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Determining the microbiological criteria for lot rejection from the performance objective or food safety objective

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Abstract

The Microbiological Criteria (MC) is a set of parameters used to determine whether a specific lot of food is acceptable or not. These parameters are the microbial test protocol and its sensitivity, the confidence level that an unacceptable lot will be detected, the number of samples to be taken and the number of positive samples that are allowed before rejecting the lot. Determining the microbiological criteria begins with knowledge of the distribution of contamination from samples within a lot, particularly within a lot that is just at the unacceptable level of the microbial hazard. The just unacceptable lot can be defined by the Food Safety Objective (FSO) or Performance Objectives (PO), the small fraction of samples that can exceed these values and the standard deviation of the samples from the lot. With this information, a microbial test protocol is chosen to have a sensitivity level that would detect between approximately 15% and 45% of the samples. A confidence level for the MC and the number of positive samples that would be acceptable (*c* value which is usually zero) are also chosen. With this information the number of samples (*n*) required can be calculated. A critical factor in setting the microbiological criteria is the sensitivity of the microbiological test (*m* value). The sample size (weight) and sampling procedure can affect the standard deviation of the samples, particularly foods with non-homogeneous distribution and low numbers of microorganisms. Sampling, sample preparation and analytical procedures that reduce the variation between the samples will affect the choice of *m* value and maximum lot mean that meets the MC.
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1. Introduction

The Food Safety Objective (FSO) is the maximum frequency and/or concentration of a microbiological hazard in a food at the time of consumption that provides the appropriate level of protection (ICMSF, 2002). Performance Objectives (PO) are the concentration limits earlier in the food processing and distribution chain that will allow the product to meet the FSO. The acceptability of a food lot is determined by the microbiological criterion (MC) (Codex, 2003; Dahms, 2004). A MC consists of a statement of the microorganisms of concern and/or their toxins/metabolites and the reason for that concern; the analytical methods for their detection and/or quantification; a plan defining the number of field samples to be taken and the size of the analytical unit; microbiological limits considered appropriate to a food at the specified point in the food chain; and the number of analytical units that should conform to these limits.

A MC may be used to indicate required microbiological status of raw materials, ingredients, and end-products at any appropriate stage of the food chain. It can also be used to establish the stringency of food control systems and process or product requirements. Three categories of application of MC were listed by ICMSF (2002): a standard that is a mandatory criterion and

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incorporated into law or regulation, a guideline used to inform what the microbial levels should be when a product is produced under control, and a specification that is part of a purchase agreement.

This paper focuses on establishing a MC from the FSO or PO. The steps from the Acceptable Level of Protection (ALOP) to the FSO and between the PO and FSO in growth supporting foods are not considered. The paper presumes the FSO and/or appropriate PO have been determined by the risk managers.

The intent of the MC is to determine whether a lot of food is "acceptable" or "unacceptable." This is a two-class attribute test which is characterized by the number of samples to be tested (n), the number of samples (c) that exceed the test criteria (in microbial testing associated with pathogenic microorganisms, c is usually zero), the lower limit of detection for the test (m) and a confidence level (e.g., 95%) that the test will identify an unacceptable lot.

The purpose of this paper is to outline the factors, assumptions and process necessary to establish a MC for a food. In demonstrating the concepts and process involved, specific values were chosen for some of the factors. Examples are the normal distribution of samples, 3 standard deviations from lot mean to PO and the 95% confidence level. While these are believed by the authors to be reasonable values to use, other values could be chosen for a specific food-pathogen case without changing the process described in this paper. The process in this paper is based on the risk assignment paradigm described in Codex Alimentarious (1999) and Codex Committee on Food Hygiene (2003, 2005), and on attribute sampling plans described in Jarvis (1989), ICMSF (2002), and Dahms (2004).

2. Method for determining the microbiological criteria

The assumption is made that the concentration of pathogens are log normally distributed within the lot, i.e., normally distributed when plotted on a logarithmic scale (Jarvis, 1989). This assumption is supported by experimental evidence (Gill et al., 1996). Another assumption is that the variance of the samples is the same for a little or highly contaminated lot. Therefore, shape and spread of the contamination distributions for a specific food-pathogen are the same and the sample distributions move right or left on the x (contamination level) axis with different mean levels of contamination.

2.1. Step 1 — determine the standard deviation of samples within a lot

Data are acquired that characterize samples within lots of the food at the FSO or PO. Sufficient quantitative data are needed from samples from multiple lots to determine the standard deviations of the within lot samples. Some lots may have samples below the detectable level of the test and the full curve may need to be inferred from the more highly contaminated samples. Fig. 1 illustrates the distribution curves for a pathogen in different lots of a food. In keeping with the assumption made above, all these lots in this example are assumed to have a standard deviation of 0.8 $\log_{10}$ cfu/g.

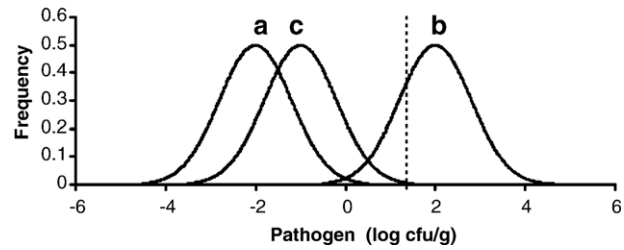

Fig. 1. Distributions of samples from lots of a food having different mean contamination levels.

2.2. Step 2 — define the 'just unacceptable lot'

The Codex Alimentarius Commission definition of the FSO or PO is a concentration that is not exceeded in the food. Because a normal curve extends by definition to plus and minus infinity, there always can be a rare high sample that exceeds the FSO or PO. Therefore, a decision must be made on the allowable fraction of samples that exceed the FSO or PO and its corresponding relationship to the lot mean. This paper will define an unacceptable lot to be a lot that has 0.13% or more of the samples exceeding the FSO or PO. This means the FSO or PO is three standard deviations above the mean. If the dotted line on Fig. 1 at 1.4 $\log_{10}$ cfu/g (25 cfu/g) represents the FSO or PO, then Lot 'a' is an acceptable lot with its mean of −2.0 $\log_{10}$ cfu/g. Lot 'b' with its mean at 2.0 $\log_{10}$ cfu/g is unacceptable. Lot 'c' with a mean of −1.0 $\log_{10}$ cfu/g and standard deviation of 0.8 $\log_{10}$ cfu/g will be designated as the 'just unacceptable lot' that the MC should reject.

2.3. Step 3—determine the needed sensitivity of the analytical procedure

Because there is heterogeneity in the concentration of the pathogen within an individual lot, some samples will be negative and others positive. If the analytical procedure would only detect positive samples that exceeded the FSO or PO, then 0.13% of the samples would be expected to be positive. A sampling plan that would identify and reject a lot having 0.13% defective samples would require 2200 samples to have 95% confidence that at least one sample would be positive ($c=0$). If the analytical procedure was sensitive to extremely low levels of contamination, lots that should be accepted would have samples test positive and be erroneously rejected. Therefore, the sensitivity of the analytical procedure must be positioned at an appropriate level so that a set of samples from a lot of food has a high probability of at least one sample being positive ($c=0$, 95% confidence). For sampling plans with five to twenty samples (n), this requires that 14% to 45% of the samples be above this level. This sensitivity level is the 'm' value and is illustrated on Fig. 2 where the lot mean, standard deviation and m value are −1.0, 0.8 and −0.5 $\log_{10}$ cfu/g, respectively. In this example, 26.6% of the samples would exceed the m value and be positive. If less than 14% of the samples were above the m value, the number of samples needed becomes excessive. If the area is more than 45% then the number of samples becomes too few and the likelihood of rejecting acceptable lots becomes large.

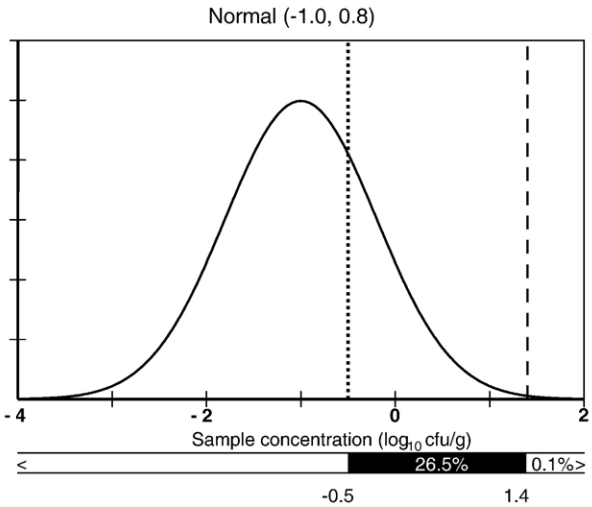

Fig. 2. Setting the *m* value for the 'just unacceptable lot'. The right vertical line (dashed) indicates the FSO, the left line (dotted) the *m* value. The lot is described by a mean of −1.0 and standard deviation of 0.80. The probabilities of a sample being in different portions of the curve as shown on the bottom bar. In this example, 26.6% of the samples would exceed the *m* value.

The percentage of samples that exceed the *m* value can be determined by a variety of ways. Statistical tables of "*z* values" (Snedecor and Cochran, 1980), software packages such as the Excell NORMDIST function (Microsoft, Redmond, WA) or the free R Project for Statistical Computing (www.r-project.org), or calculations (Shah, 1985) can determine the relative areas under different portions of the standard normal curve. The probability of exceeding the *m* value is

$$z = (m - \text{mean})/\text{std.dev.}$$

$$p = 0.5 - (z(4.4 - z)/10$$

All parameters are in their $\log_{10}$ values. *Note:* The formula provides a very good approximation (error < 0.0058) for *p* when $0 < z \leq 2.2$. *For* $z > 2.2$, *do not use this formula*; use a table or software for the Standard Normal Distribution to determine *p*.

2.4. Step 4 — determine the number of samples

The probability of lot rejection $P_{(R)}$ for a two-class sampling plan with sample size *n* and $c = 0$ follows a binomial distribution and can be calculated as follows:

$$P_{(R)} = 1 - (1 - p)^n$$

Rearranging this formula, the required number of samples (*n*) can be calculated as follows:

$$n = \log_{10}(1 - P_{(R)})/\log_{10}(1 - p)$$

For the above example and letting $P_{(R)} = 0.95$:

$$n = \log_{10}(1 - 0.95)/\log_{10}(1 - 0.266)$$
$$= 9.7 \text{ samples (rounded to 10 samples)}$$

Therefore, for this example the MC would be:

Lot mean = −1.0 $\log_{10}$ cfu/g
Standard deviation = 0.8 $\log_{10}$ cfu/g
m = −0.5 $\log_{10}$ cfu/g
$n = 10$
$c = 0$
≥95% confidence of lot rejection when contamination greater than or equal to the lot mean of −1.0 $\log_{10}$ cfu/g and area above $m \geq 26.6\%$.

A lot with a mean contamination equal to −1.0 $\log_{10}$ cfu/g would have 95% chance of being rejected with this MC with 10 samples or, conversely, a 5% chance of being accepted. Lots with greater contamination would have a greater chance of being rejected and a lower chance of being accepted. Lots with lesser levels of contamination which are below the FSO or PO would have a lower chance of being rejected and greater chance of being accepted. Type I error occurs when a lot that should be accepted is rejected (i.e., "false positive"). Type II error occurs when a lot that should be rejected is accepted (i.e., "false negative"). The range of contamination levels where types I and II errors occur is minimized by a small standard deviation in the sampling-testing protocol. It would be desirable to have a food process designed and controlled so that most food lots would have contamination levels significantly below the *m* value and the incidence of a false unacceptable determination would be rare.

This example also presumes that the best sampling plan would have $c = 0$, e.g., none of the samples can be positive for the lot to be accepted. Sampling plans with $c = 1$, 2, etc. and Operating Characteristic Curves (ICMSF, 2002; Dahms, 2004) with equivalent or higher confidences in rejecting a contaminated lot can be created. However this implies that the lot is known to have some level of contamination. From a risk communication perspective, a better message would be that a sampling protocol designed to assure the necessary level of public health protection did not detect contamination in any sample. It would be more difficult to communicate that the sampling protocol designed to assure the appropriate level of protection allows a certain number of samples known to be contaminated with a pathogen.

An *m* value may be picked based on an existing test protocol if the *m* falls at an appropriate place on the distribution for the 'just unacceptable lot'. If the protocol is too sensitive, i.e., the *m* value is too low, dilutions can be added to the sample preparation to decrease the test sensitivity. Conversely, if the *m* value is not sufficiently sensitive, a larger sample (greater weight) may be taken or a concentration step, e.g., immunomagnetic separation, can be added to the protocol to increase the sensitivity.

Taking a larger sample, homogenizing or compositing samples may reduce the variation between samples within the lot, particularly in non-homogeneous foods. This may reduce the standard deviation of the samples and will allow a shift in the distribution of the 'just rejectable lot' to a higher lot mean and *m* value which may be easier to detect (see example 3 below). The variation in the sampling protocol is the net result of non-homogeneity in the sample, sampling and assay errors. In a homogeneous sample, most of the standard deviation results from the assay error and can't be reduced. For non-homogeneous or low levels of contamination, modifications in the sampling and sample manipulation can reduce the variation.

Because the FSO concept states that no samples (in this paper defined as 0.13%) can exceed the FSO or PO, if the sampling protocol can reduce the standard deviation of the samples, the lot mean can be closer to the FSO or PO and still be an acceptable lot. The *m* value could correspondingly increase as well. This means that a MC with a test protocol that has a lower standard deviation can have a higher contamination level (lot mean) than a test protocol that has a larger standard deviation and still meet the FSO or PO. Prior considerations of microbial attribute testing plans have not placed much importance on specifying the testing protocol and *m* value and these are weaknesses in the design of these plans.

3. Discussion of MC parameter sensitivity

The following three examples show how variation in a selected parameter affects another parameter in the sampling protocol. The fixed parameter values and the varied parameters in bold font are the values (with rounding) used previously.

Example 1. How the number of samples would need to be adjusted for varying test sensitivity (*m*).

Standard deviation = 0.8 $\log_{10}$ cfu/g
Lot mean = −1.0 $\log_{10}$ cfu/g
95% confidence of lot rejection
c = 0 positive samples

If the test sensitivity (*m*) is ($\log_{10}$ cfu/g)	Then *n* would be[a]
−0.7	6.86
−0.6	8.12
−0.5	**9.69**
−0.4	11.66
−0.3	14.15

[a] *n* = number of samples (not rounded).

As the test sensitivity decreases, i.e., *m* increases, more samples must be tested to have the same confidence of detecting an unacceptable lot. This is because the *m* value is further from the mean concentration and a smaller proportion of the samples will test positive.

Example 2. How the desired confidence of lot rejection affects the lot mean when the test sensitivity, standard deviation and *n* remain constant.

Standard deviation = 0.8 $\log_{10}$ cfu/g
m = −0.5 $\log_{10}$ cfu/g
n = 10 samples
c = 0 positive samples

If the confidence level of rejecting the lot is (%)	Then the lot mean would be ($\log_{10}$ cfu/g)
99.9	−0.502
99	−0.768
95	**−1.017**
90	−1.157
80	−1.336
60	−1.585
40	−1.817
25	−2.024

The confidence level is related to the area of the sample distribution that exceeds *m*. To increase the confidence that a lot is recognized as being contaminated with no change in the number of samples (*n*), this area must increase. To increase this area, the distribution must shift to a higher level of contamination. This example can also be interpreted to show the likelihood of a sample slightly less contaminated than the 'just unacceptable lot' being rejected. If the lot mean was −1.2 $\log_{10}$ cfu/g instead of −1.0 $\log_{10}$ cfu/g, the former lot would have a 90% chance of being rejected instead of a 95% chance. This table shows that there is a probability (25%) of rejecting an acceptable lot that has a mean as much as one log below the MC (−2.0 $\log_{10}$ cfu/g compared to −1.0 $\log_{10}$ cfu/g). This example reflects the trade off between type I and type II errors.

Example 3. How the mean of a just rejectable lot would need to be related to the standard deviation between the samples.

m = −0.5 $\log_{10}$ cfu/g
n = 10 samples
c = 0 positive samples
95% confidence of lot rejection

If the standard deviation is ($\log_{10}$ cfu/g)	Then the just rejectable lot mean would need to be ($\log_{10}$ cfu/g)
0.6	−0.888
0.7	−0.953
0.8	**−1.017**
0.9	−1.082
1.00	−1.147

This example shows that as the variation between samples within a lot increases, the lot mean must move to a lower level of contamination to have the same confidence of rejecting the lot. For a heterogeneous food, a sampling and testing protocol that minimized the within lot variation would allow a lot with a larger mean to meet the FSO compared to lot samples with more variation. The variation between samples in non-homogeneous foods may be reduced by taking larger samples or compositing/homogenizing a set of subsamples. If this were done, the distribution of the samples from a lot at Step 1 would change and the process of determining the MC would need to be repeated.

4. Conclusions

A procedure is described to determine the MC after a risk management decision has been made of the appropriate FSO or PO. The characteristics of the lot that should be rejected, including the mean, standard deviation and fraction of samples that exceed the FSO or PO must be determined. An analytical protocol must be selected that has the appropriate sensitivity. For a microbial pathogen, the number of samples that could be positive in an acceptable lot would probably be set at zero. A confidence level that the just acceptable lot will be detected is chosen and, with the above information, the number of samples can be calculated. Thus for a specific pathogen and food process, this procedure links the acceptance/rejection criteria of the MC to the public health objectives.

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