Microbiological sampling plans – Statistical aspects*

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Introduction

It is nearly 30 years ago that the International Commission on Microbiological Specifications for Foods (ICMSF) provided urgently needed guidance on the use of sampling plans and microbiological criteria for foods in international trade. With publications like "Microorganisms in Foods 2: Sampling for Microbiological Analysis: Principles and Specific Applications" (1) and now "Microorganisms in Foods 7: Microbiological Testing in Food Safety Management" (2) ICMSF introduced concepts of probability and sampling into microbiological criteria and developed a scheme for selection of cases and attributes plans in order to establish criteria for food lot acceptance. Dependent on the conditions in which food is expected to be handled and consumed in the usual course of events and on the degree of concern relative to food utility and health hazard, 15 cases have been distinguished by ICMSF that require increasing stringency of acceptance sampling.

Two general types of sampling plans, attributes sampling plans and variables sampling plans, are used in microbiological testing to make decisions concerning the safety or quality of foods. Attributes plans are used to evaluate qualitative data (presence-absence) or quantitative data that have been grouped (e.g., <10 cfu, 10 to 100 cfu, >100 cfu), whereas variables plans evaluate non-grouped quantitative data.

However, despite their wide use and adoption, microbiological criteria and sampling plans are not fully understood, especially with regard to their statistical background, and in relation to other risk management approaches such as HACCP or Food Safety Objectives. This paper gives an overview on the design of sampling plans forming part of microbiological criteria for foods and on characteristics that determine their reliability and performance.

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Attributes sampling plans

Two-class attributes sampling plans

A simple way to decide whether to accept or reject a food lot may be based on some microbiological test performed on several sample units. For pathogens this will usually be a test for the presence (positive result) or absence (negative result) of the organism. Concentrations of microorganisms can be assigned to a particular attribute class by determining whether they are above (positive) or below (negative) some preset concentration.

The decision making process of a two-class plan is essentially defined by two numbers. The first, denoted as *n,* determines the number of sample units that are to be drawn independently and randomly from the lot. The second number, denoted as *c,* is the maximum allowable number of sample units yielding unsatisfactory test results, for example, the presence of the organism. In case of a two-class plan applied to grouped quantitative data there is one microbiological limit, denoted by *m,* which separates good quality from non-acceptable or defective quality. In this case the maximum allowable number of sample units exceeding this limit is given by *c*, which is usually set to zero for pathogens.

To visualize and study the performance of a sampling plan a graphical representation of its Operating Characteristic (OC) curve or function is useful. For a two-class plan this curve has two scales, the horizontal scale showing a measure of lot quality like the fraction or percentage of positive ("defective") units in the lot being tested, the vertical scale giving the probability of acceptance. The OC curve shown in figure 1, for example, depicts acceptance probabilities for lots in relation to the fraction of defective units when a two-class plan is applied specifying that a number of $n=5$ sample units are to be drawn and none of them $(c=0)$ are allowed to be positive.

If evaluated for lots containing proportions defective that are regarded as not acceptable, for instance in a risk analysis context, acceptance probabilities characterize the risk that non-conforming lots will be falsely accepted. On the other hand, rejection probabilities, or one minus the according acceptance probabilities, that are derived for actually conforming lots describe the so-called producer's risk.

When sampling plans are compared and their stringency in making decisions is considered, different aspects of their performance can be addressed. In an idealized situation the OC curve would fall down from a 100 % probability of acceptance to a 100 % probability of rejection just at the limit proportion defective that distinguishes between conforming and non-conforming lot quality. In practice, no sampling plan can achieve this ideal, but the steeper the curve, the closer the sampling plan comes to approaching the ideal. In general steeper curves can only be achieved by increasing the number of sample units *n* to be drawn from a lot. This should be distinguished from a shift of the OC curve that is achieved by decreasing the acceptance number *c*. A lower value for *c* will result in a general reduction of consumer's risk by stating a different limit of acceptability, whereas the producer's risk will be increased.

There are two additional vertical lines in figure 1 highlighting lot qualities that may be referred to as characterizing sampling plan performance. From a consumer's or regulator's point of view the prospects to ensure food safety by applying a sampling plan can be evaluated by examining which lot quality would be rejected with high probability, for example 95 % (or accepted with low probability). Food producers, however, will be more interested in examining which lot quality would be accepted with high probability, say 95 %, to adjust their production processes accordingly.

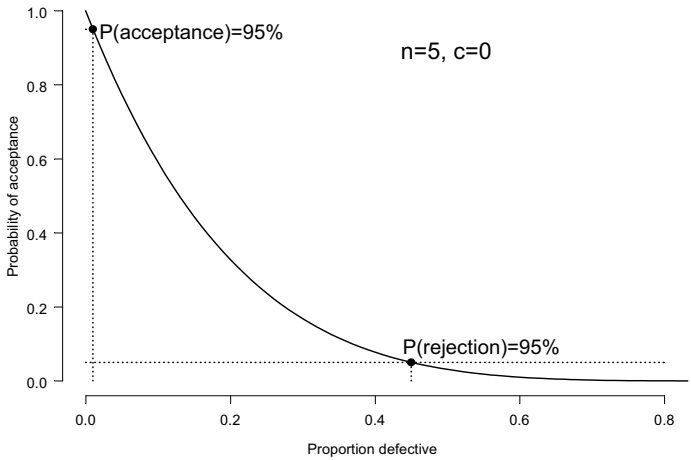

Figure 1 **OC-curve for a two-class sampling plan in relation to proportion defective**

Thus, performance of the sampling plan in general, lot qualities that are actually rejected or accepted with high probability, and steepness of the OC curve depend on sampling plan specifications *n* and *c*. Figure 2 gives an impression how much these characteristics change in case the number of sample units is increased to *n=10* or *n=20* resulting in more steeply falling curves and lower acceptance probabilities, and thus in better assurance that lots with high proportions defective will be rejected.

Three-class attributes sampling plans

In situations where decisions are not based on results of presence-absence tests but on quantitative analytical results, three-class plans can be applied as an alternative to two-class plans working with data grouped according to a single microbiological limit *m*.

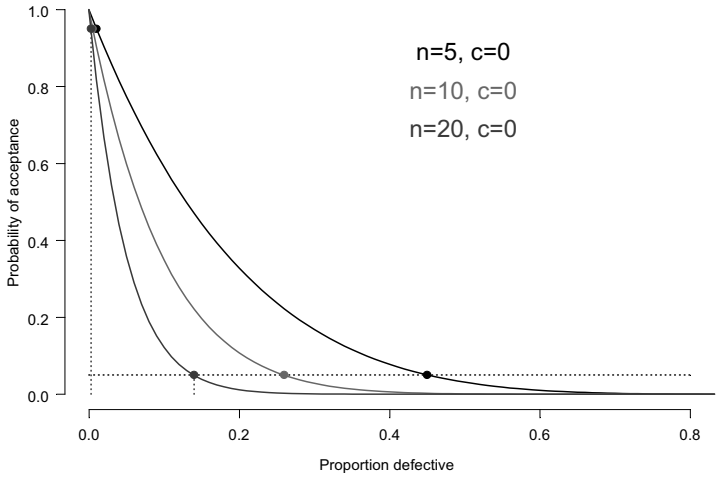

Figure 2 **OC-curves for two-class sampling plans in relation to proportion defective with varying number of sample units**

Three-class plans were devised for situations where the quality of food lots can be divided into three attribute classes. As in two-class plans based on quantitative analytical results, sample results above a concentration *m*, which in a three-class plan separates good quality from marginally acceptable quality, are not desirable, but a certain number, denoted as *c*, can be accepted. However, sample results above a second microbiological limit *M* are unacceptable (or defective), and usually a lot is rejected if any analytical result for the *n* sample units drawn from the lot being tested exceeds *M*.

For three-class plans acceptance probabilities for lots being tested depend on two fractions describing lot quality, the percentage of marginally acceptable units with microbiological concentrations between *m* and *M*, and the percentage of unacceptable units with concentrations exceeding *M*. Therefore, depiction of OC functions for three-class plans results in three-dimensional graphs, which are difficult to compare with two-dimensional OC curves visualizing performance of two-class plans as described earlier.

Attributes sampling plans for assessment of mean microbiological concentrations

Only when the result of a microbiological analysis is given in a quantitative manner, for instance as a count, there is a choice between types of sampling plans like two- or three-class plans, and thus need for some way to compare their per-

formance. The decision for a suitable sampling plan depends on the given purpose and on available prior information on production processes. When dealing with quantitative analytical results for sample units in a lot, questions arise concerning the frequency distributions of sample results and whether there is any previous information on shape, location, and spread of these distributions.

The following considerations are restricted to a situation where the production process is known and well documented showing some evidence that log-transformed sampling results from food lots follow a normal distribution. Based on this assumption sampling plans can be compared by means of their OC function, which is again calculated and plotted for various lot qualities, but now lot quality is described by the mean concentration of microbes for all units in the lot and their standard deviation. To relate the performance of attributes sampling plans to concentration the frequency distribution of analytical results in sample units is used to establish the proportion of defective samples in the lot, as proposed by *Hildebrandt et al.* (3). Assuming a normal distribution for log-concentrations of microbes, the area under the normal density function above *m,* as shown in figure 3, is used to define the value for the proportion defective for a two-class sampling plan. For a three-class sampling plan the area between *m* and *M* defines the value for the proportion marginally acceptable, as shown in figure 4, and the area above *M* defines the value for the proportion defective. With given frequency distribution for the lot, these proportions can be derived, making it possible to calculate acceptance probabilities for both types of attributes plans and to relate them to mean concentration in the lot being sampled.

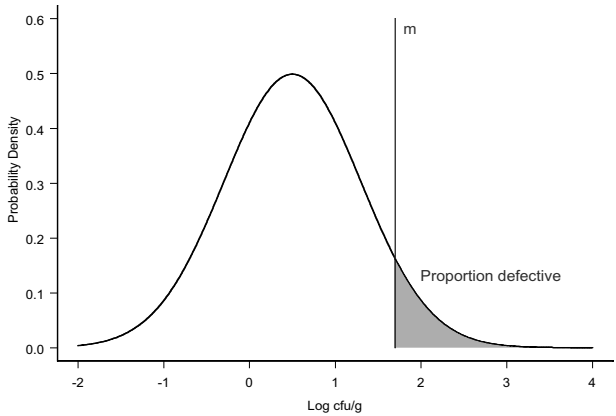

Figure 3 **Frequency distribution (log-normal) describing lot quality and proportion defective for a two-class sampling plan**

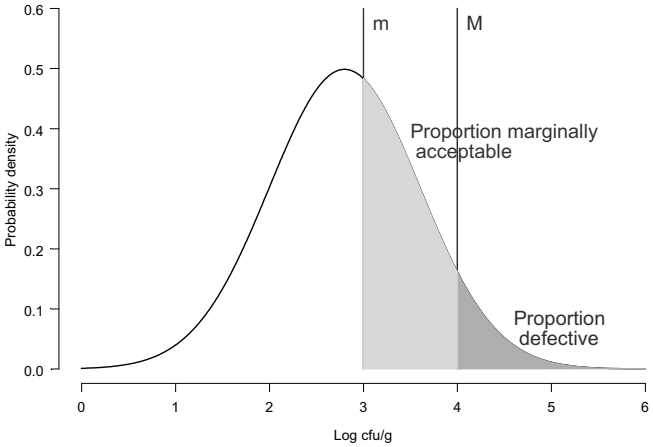

Figure 4 **Frequency distribution (log-normal) describing lot quality and proportions marginally acceptable and defective for a three-class sampling plan**

OC curves related to mean concentrations then can be developed by

- increasing the mean of a normal distribution with fixed standard deviation through a range of values,
- deriving the corresponding proportions defective (and marginally acceptable) for each distribution,
- calculating acceptance probabilities according to prescriptions for *n* and *c*,
- and plotting them against the normal distribution means.

A spreadsheet to facilitate these calculations has been developed by *Legan et al.* (4) and can be downloaded from the ICMSF homepage (see references).

As an example, figure 5 shows the resulting OC curve for a two-class plan with $n=5$, $c=0$, and a microbiological limit set at $m=100$ cfu/g (or $m=2$ in log-units), assuming log concentrations in sample units are normally distributed with a standard deviation of 0.8 log-units.

Mean concentrations characterizing lot qualities that will be rejected or accepted with high probability, say 95 %, can be highlighted for this type of plot as well (figure 5). Likewise, the effects of changes in sampling plan prescriptions can be studied. Figure 6, for example, visualizes shifts of the OC curve gained by increasing the number of sample units to $n=10$ and $n=20$.

However, when dealing with quantitative analytical results, performance of the sampling plan does not only depend on *n* and *c,* the number of sample units and the maximum allowable number of non-acceptable units, but on the microbiological limits *m* and *M* as well. Furthermore, calculation of acceptance probabilities

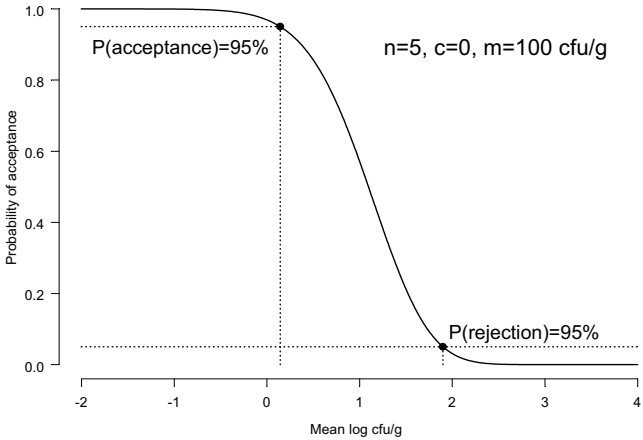

Figure 5 **OC-curve for a two-class sampling plan in relation to mean microbial concentration**

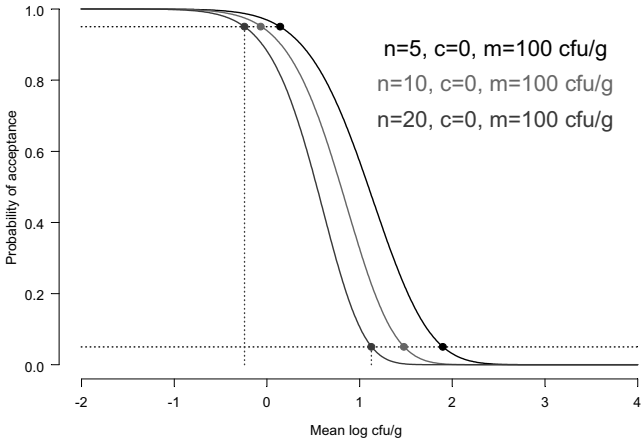

Figure 6 **OC-curves for two-class sampling plans in relation to mean microbial concentration with varying number of sample units**

requires assumptions be made regarding the shape and spread of the frequency distribution of sample results. Thus, the effect of using an attributes plan is also dependent on the validity of the underlying assumptions for the frequency distribu-

tion, especially with regard to its standard deviation. However, with prior experience substantiating the assumptions made, even attributes plans can be used to assess mean microbiological concentrations in lots of food.

For two-class plans suggested by ICMSF for situations classified as cases 10 to 15 (serious to severe concern) mean microbial concentrations that are rejected with 95 % probability are given in table 1, assuming a lot standard deviation of 0.8 log-units and a microbiological limit of *m=0 cfu/25 g* (i.e., 25 g samples are drawn that should be negative with regard to the target microorganism). Likewise, table 2 is listing mean microbial concentrations that are accepted with 95 % probability. With respect to a scale expressed in cfu/g these means should be interpreted as geometric means, as they are derived by taking arithmetic means on the log-scale to base 10.

Table 1
Mean cfu/g rejected with 95 % probability for ICMSF two-class attributes sampling plans (geometric mean)

Type of hazard	*Conditions reduce hazard*	*Conditions cause no change in hazard*	*Conditions may increase hazard*
Serious	Case 10: n=5, c=0 1 cfu/32 g	Case 11: n=10, c=0 1 cfu/83 g	Case 12: n=20, c=0 1 cfu/185 g
Severe	Case 13: n=15, c=0 1 cfu/135 g	Case 14: n=30, c=0 1 cfu/278 g	Case 15: n=60, n=0 1 cfu/526 g

m=0 cfu/25 g
Assumed frequency distribution: log-normal with a standard deviation of 0.8 log-units

Table 2
Mean cfu/g accepted with 95 % probability for ICMSF two-class attributes sampling plans (geometric mean)

Type of hazard	*Conditions reduce hazard*	*Conditions cause no change in hazard*	*Conditions may increase hazard*
Serious	Case 10: n=5, c=0 1 cfu/1515 g	Case 11: n=10, c=0 1 cfu/2439 g	Case 12: n=20, c=0 1 cfu/3846 g
Severe	Case 13: n=15, c=0 1 cfu/3125 g	Case 14: n=30, c=0 1 cfu/4761 g	Case 15: n=60, n=0 1 cfu/7142 g

m=0 cfu/25 g
Assumed frequency distribution: log-normal with a standard deviation of 0.8 log-units

Variables sampling plans

When the underlying distribution of microbial concentrations within lots is known, or can be assumed, an alternative option is to use variables sampling plans. As such plans make full use of the microbial counts, rather than ascribing them to categories or classes, variables plans can be more useful under some conditions than attributes plans. The following is an example of the way in which a variables plan

may be designed. In this case, the decision rule is based on the assumption that the underlying distribution of microbial concentrations in the lot is log-normal, as assumed for attributes plans.

Design of a variables plan involves several decisions to be made. The first is to define acceptable limits for a lot to be tested, in terms of an acceptable microbiological quality limit V and the maximum proportion p_0 of the lot, that can be accepted with concentrations above the limit V. Based on these values and information on the expected standard deviation within lots a limit for the mean concentration can be derived as shown in figure 7. In addition a value α must be chosen which represents the maximum probability – or risk – to accept a non-conforming lot. This corresponds to $1\text{-}\alpha$ being the desired probability to reject a non-conforming lot.

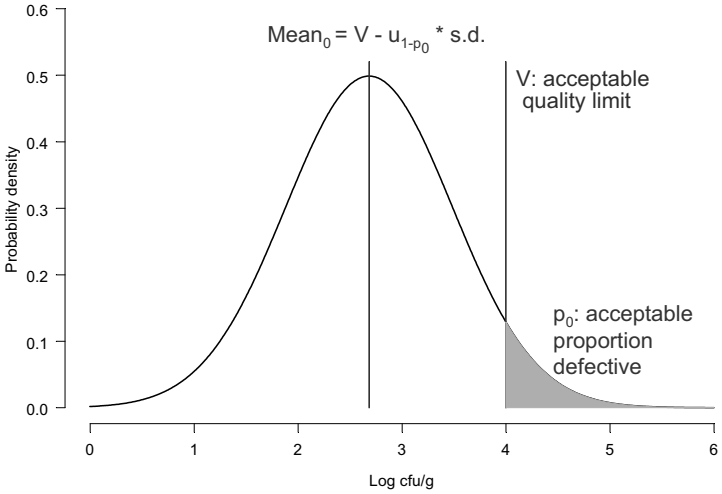

Figure 7 **Frequency distribution (log-normal) describing lot quality and acceptability limits for a variables sampling plan**

Using these prescriptions the decision rule of the variables plan can be based on the arithmetic mean of log-transformed analytical results for a specified number n of sample units. When acceptable or conforming lots are tested this mean is expected to be lower than the limit mean concentration derived from V and p_0. Therefore a lot is rejected if the arithmetic mean calculated for the sample units, plus the standard deviation multiplied by a factor k, exceeds the limit V, where k is dependent on the given values for n, p_0, and α. For more details, and some tables for selection of k, see (2).

Though formulation of the decision rule appears to be more complex for variables plans than for attributes plans, this procedure has the advantage that definition of a conforming lot and the desired confidence in decision making become more transparent.

Microbiological sampling plans and Food Safety Objectives

To illustrate points to consider when relationships between microbiological criteria or sampling plans and Food Safety Objectives (FSOs) are discussed, a published proposal for *Listeria monocytogenes* may serve as an example.

With regard to the limited data on the occurrence of *Listeria monocytogenes* in foods and of listeriosis in humans and difficulties in performing sound risk analyses for food-born diseases, ICMSF has used an FSO of 100 cfu/g at time of consumption just as an example for illustration purposes (5). Using the cases as classified by ICMSF (2) to decide on a sampling plan to be applied as a control measure for *Listeria monocytogenes* in cold-smoked salmon, case 11 would apply when no inactivation and no growth is expected to occur before consumption. For this situation ICMSF has suggested a two-class plan for grouped quantitative analytical results with $n=10$ and $c=0$, choosing a microbiological limit m that corresponds to the tentative FSO of 100 cfu/g (6).

Performance of this sampling plan now can be assessed with respect to characteristics outlined for attributes plans. Assuming a log-normal distribution of *Listeria monocytogenes* concentrations with a standard deviation of 0.8 log-units within lots being tested, lots with a mean concentration back-transformed to a geometric mean of 30 cfu/g would be rejected with 95 % probability; lots with a mean concentration corresponding to 1 cfu/g cold-smoked salmon would be accepted with 95 % probability. At first glance this seems to be appropriate to meet the FSO.

However, if these lot qualities are depicted as frequency distributions, as in figure 8, it becomes obvious that a lot that is rejected with 95 % probability (and hence accepted 5 % of the times such a lot is tested) still contains a proportion of about 26 % exceeding the FSO. Only in case all producers would process the food according to a quality level that would be accepted with 95 % probability, the FSO would represent the upper limit of concentrations in terms of the 99.5-percentile of their frequency distribution.

With such calculations it can be demonstrated that a simple translation of an FSO into a microbiological limit for a sampling plan is not appropriate, as uncertainties and confidence requirements usually accounted for with statistically based decision rules would be neglected. Precise definition of what is meant with an FSO and prescription of desired confidence in decision making still need more discussion, in order to develop suitable sampling plans and microbiological criteria that are in accordance with stated Food Safety Objectives.

Effective use of microbiological sampling requires a firm understanding of the statistical basis for subsequent decision-making, providing the conceptual frame-

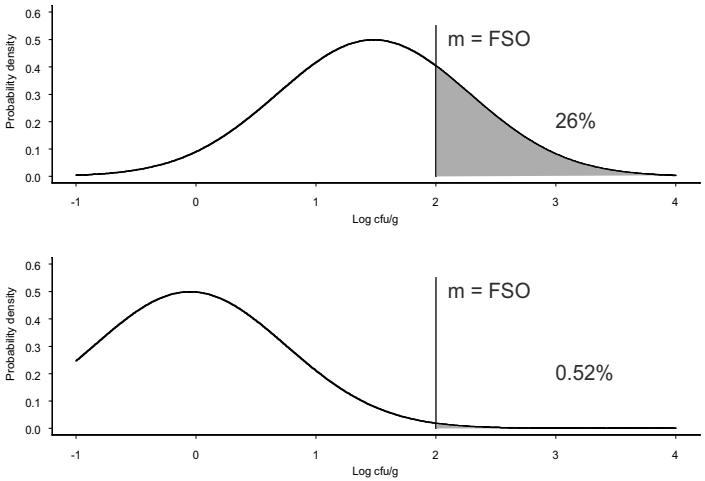

Figure 8 **Critical lot qualities in relation to tentative FSO for a sampling plan suggested for *Listeria monocytogenes***

work for the development and implementation of microbiological testing programs. These concepts are critical if microbiological testing is to remain an important tool for evaluating the quality or safety of foods within a risk analysis context.

An extended version of this lecture with more comprehensive slides can be downloaded from www.foodscience.afisc.csiro.au/icmsf/SD2002_website.pdf.

Acknowledgements

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Summary

Two types of microbiological sampling plans, attributes sampling plans and variables sampling plans, are outlined in this paper. Attributes plans are used to evaluate qualitative data (presence-absence) or quantitative data that have been grouped, whereas variables plans evaluate non-grouped quantitative data. Operation Characteristic (OC) curves are used to visualize lot acceptance probabilities both in relation to the fraction of defective units and the mean concentrations of a target microorganism in food lots being sampled. In the latter case calculation of acceptance probabilities requires assumptions be made regarding the shape and spread of the frequency distribution of sample results. Thus, the effect of using a sampling plan to assess mean microbial concentration in a lot is not only dependent on the microbial limits set and the number of samples required, but as well on the validity

of the underlying assumptions for the frequency distribution. However, with prior experience substantiating the assumptions made, even attributes plans can be used to assess mean microbiological concentrations in lots of food. Taking this approach the performance of microbiological sampling plans as a risk management option can be evaluated in relation to Food Safety Objectives.

Zusammenfassung

Dieser Beitrag behandelt zwei Arten mikrobiologischer Stichprobenpläne: Attributpläne, die verwendet werden, um qualitative Daten, wie Ergebnisse von Presence-Absence-Tests, oder gruppierte quantitative Daten auszuwerten, und Variablenpläne, die zur Bewertung von ungruppierten quantitativen Analyseergebnissen herangezogen werden. Mit Hilfe von Operationscharakteristiken lassen sich in Abhängigkeit von der Chargenqualität die Wahrscheinlichkeiten darstellen, mit denen beprobte Lebensmittelchargen angenommen werden. Chargenqualität lässt sich dabei als Anteil defekter Einheiten in der Charge beschreiben oder durch die mittlere Konzentration, mit der ein interessierender Mikroorganismus in der Charge vorkommt. Im zweiten Fall sind zur Berechnung von Annahmewahrscheinlichkeiten Verteilungsannahmen hinsichtlich der Keimkonzentrationen in der Charge zu treffen. Die Zuverlässigkeit eines Stichprobenplanes hängt dann aber nicht nur von den festgelegten mikrobiologischen Grenzwerten und der Zahl zu ziehender Stichprobeneinheiten ab, sondern auch von der Validität der getroffenen Annahmen. Wenn sich diese jedoch durch Vorinformationen untermauern lassen, können sogar Attributpläne zur Bewertung mittlerer Keimkonzentrationen in Lebensmittelchargen verwendet werden. Solch ein Ansatz ermöglicht es, die Leistungsfähigkeit mikrobiologischer Stichprobenpläne als Option des Risikomanagements hinsichtlich so genannter «Food Safety Objectives» zu untersuchen.

Résumé

Cette contribution discute deux types de plans d'échantillonnage microbiologique : des plans à attributs utilisés pour évaluer des données qualitatives comme les résultats de tests présence/absence ou des données quantitatives groupées. Les plans à variables eux sont utilisés pour l'évaluation de résultats analytiques non groupés. A l'aide de caractéristiques opératoires il est possible de déterminer, en fonction de leur qualité, les probabilités d'acceptation de lots de produits. La qualité du lot peut, dans ce cas là, être décrite comme nombre d'unités défectueuses dans ce lot ou par la concentration moyenne du germe recherché dans ce lot. Afin de calculer les probabilités d'acceptation il est nécessaire, dans le deuxième cas, de faire des estimations de la distribution des germes dans le lot. La fiabilité d'un plan d'échantillonnage dépend alors non seulement des limites microbiologiques fixées et du nombre d'échantillon, mais également de la validité des estimations faites. Si ces estimations se laissent confirmer par des informations complémentaires, alors même les plans à attributs peuvent être utilisés pour l'évaluation de la charge microbienne moyenne

d'un lot. Une telle approche permet d'examiner la performance de plan d'échantillonnage microbiologique en tant qu'option dans le cadre d'une gestion des risques en relation avec des « Food Safety Objectives » établis.

Key words
Sampling plans, microbiological criteria, attributes plans, food safety objectives, operating characteristic curve

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