67 log_{10} CFU g^{-1} to ensure that 99% of units from the lot did not exceed the FSO/PO, or 2.77 log_{10} CFU g^{-1} to ensure that 99.5% of units were below the FSO/PO.

If experience showed that an s.d. of 0.2 log_{10} units was more appropriate to describe the distribution, the situation would be as depicted in Fig. A3.2. In that case lots with mean log-concentrations at 1.87 will be rejected with 95% probability when the same sampling plan is applied (consumer’s ALS). The producer’s ALS, has a mean concentration of 1.49 log_{10} CFU g^{-1}. The 99%-point reflecting the FSO/PO is at 2.34 and the 99.5%-point is at 2.39 log_{10} CFU g^{-1} as depicted in the second graph.

Experience may also show that an s.d. of 0.8 log_{10} CFU g^{-1} is more appropriate to describe the distribution, as depicted in Fig. A3.3. In this case, lots with mean log-concentrations at 1.48 will be rejected with a 95% probability, and those with a mean log-concentration of −0.054 log_{10} CFU g^{-1} accepted with a 95% probability. The 99%-point would be at 3.34 and the 99.5%-point would be at 3.54 log_{10} CFU g^{-1}.

These examples show the implied PO/FSO given a certain sampling plan and a given standard deviation. For a specified PO/FSO the procedure would be the other way around, to calculate the microbiological criterion based on the PO/FSO and the standard deviation.

References


