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MICROORGANISMS IN FOOD 7

MICROBIOLOGICAL TESTING IN FOOD SAFETY MANAGEMENT

Continuing this excellent series, *Microorganisms in Foods 7* describes the role of microbiological testing in modern food safety management systems. It explores how risk assessment and risk management can be used to establish goals—known as "tolerable levels of risk" or "food safety objectives"—for use in controlling foodborne illness, and provides guidelines for establishing effective management systems to control specific hazards in foods. It describes the respective roles of industry and government, recognizing that it is through their collective actions that effective food safety systems are developed and verified, and helps countries determine whether imported foods have been produced with an equivalent level of protection.

This groundbreaking book will be of interest to food microbiologists, researchers, and others in the food industry, regulatory agencies and academia worldwide.

The information has been prepared by the International Commission on Microbiological Specifications for Foods (ICMSF). The ICMSF was formed in response to the need for internationally acceptable and authoritative decisions on microbiological limits for foods moving in international commerce. Currently the membership consists of eighteen food microbiologists from eleven countries, drawn from governmental laboratories in public health, agriculture, and food technology, from universities and from the food industry."

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MICROBIOLOGICAL TESTING IN FOOD SAFETY MANAGEMENT

ICMSF

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ICMSF

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Introduction

Microorganisms in Foods 7: Microbiological Testing in Food Safety Management was written by the International Commission on Microbiological Specifications for Foods (ICMSF/the Commission) with assistance from a limited number of consultants.

Microorganisms in Foods 7 is based upon Part I of *Microorganisms in Foods 2: Sampling for Microbiological Analysis: Principles and Specific Applications (2nd ed. 1986)*. In the 1980s, control of food safety was largely by inspection and compliance with hygiene regulations, together with end product testing. *Microorganisms in Foods 2* put such testing on a sounder statistical basis through sampling plans, which remain useful at port-of-entry when there is no information on the conditions under which a food has been produced or processed. At an early stage, the Commission recognized that no sampling plan could ensure the absence of a pathogen in food. Testing foods at ports-of-entry, or elsewhere in the food chain, could not guarantee food safety.

This led the Commission to explore the potential value of Hazard Analysis Critical Control Point (HACCP) for enhancing food safety, particularly in developing countries. *Microorganisms in Foods 4: Application of the Hazard Analysis Critical Control Point (HACCP) System to Ensure Microbiological Safety and Quality (1988)*, illustrated the procedures used to identify the microbiological hazards in a practice or a process, to identify the critical control points at which those hazards could be controlled, and to establish systems by which the effectiveness of control could be monitored. In addition, recommendations were included for the application of HACCP from production/harvest to consumption, together with examples of how HACCP could be applied at each step in the food chain.

Effective implementation of HACCP requires knowledge of the hazardous microorganisms and their response to conditions in foods (e.g., pH, a_w, temperature, preservatives). The Commission concluded that such information was not collected together in a form that could be assessed easily by food industry personnel in quality assurance, technical support, research and development, and by those in food inspection at local, state, regional, or national levels. *Microorganisms in Foods 5: Characteristics of Microbial Pathogens (1996)* is a thorough, but concise, review of the literature on growth, survival, and death responses of foodborne pathogens. It is intended as a quick reference manual to assist in making judgements on the growth, survival, or death of pathogens in support of HACCP plans and to improve food safety.

Microorganisms in Foods 6: Microbial Ecology of Food Commodities (1998) is intended for those primarily involved in applied aspects of food microbiology such as

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food processors, food microbiologists, food technologists, veterinarians, public health workers, and regulatory officials. Addressing 16 commodity areas, it describes the initial microbial flora and the prevalence of pathogens, the microbiological consequences of processing, typical spoilage patterns, episodes implicating those commodities with foodborne illness, and measures to control pathogens and limit spoilage.

This book, *Microorganisms in Foods 7: Microbiological Testing in Food Safety Management (2002),* illustrates how systems such as HACCP and good hygiene practices (GHP) provide greater assurance of safety than microbiological testing, but also identifies circumstances where microbiological testing still plays a useful role in systems to manage food safety. It continues to address the Commission's objectives to: (a) assemble, correlate, and evaluate evidence about the microbiological safety and quality of foods; (b) consider whether microbiological criteria would improve and ensure the microbiological safety of particular foods; (c) propose, where appropriate, such criteria; (d) recommend methods of sampling and examination; (e) provide guidance on appraising and controlling the microbiological safety of foods.

This book introduces the reader to a structured approach for managing food safety, including sampling and microbiological testing. The text outlines how to meet specific food safety goals for a food or process using GHP and the HACCP system.

The concept of a food safety objective (FSO) is recommended to industry and control authorities to translate "risk" into a definable goal for establishing food safety management systems that incorporate the principles of GHP and HACCP. FSOs provide the scientific basis for the industry to select and implement measures that control the hazard(s) of concern in specific foods or food operations, for control authorities to develop and implement inspection procedures to assess the adequacy of control measures adopted by industry, and for quantifying the equivalence of inspection procedures in different countries.

Microbiological testing can be a useful tool in the management of food safety. However, microbiological tests should be selected and applied with knowledge of their limitations, as well as their benefits and the purposes for which they are used. In many instances other means of assessment are quicker and more effective.

The need for microbiological testing varies along the food chain. Points in the food chain should be selected where information about the microbiological status of a food will prove most useful for control purposes. Similarly, in a food operation, samples may be collected from different points in a process for control purposes.

Finally, a framework is provided by which importing countries can assess whether foods from other countries have been produced in a manner that provides a level of protection equivalent to that required for domestically produced foods.

This book illustrates the insensitivity of even statistically based sampling plans and encourages a rational approach to the use of microbiological testing in systems that manage food safety through GHP and HACCP. Several new chapters are based on the experience of the food industry in controlling salmonellae, *Listeria monocytogenes* and *Escherichia coli* O157:H7, on tightened or investigational sampling, on microbiological testing of the processing environment, and the use of statistical process control to detect trends and work toward continuous improvement.

The book is intended to be useful for anyone who is engaged in setting microbiological criteria for the purpose of governmental or industry food inspection and control. For students in food science and technology it offers a wealth of information on food safety management and many references for further study.

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Concepts of Probability and Principles of Sampling

6.1 Introduction
6.2 Probability
6.3 Population and Sample of the Population
6.4 Choosing the Sample Units
6.5 The Sampling Plan
6.6 The Operating Characteristic Function
6.7 Consumer Risk and Producer Risk
6.8 Stringency and Discrimination
6.9 Acceptance and Rejection
6.10 What Is a Lot?
6.11 What Is a Representative Sample?
6.12 Confidence in Interpretation of Results
6.13 Practical Considerations
6.14 References

6.1 INTRODUCTION

It is important to recognize that the management of food safety using the approaches outlined in the first five chapters, based on controlling hazards through Good Hygienic Practices (GHP) and Hazard Analysis Critical Control Point (HACCP), is much more effective than trying to ensure safety by lot acceptance through end-product testing. This chapter discusses the concepts of probability and sampling that determine the limitations of end-product testing. Those concepts form the basis of the design of statistically based sampling plans (Chapter 7) and the establishment of cases (Chapter 8).

In Chapters 6 and 7, it is assumed that the microbiological test will give an absolutely accurate result, with no false positives or false negatives. This is not a realistic assumption, and problems arising from sampling, sample transport and preparation for analysis, and the imprecision of microbiological methods are addressed in Chapters 10 and 12 and elsewhere in the text.

6.2 PROBABILITY

Consider a trial or a test, the outcome of which is doubtful, such as a test for the presence of an organism in a food. After the standard procedure has been followed, the test either shows the presence of the organism or it does not; thus we have a positive or a negative result. If many of the organisms were present in the food sampled, we would expect relatively many such tests to yield a positive result. But if there were few organisms pres-

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ent, we would expect that fewer tests would yield a positive result. In the two cases, the probability of a positive result would be high and low, respectively.

The probability of a positive result is, in fact, the long-run proportion of times a positive result occurs out of all the times we test the food. Thus, if a positive result occurs 112 times in 1000 tests, we estimate the probability to be 112/1000 = 0.112, while if a positive result occurs 914 times in 1000 trials, we estimate the probability to be 914/1000 = 0.914. The word "estimate" is used because if we were to run 1000 trials again on the same material and using the same procedure, we could not be sure of observing 112 and 914 positive results, respectively. The results should, however, be close to this proportion because 1000 is a large number of trials. Thus, the estimated probability of a positive result, or any other outcome in some other type of test, is the proportion of times a positive outcome occurred among the trials or tests actually made. A probability can be anywhere from 0 to 1. It will be zero if the organism is absent from the food (assuming a test procedure that will detect any organism present) and 1 if every one of a large number of tests provides a positive result.

What does an observed proportion of trials such as 0.112 signify? Suppose we divided the entire lot of food into small sample units, say perhaps 10,000,000 one-gram units, and then went through the test procedure on each of these sample units. Suppose 1,051,200 gave positive results. Then the ratio 1,051,200/10,000,000 (the actual proportion of positives) = 0.10512 is the measure of the probability. But what kind of measure? This is no longer an estimate of the probability of a positive result. Instead, it is the *true probability* or *population probability*. Of course, this approach is not practical, because of the test time needed, and because there would be no food left to eat! But it is useful to have this concept in mind. The population probability determines the kind of sample probabilities or estimated probabilities we may expect from a given number of sample units examined. If that number is low, the estimated probability is not likely to be precise. In general, we never know the true or population probability. But we do know that the larger the number of units included in the sample of the population, the closer the estimated probability is likely to be to the population probability.

6.3 POPULATION AND SAMPLE OF THE POPULATION

The preceding section introduced the two basic concepts: (i) population as a whole and (ii) sample of the population. In relation to results of an experimental test, these are, respectively, the whole set of results of the test made on each and every unit in the lot of food, and the partial collection of such results, derived only from the group of sample units actually examined. In terms of standard plate counts, for example, these concepts would be represented by (i) all the counts that could be made by examining every unit in the lot and (ii) those counts actually made on the few sample units examined. Statisticians use the word *sample* for the group of units which is withdrawn to estimate the character of a population, while analysts or bacteriologists would call any one of these units a sample. To try to minimize the confusion, we will speak of the sample of the population, being the whole group of units withdrawn, and the *sample units* of which the sample is composed. The assumption is also made that a sample unit is an identifiable unit that can be repeatedly recognized (e.g., a batch of uniform sized beef burgers or a unit of packaging, or the contents of a defined sampling tool). The *analytical unit* may then be a portion of the sample unit. In this chapter, the sample unit and the analytical unit are treated as though they are the same.

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6.4 CHOOSING THE SAMPLE UNITS

Sections 6.10 and 6.11 describe how to choose the material to be tested from the total amount in the lot or shipment, and Chapter 12 describes the practical aspects of collecting and handling samples. The important point is to avoid bias, so that the sample of the population may represent the lot as well as possible. Random choice is one way of achieving this. Thus, if we can think of the lot as made up of a population of 10 g blocks called sample units, and we decide upon a sample from this population of 10 such units, then we should choose these units in such a way that each sample unit in the lot has the same chance of being included among the sample units chosen. In practice, it is often difficult to ensure such random sampling, and this can be particularly significant for populations with incomplete mixing or of unknown origin. At the very least, however, we should try to draw test material from all parts of the lot.

In this book, all statistical calculations assume that the sample has been drawn randomly, unless otherwise stated.

6.5 THE SAMPLING PLAN

The sample units so drawn will, after examination, yield results that will be compared with certain criteria to reach a decision as to whether the entire lot should be accepted or rejected (see section 6.9 for explanation of rejection). The particular choice of sampling procedure and the decision criteria make up the *sampling plan*. Proper drawing of samples is essential if the decision making criteria of the sampling plan are to give unbiased results. By sampling randomly, we are able to reduce the risk of making biased decisions.

A simple example of a sampling plan follows. Take 10 sample units of a food from a lot and subject them to appropriate laboratory procedure to test for the presence or absence of a microorganism. If two or fewer of the 10 sample units show presence of the organism, that is, give a positive result, then the whole lot of food is acceptable (relative to this organism). But if three or more give a positive result, the whole lot is to be rejected. This plan is described by the use of two terms $n = 10$ and $c = 2$, where n is the number of sample units chosen separately and independently, and where c is the maximum allowable number of sample units yielding a positive result.

6.6 THE OPERATING CHARACTERISTIC FUNCTION

In section 6.5, we described what we mean by a sampling plan and gave an example of a simple sampling plan based upon positive and negative indications of a microorganism. This plan was described by the two numbers $n = 10$, $c = 2$. If we are going to use this plan, we want to know what assurance it will give us. How discriminating is it? It is possible when using such a plan that it will sometimes accept a poor lot; it is also possible for the plan to reject a good lot. There is no way to avoid some degree of risk in each acceptance and in each rejection (see also section 6.7), unless we test the entire lot, in which case no edible food will be left. We can make these risks smaller by testing more sample units; that is, by using a larger number of samples (larger n). In fact, we can reduce the risks to any desired level by making n sufficiently large. But in practice, we usually have to seek a compromise between (a) large n (many sample units) and small risks, and (b) small n (few sample units) and large risks.

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We normally use an *operating characteristic* function to describe the performance of a sampling plan. This is often depicted as an operating characteristic curve (OC curve). Such a curve has two scales. The horizontal scale shows a measure of lot quality. The most common measure of lot quality is the true probability or percentage of times that a test on a sample unit from a lot of this quality would show an unsatisfactory result (e.g., a positive, or a count above some number m). This is called p, and it can be anywhere between 0 and 100%. This probability is commonly expressed as percentage of *defectives*. The vertical scale gives the probability of acceptance, P_a; that is, P_a is the expected proportion of occasions that the results will indicate that the lot is acceptable out of the number of times a lot of this given quality is sampled to find a decision. Let us see how the probability runs $n = 10$, $c = 2$ (the calculations are carried out by using the so-called binomial distribution):

p(%)	0	10	20	30	40	50	60
P_a	1.00	0.93	0.68	0.38	0.17	0.05	0.01

Figure 6–1 shows the full curve. We see that the greater the probability (p) that a test unit from a given lot yields a positive result to indicate the presence of the target microorganism, the lower is the probability of acceptance (P_a) of that lot. If, for example, we set a limit of 20% defectives (i.e., $p = 20\%$), then $P_a = 0.68$. This means that on 68 of every 100 occasions when we sample a lot containing 20% defectives, we may expect to have 2 or fewer of the 10 tests showing the presence of the organism, and thus calling for acceptance, while on 32 of every 100 there will be 3 or more positives, calling for nonacceptance. But if $p = 10\%$ (i.e., 10% positive units in the lot), such lots will be accepted 93 out of 100 times; if $p = 40\%$, such lots will be accepted 17 out of 100 times. Thus

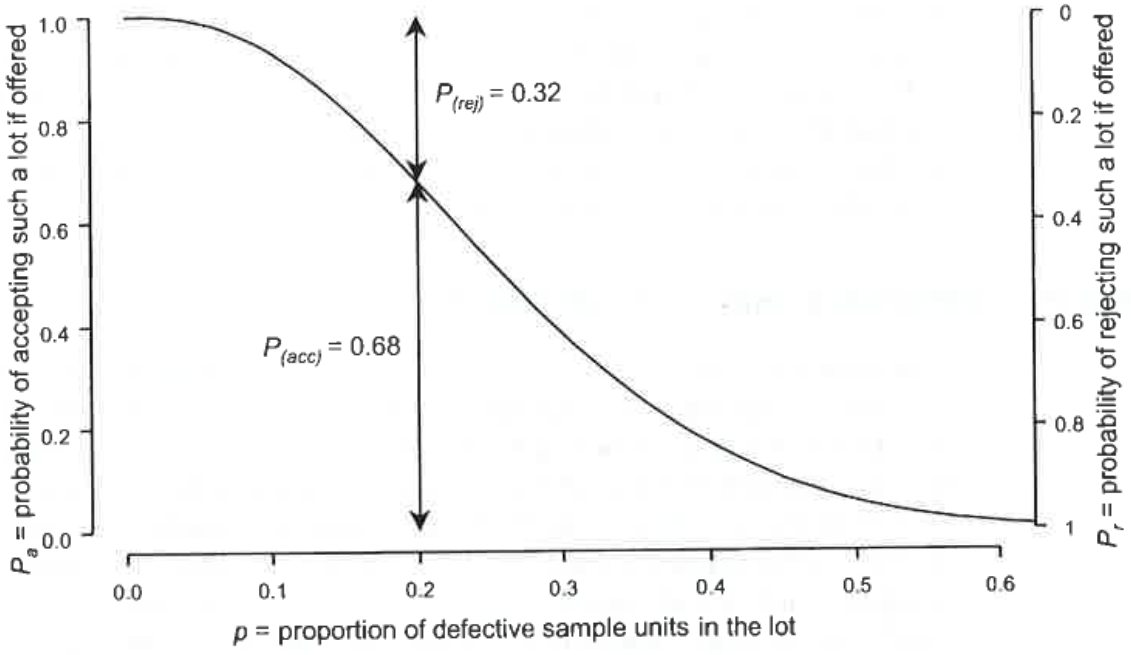


Figure 6–1 The operating characteristic curve for a sampling plan with $n = 10$, $c = 2$, i.e., the probability of accepting lots, in relation to the proportion defective among the sample units in the lot being examined. If, for example, the lot comprises 20% ($p = 0.2$) defective sample units, the lot will be accepted with a probability of 0.68 and rejected with a probability of 0.32.

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lots with 10% of the sample units defective will be accepted most of the time, but with 40% defective, rather seldom.

6.7 CONSUMER RISK AND PRODUCER RISK

Since decisions to accept or reject lots are made on samples drawn from the lots, occasions will arise when the sample results do not reflect the true condition of the lot. The *producer's risk* describes the probability that an acceptable lot if offered will be falsely rejected. The *consumer's risk* describes the probability that an unacceptable lot when offered will be falsely accepted. Consumer's risk, for the purpose of this text, is considered to be the probability of accepting a lot whose actual microbial content is substandard as specified in the plan, even though the determined values indicate acceptable quality. This is expressed by the probability of acceptance (P_a). The producer's risk is expressed by the probability of rejection $1 - P_a$ (Figure 6–1).

6.8 STRINGENCY AND DISCRIMINATION

When sampling plans are compared and their stringency in making decisions is considered, different aspects of their performance can be addressed.

Figure 6–2 illustrates an OC curve for an idealized sampling plan. This allows for total discrimination between acceptable and unacceptable lots as acceptance probabilities fall from 100% to 0 just at the chosen limit of acceptability. In practice, no sampling plan can achieve this ideal, but the steeper the curve, the closer the plan comes to approaching the ideal. Generally steeper curves can only be achieved by increasing the number of sample units (n) to be drawn from a lot (Figure 6–3).

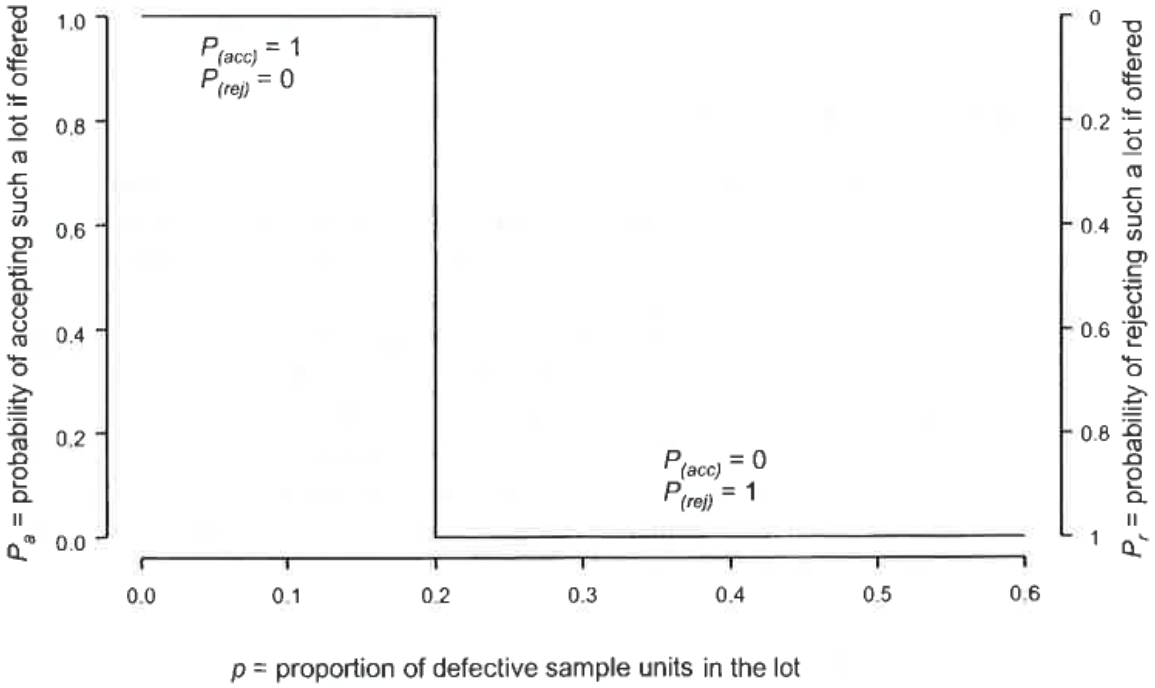


Figure 6.2 The operating characteristic curve (OC) for the idealized situation of complete discrimination between lots with a proportion of defective sample units below 20% and lots with such a proportion above 20%.

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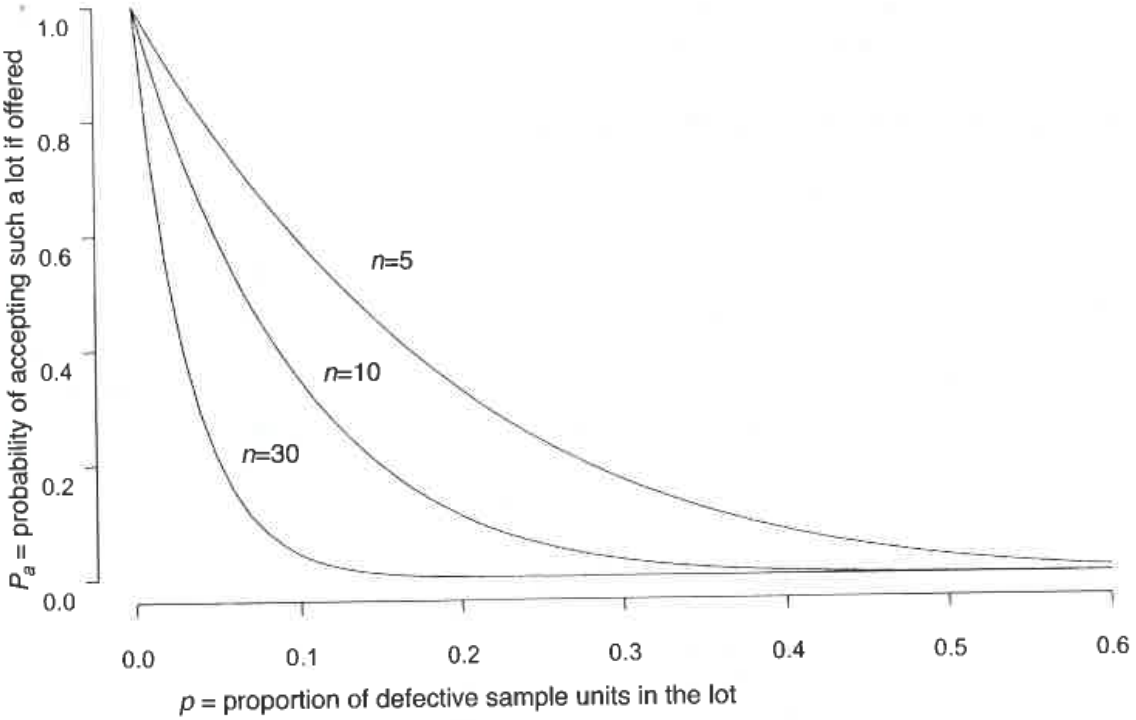


Figure 6–3 Operating characteristic curves for sampling plans with $n = 5$, $n = 10$, $n = 30$ and $c = 0$. With increasing numbers of sample units examined (n), the slope of the OC curve becomes steeper.

The ability to discriminate between acceptable and unacceptable lots should be clearly distinguished from a shift of the OC curve that is achieved by decreasing the acceptance number c. A lower acceptance number will result in a general reduction of consumer's risk by stating a different limit of acceptability, although the producer's risk will be increased.

6.9 ACCEPTANCE AND REJECTION

We have been speaking of acceptance or rejection of a lot on the basis of a sampling plan associated with some particular microbiological test. This judgment, of course, applies only to the purpose for which that test (or several such tests) was performed. A food unsuitable for one purpose may still be suitable for another; for example, if rejected for humans, it might still be suitable for animals. Or a rejected food might even, if sorted to remove objectionable material or if reprocessed, be so improved as to pass the test and become acceptable for the original purpose. Normally, therefore, a rejected lot will simply be withheld while the responsible authority decides what to do with it: return it to the producer, order reprocessing, forbid its use for human consumption, or order its destruction, according to circumstances. Throughout this text, the terms *accept* and *reject* are used in this limited sense.

6.10 WHAT IS A LOT?

Ideally, a lot is a quantity of food or food units produced and handled under uniform conditions. Under certain circumstances, it has been shown that the logarithms of the counts from a batch of food are likely to be normally distributed (Baird-Parker, 1980; Kilsby & Baird-Parker, 1983 [their Table 2]; Ingram & Roberts, 1976). The implication

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is that there is homogeneity within a lot, which, when considering microbial levels and distributions, rarely occurs in practice. In most instances, the distribution of microorganisms within lots of food is heterogeneous. Even in food factories, this heterogeneity makes the interpretation of sample results difficult, and it becomes even more so in circumstances where a lot is less well defined, such as in a consignment of a very large quantity of food.

It is therefore helpful if suppliers can be persuaded to give identifiable code numbers to batches (lots) of food produced over short time periods (e.g., a day or part of a day) for particular processes. The choice of coding system will vary from process to process depending on the type of process and degree of homogeneity within the batch. For example, a continuous process may produce a relatively homogeneous product, in which case a lot could include units produced continuously over a relatively long time, whereas a batch process, such as batch retorting, may require the coding of relatively few product units as one lot (e.g., one retort load).

If a consignment consisting of a mixture of production batches is treated as one lot, a rejection of an acceptable lot (i.e., the situation the producer's risk refers to) can have severe consequences, as the whole lot will be affected by this decision, even though only a few of the production batches within the lot may be of poor quality. Treating the individual production batches as lots, and coding appropriately, permits more precise identification of poor quality food and, at the expense of more analyses, can result in the rejection of less units from the whole consignment.

A lot should be composed of food produced with as little variation as possible for a given process or commodity. Because of the uncertainties in identifying a lot commercially, however, the use of the word *lot* in this book is usually in its statistical sense, as a collection of units of a product, the acceptability of which is determined by examining a sample drawn from it.

6.11 WHAT IS A REPRESENTATIVE SAMPLE?

A *representative sample* reflects, as far as is possible, the composition of the lot from which it is drawn.

How then should a representative sample be drawn? It is important to avoid bias and to draw a sufficient number of sample units to confidently make a judgment about a lot. Sampling at random is the universally recognized way of avoiding bias and is more reliable than consciously trying to draw the sample units from the various parts of a lot. The units (cartons or containers, particular weights of solid, or volumes of liquid) are drawn by using random numbers. There is, of course, no guarantee that a random sample has characteristics identical with those of the lot, but the randomness of sampling is the basis for calculation of the probability that a sample will give a biased result.

It is also possible, and may be desirable, to use a *stratified random sampling* approach, drawing a random sample of a given number of sample units from each *stratum* (e.g., each sublot or carton). The distribution of sample units on strata should correspond to the distribution of lot units on strata. This means that if each stratum contains the same quantity as every other one, the number of sample units per stratum should be the same. Otherwise, they should differ just as the proportions of the lot contained in the strata differ. Stratification is a device for handling known sources of variation and may be used where one has prior knowledge that the consignment is potentially not of uniform quality. A consignment might not be of uniform quality if portions of it are shipped in different vehicles or in different holds of a vessel, or if it is known that it is really composed

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of several lots representing, for example, different days of production from the same plant or different plants of the same company. The results for different strata should be assessed separately and then pooled if they appear to be homogeneous.

The calculation of acceptance probabilities is based on the assumption that random samples have been drawn and analyzed. Where physical or practical constraints prevent random sampling, such calculated probabilities may not be valid. Their reliability depends on how close reality approaches the ideal situation of random sampling. This fact should be borne in mind when subsequently interpreting results.

6.12 CONFIDENCE IN INTERPRETATION OF RESULTS

Decisions based on sampling plans are not suited to give total assurance that the decision will be correct. There are many factors that can affect our confidence in the sampling results obtained. Many of these are related to the methodologies we use. In this chapter, it is assumed that the analytical procedure is certain to detect a contaminant if present, and that it will give no false positives. In practice, these assumptions are never correct and, even for standard microbiological methods, precision values for sensitivity and specificity or repeatability and reproducibility of the analytical procedure may still be missing. Knowledge of these values sometimes would lead to a modified calculation of the OC function or to different critical limits.

The way in which the sample is handled and manipulated can also have an effect on our ability to identify accurately any contaminants present in it. The choice of handling procedure will vary according to the material under examination, the preservation system used, and the method of analysis. The choice of such handling procedures is a microbiological judgment to be made only by experts.

The statistical considerations are related to our sampling plan assumptions, as outlined previously. Particular attention should be given to the sampling plan OC curve (stringency and discrimination) and to the method of sampling, since the calculation of acceptance probabilities is only reliable if sample units are taken at random. With regard to quantitative analytical results, the design of sampling plans relies on knowledge about underlying distributions and the typical variability between sample units. This is reflected either by the calculation of acceptance probabilities or by the choice of critical limits. For further discussion of these issues, see Chapter 7. In addition, it is important to consider whether the whole sampling protocol and assumptions implied are suitable for the intended or desired decision to be made about the lot.

Finally, it is important to remember that many nonstatistical constraints will be brought into play. Issues, such as the economic cost of sampling and the need for rapid results with perishable or commercially sensitive foods, can result in selection of methods that are not statistically ideal or sampling plans with little discrimination. The usual numbers of sample units that can be found in many sampling plans in food microbiology, e.g., $n = 5$ or even $n = 1$, are mainly motivated by such issues. These low numbers represent self-imposed, nonstatistical constraints that limit the confidence we can have in the correctness of decisions based on the according sampling plans.

To be fit for the intended purpose, a sampling plan should be designed with regard to these constraints and the validity of assumptions made. The choice of the sampling plan type, critical limits, and the number of sample units to be analyzed should be chosen according to the intended purpose. Especially the last aspect, choice of n, should be reflected and guided in the light of the desired stringency of the decision-making process.

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The recommended procedure would be to fix desired acceptance and rejection probabilities for lots of defined acceptable and unacceptable qualities first, and then to derive the number of sample units required for this purpose.

6.13 PRACTICAL CONSIDERATIONS

To use available resources to the best effect, all food cannot receive the same attention. The most important foods and lots should receive the most time and effort, which means that they should be subjected to the most intensive sampling. What factors govern this decision?

Hazard. The most important factor is the hazard involved. How hazardous is the type (or types) of microorganisms present? And how hazardous are the numbers likely to be present? These questions are discussed more fully in Chapter 8. The probability of accepting a lot that should be rejected should be reduced with increasing hazard.

Uniformity. If the food has relatively few loci of contamination, even if the number of contaminants is very high, then the chance of detection is low. On the other hand, if the same food is thoroughly mixed, and the contaminants are more uniformly spread, they will appear in more sample units. The chance of detection is then higher with any given sampling plan.

Stratification. If it is known that there is stratification within lots of the food (see section 6.11), a corresponding stratification can be used in selecting sampling units.

Record of consistency. A consistently good record for a food from a specific source indicates that its control is good and reliable, and hence may justify reduced sampling or even omission of sampling on occasional lots. Discretion must be used in making this decision, with increasing confidence as the record accumulates. Confidence will be maintained if the producer provides detailed records of control procedures. Skip-lot sampling is a statistical procedure that can be used under such circumstances.

Practical limitations. Since regulatory agencies never have the resources to test all imported lots, they must reduce the sampling plans to a feasible level. Most microbiological tests are laborious and slow; yet regulatory agencies cannot hold highly perishable foods pending results of analysis. Political or administrative pressures to reduce sampling will increase the probability of error.

In considering the above factors, it must be remembered that the ability to distinguish between acceptable and unacceptable lots often improves relatively little compared with the increase in the number of sample units withdrawn from the population. Indeed, in many instances, reliability only increases roughly as the square root of the number of sample units, so that multiplying the sample number by four will only halve the likelihood of making wrong decisions.

6.14 REFERENCES

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Ingram, M. & Roberts, T. A. (1976). The microbiology of the red meat carcass and the slaughterhouse. *Royal Soc Health J* 96, 270–276.

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Experience in the Use of Two-Class Attributes Plans for Lot Acceptance

10.1 Introduction
10.2 The Concept of Zero Tolerance
10.3 The Need for Compromise
10.4 Application of Two-Class Sampling Plans for Pathogens Such as *Salmonella*
10.5 Problems in the Implementation of Stringent Sampling Plans
10.6 Relation to Commercial Practice
10.7 Discrepancies between Original and Retest Results
10.8 Summary
10.9 References

10.1 INTRODUCTION

The first portion of this chapter is slightly updated from Chapter 6, Book 2 of the International Commission on Microbiological Specifications for Foods (ICMSF, 1986). Very little of the text has been changed because the principles still hold true. Although the text is largely concerned with *Salmonella* in a variety of foods, the concepts also apply to other pathogens where presence/absence tests are applied and $c = 0$. The chapter from Book 2 is historically significant because when it was written, there was an increasing awareness and concern for *Salmonella* in a wide variety of foods, many of which were ready-to-eat. In response to this concern, a *zero tolerance* policy was applied by control authorities. Some companies buying ingredients from suppliers subsequently adopted this practice. As methods improved and the quantity of food analyzed increased, lower levels of contamination could be detected.

There has been an erroneous, but common, perception that *zero tolerance* equates to zero risk. This perception is not valid, as is evident in the following sections and elsewhere in this book. The concept of *zero tolerance* provoked questions about procedures to reduce the analytical workload associated with large numbers of samples and whether a positive lot could be retested. Some procedures are fallacious and do not provide the expected level of confidence. One procedure, compositing (pooling or bulking), proved highly successful and is now commonly applied for the analysis of *Salmonella* and certain other pathogens when research demonstrates no loss of test sensitivity.

Judgments about hazards from pathogens are based largely on microbiological analysis, but the results and conclusions are influenced by many factors, including the analytical method. For example, until after the late 1950s, methods of detecting *Salmonella* in

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food were inadequate because the method in use had been developed to analyze for the relatively large numbers of *Salmonella* in feces or blood of a diseased person. These methods could not detect the very low numbers of *Salmonella* expected to be present in foods. As more sensitive methods were developed, some foods previously thought to be generally free of *Salmonella* proved to be contaminated.

Today, highly sensitive methods have yet to be developed for *Campylobacter jejuni* and a variety of pathogens such as *Cyclospora cayetanensis* and viruses in foods. With each newly recognized or emerging pathogen, several years may elapse during which improvements are made in methodology, and the ecology of the pathogen becomes elucidated. Hence, opinions on frequency and levels of contamination by such pathogens need to be reviewed as more sensitive procedures become available.

Increasing the quantity of material examined has further improved the sensitivity of testing. Initially, for *Salmonella*, it was customary to examine samples of food weighing 1 or 10 g; but as methodology improved and the desire to detect defective lots increased, larger quantities of material were analyzed (e.g., 25 g or 50 g and occasionally more). The importance of this change will become evident in the following sections. A similar evolution occurred after about 1990 with *Listeria monocytogenes* and *Escherichia coli* O157:H7.

10.2 THE CONCEPT OF ZERO TOLERANCE

One objective of this book is to facilitate the adoption of realistic microbiological criteria. For example, some governments and companies have established criteria stating that certain pathogens "shall be absent," with no numerical tolerance being expressed. This practice is to be discouraged since it is not compatible with the use of food safety objectives and performance criteria. Microbiological criteria should include the parameters described in Chapter 5. Furthermore, at least four considerations challenge the ideal of *zero tolerance*:

1. No feasible sampling plan can ensure complete absence of a pathogen. Even when $c = 0$, it cannot be guaranteed that the lot is completely free of the organism, no matter how large the number of sample units, n. However, what can be stated is the probability of acceptance (P_a) for lots of various qualities, as a function of the given n and c. For example, when $n = 60$ and $c = 0$, then $P_a = 0.5$, meaning there is one chance in two that a lot will be accepted when 1% of the sample units are defective (Figure 10–1).
2. Plans in which $c = 0$ are not necessarily the most exacting. For example, if one sets a limit of 5% defectives in a lot, the plan $n = 95$, $c = 1$ will accept unacceptable lots less often, and acceptable lots more often than will the plan $n = 60$, $c = 0$. In other words, the former plan is the more discriminating, even though it admits the presence of the pathogen. Preference for $c = 0$ is influenced by the wish to emphasize that absence is the desired objective (although it cannot be guaranteed) and by the knowledge that pathogens such as *Salmonella* may be found by direct examination of certain foods and, having been demonstrated, the finding cannot be ignored. In addition, the work and cost associated with collecting, transporting, and analyzing 60 samples, even with compositing, is already a significant burden without increasing the number to 95 samples.
3. The sampling plans typically used for lot acceptance testing assume that defects (e.g., *Salmonella*) are randomly distributed through the lot. Section 10.7 and exam-

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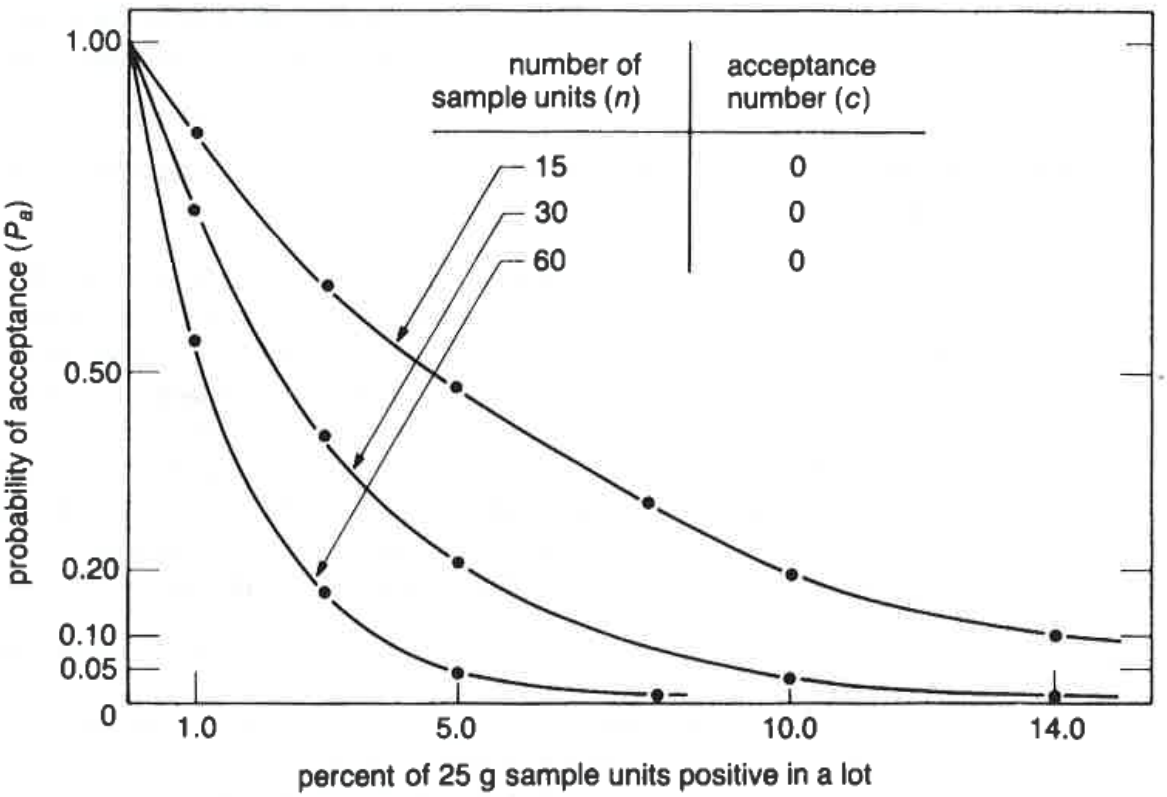


Figure 10–1 Relations between the sampling plan and the degree of security conferred—operating characteristic curves for three sampling plans proposed for *Salmonella*.

ples discussed elsewhere in the book demonstrate that this is often not the case. In addition, the plans assume that sample units are randomly drawn from the lot but, for practical reasons, this may be difficult, or impossible, to achieve.

4. It is not yet commercially possible to market some foods completely without pathogens. For example, most raw meats and poultry occasionally contain *Salmonella*. A sampling plan adjusted to this situation would seem more realistic and satisfactory than one based on an unattainable ideal of complete absence (i.e., zero tolerance).

10.3 THE NEED FOR COMPROMISE

The foregoing illustrates the difficulty of trying to make a reasonable compromise between a desire to eliminate pathogens completely from the food supply to protect consumers and current practicable methods of food production. In this respect, past decisions have often been arbitrary, and sometimes illogical. In most regions of the world, no restriction is enforced on the enormous quantities of poultry and carcass meats that are regularly contaminated with *Salmonella*, though these foods have been clearly shown by epidemiologic evidence to be major vehicles for outbreaks of salmonellosis. The risk entailed by regularly contaminated foods is probably as great as with an infective carrier whose detection would lead to his/her removal from a food operation. Sampling procedures are needed that remove the more highly contaminated lots and result in continuous improvement without completely disrupting the food supply.

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To encourage a realistic choice of sampling plans for hazardous pathogens in foods, the problem is considered in further statistical detail with particular reference to *Salmonella*.

10.4 APPLICATION OF TWO-CLASS SAMPLING PLANS FOR PATHOGENS SUCH AS *SALMONELLA*

Any member of the genus *Salmonella* presents some degree of hazard to human health. Some, like *S. gallinarum* and *pullorum*, are of minor significance for humans; others, notably *S. typhi*, are severe hazards. But the majority, to which this discussion relates, represent a moderate direct hazard that, through cross-contamination and multiplication, can readily become widespread among a population via foods.

The numbers and prevalence of these organisms in food can, and should, be low. Foods presenting a minimal risk are examined for *Salmonella* only when there is unusual cause for concern. Under certain circumstances, sampling and testing may be needed to determine whether a lot of food is acceptable, such as when there is:

1. no knowledge of the manufacturing process or of earlier consignments from the manufacturer and
   —the food is of a kind commonly involved in salmonellosis or
   —the food is intended for a sensitive population.
2. special reason to suspect that the product from a particular factory may contain *Salmonella*.

Because most food control efforts should be directed to the areas of greatest risk, foods are assigned to categories according to the degree of risk they present. To each category is attached an appropriately stringent method of deciding the acceptability of the finished product. Case 10 (see Chapter 8) recognizes a hazard with reduced risk with normal preparation, such as exists when a food may contain *Salmonella* but will be consumed by ordinary consumers after cooking (e.g., red meats, poultry). Case 12 expresses a greater risk, where the conditions of use may favor spread and/or multiplication of the microorganism, and hence requires more stringent sampling. Case 11 is intermediate, reflecting no likely change in risk during normal conditions of storage, distribution, and preparation for serving. Because absence of *Salmonella* is the preferred criterion of acceptance ($c = 0$), the stringency of examination normally is increased by raising the number of sample units n. Table 10–1 shows recommended sampling plans for the various categories, with degrees of stringency related to hazard expressed by cases.

The ICMSF recommendations in Table 10–1 are similar in principle to those of the report of the *Salmonella* Committee of the National Academy of Sciences-National Research Council (NAS-NRC, 1969), in that both increase the stringency of the sampling plan with increase in the degree of risk caused by the conditions of food use. An obvious difference is that the proposals presented in Table 10–1 also involve less stringent plans for routine use. There are two reasons for this:

1. The sampling plans proposed by NAS-NRC were not intended for routine examinations, but for use when a question arises whether a product contains *Salmonella*. Routine inspections or audits will from time to time raise this question, and the NAS-NRC plans were intended for the special investigation it entails. For such investigations, the ICMSF agrees that plans are desirable in which the degree of stringency is as great as the NAS-NRC recommended (Table 10–1).

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Table 10–1 Stringency of Sampling for *Salmonella* for Foods with Different Categories of Risk (All Two-Class Plans with $c = 0$)

	ICMSF			NAS/NRC*	
		n values†			
Hazard in use	*Case*	*Normal routine*	*Special‡*	*Category*	*n values†*
Reduced	10	5	15	B	13
Unchanged	11	10	30	C	29
Increased	12	20	60	D	60

*Based on the Report of the *Salmonella* Committee (NAS-NRC, 1969).
†n = number of sample units.
‡e.g., where the food will be eaten by susceptible consumers or for tightened inspection.

2. If a few highly pathogenic types, such as *S. typhi*, are regarded separately, the remaining *Salmonella* types that commonly occur in foods (to which Table 10–1 refers) usually involve mild to moderate disease risks. The NAS-NRC report does not make this distinction and considers all types of *Salmonella* together, implying a high degree of risk for all types. The ICMSF agrees that where circumstances (e.g., unusual susceptibility in the probable consumers) create a higher risk, sampling plans of higher stringency are desirable.

Figure 10–1 gives the operating characteristic curves that express the relationship between the degree of security conferred and the sampling plans proposed. For instance, consider the curve for the plan $n = 30$, $c = 0$. If the proportion of sample units defective (p) is 0.05 (i.e., 5% are defective), the probability of acceptance (P_a) is about 0.25 (i.e., 1 in 4), hence three out of four such lots would be rejected. Suppose the units are 25 g samples—a realistic supposition with a food such as dried or frozen egg. They are defective if examination shows them to contain any *Salmonella*. Hence, if 5% of the units are defective (i.e., 1 defective in 20), there is, on average, *Salmonella* contamination in 20×25 g = 0.5 kg of food. That is to say, the plan $n = 30$, $c = 0$, with analytical units of 25 g, will reject three out of four lots in which the prevalence of contamination is about two analytical units positive for *Salmonella* per kg.

To reduce laboratory effort while maintaining the stringency of the sampling plan, it would be helpful to combine either groups of analytical units or the enrichment cultures therefrom, using plans in which the presence of a single positive rejects the consignment. A laboratory investigation undertaken for ICMSF (Silliker & Gabis, 1973) indicated that different sized analytical units drawn from a single well-mixed sample unit of dried food give very similar results. A subsequent investigation (Gabis & Silliker, 1974) using foods of high moisture content (including eggs, poultry meat, meat, and meat products) showed comparable reliability in the use of composite analytical units; for example, examination of three composites of 20×25 g analytical units gave the same result as examination of all 60 analytical units individually (the total amount examined being 1500 g in both instances). This finding suggests that considerable reductions may be made in the cost of laboratory analyses by compositing (pooling) analytical units. Such compositing offers the prospect of large increases in the number of analytical units, n, with corresponding increase in the stringency of examination, without correspondingly increasing laboratory effort.

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Numerous studies conducted since 1974 have continued to demonstrate the merits and limitations of compositing. Experience may vary for different food-pathogen combinations and analytical methods, indicating a need for caution and validation that compositing is acceptable. No reduction in the cost of collecting the necessary number of sample units can be expected, since analytical units to be composited would still have to be chosen randomly.

10.5 PROBLEMS IN THE IMPLEMENTATION OF STRINGENT SAMPLING PLANS

10.5.1 Conceivable Alternative Plans

As indicated above, for an infectious agent such as *Salmonella*, there are strong reasons for adopting sampling plans in which $c = 0$:

1. It is philosophically objectionable to some people to accept a lot containing a recognized pathogen, regardless of how the food is to be used in a manufacturing process or by the consumer.
2. There is the very great technical advantage of combining many analytical units for bacteriological examination in systems with $c = 0$, because a single positive decides the outcome.
3. The plan with $c = 1$ (or more) would always require the examination of (many) more analytical units, for equal probability of acceptance, than a plan with $c = 0$.
4. Even if the indicated probabilities of plans with $c = 0$ versus $c = 1$ are the same, their implications are not. For example, when a lot is accepted with the plan $n = 60$, $c = 0$, it is possible (though not certain) that the lot may not be contaminated at all. But using the plan $n = 95$, $c = 1$ and accepting one positive, it is known that the lot certainly is contaminated (though the probability that a lot with 5% of analytical units contaminated would be accepted is in both instances $P_a = 0.05$).

10.5.2 Fallacious Procedures

It might be suggested, for example, that if the plans $n = 60$, $c = 0$ and $n = 95$, $c = 1$ provide equivalent probability (for lots having, for example, 5% of units defective), an operator finding one positive in 60 analytical units might then proceed to examine another 35 (total $n = 95$) in the hope of clearing the lot if all the latter proved negative. But such a procedure is in reality a two-stage plan $n_1 = 60$, $c_1 = 1$, plus $n_2 = 35$, $c_2 = 0$, which has a greater probability of accepting an unsatisfactory lot than $n = 95$, $c = 1$. In fact, the probability P_a of this two-stage plan is 0.07, compared with 0.05 for the one-stage plan. Although in this example the difference is not great, there are situations where such two-stage procedures cause more serious error. Where two-stage sampling plans are actually being used, their operating characteristic curves should be computed and the resulting probabilities of acceptance evaluated.

A similar fallacy can arise where a plan requires a large number of analytical units, which would be unusually costly. Suppose the plan is $n = 95$, $c = 1$, but for economy, a group of only 20 units is tested initially. If one unit should fail in this initial group, an analyst might examine the remainder (in this case 75) with the idea that if a defective is not found in the second group, the first may be ignored. Nevertheless, the sampling plan that has been applied actually corresponds to $n_1 = 20$, $c_1 = 1$ and $n_2 = 75$, $c_2 = 0$. No justification exists for preferring the results from the second series. Similarly, if the plan

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were $n = 60$, $c = 0$ and an analyst felt uncertain about finding a defective unit in the first 20 samples, and the lot was accepted on the basis of the remaining 40 samples being negative, then the actual plan applied would be the two-stage sampling plan: $n_1 = 20$, $c_1 = 1$; $n_2 = 40$, $c_2 = 0$, which actually accepts more defective lots than would $n = 60$, $c = 0$.

10.6 RELATION TO COMMERCIAL PRACTICE

Stringent sampling plans like those just suggested represent an ideal not yet attainable for products such as poultry or carcass meats, which are found to be contaminated by *Salmonella* when specially sensitive methods of detection are used. At present, it is often recommended to use procedures such as rinsing or swabbing of the whole carcass, where in effect the analytical unit is most of the carcass surface and internal cavity, and a positive may correspond to levels of contamination on the order of one *Salmonella* cell per 2 kg for poultry or even one per 100 kg for carcass meats. Using such criteria, one finds slaughter lines producing contaminated carcasses with a frequency of 10% or more. For lots with 10% defectives, the relations shown in Section a of Table 10–2 are valid. The sampling plan $n = 60$, $c = 0$ would reject virtually all such material; $n = 5$, $c = 0$ would reject one-third of it (Table 10–2). Even the plan $n = 5$, $c = 1$ would reject one in twelve lots, a commercially severe rate of rejection. If, as a result of rejections on the basis of $n = 5$, $c = 1$, the quality of lots improved to the point where only 2% instead of 10% of carcasses were found to be contaminated, the relations would be as in Section b of Table 10–2. If 2% of carcasses are contaminated, the indicated average concentration of *Salmonella* cells is on the order of one per 50 kg for poultry, and one per 5000 kg or more for beef. If a 2% frequency of contamination was still thought to be too great, a similar pressure for further improvement (rejecting about 1 in 10 such contaminated lots) could be reintroduced by changing the sampling plan from $n = 5$, $c = 1$ to $n = 5$, $c = 0$.

The above illustrates how, by periodic adjustment of the stringency of the sampling plan, a steady pressure to improve could be applied without placing catastrophic restrictions upon the supply of the commodity at any time.

The sampling plan $n = 5$, $c = 1$ has P_a of about 0.65 for lots with $p = 0.24$ (Figure 10–2); that is, this plan would reject 1 out of 3 lots in which one-quarter of the units were

Table 10–2 Comparison of Some Operating Characteristics of Several Sampling Plans Relevant to Examination for Pathogens

	a. For lots with p = 0.10, i.e., 10% of samples contaminated			*b. For lots with p = 0.02, i.e., 2% of samples contaminated*		
Sampling plan	P_a*	P_r†	*Approximate proportion of lots rejected*	P_a*	P_r†	*Approximate proportion of lots rejected*
$n = 60$, $c = 0$	<0.005	0.995	199/200	0.30	0.70	2/3
$n = 10$, $c = 0$	0.35	0.65	2/3	0.82	0.16	1/6
$n = 5$, $c = 0$	0.59	0.41	1/3	0.90	0.10	1/10
$n = 3$, $c = 0$	0.73	0.27	1/4	0.94	0.06	1/16
$n = 5$, $c = 1$	0.92	0.08	1/12	1.00	<0.005	<1/200

*P_a probability of acceptance of lot
†P_r probability of rejection of lot

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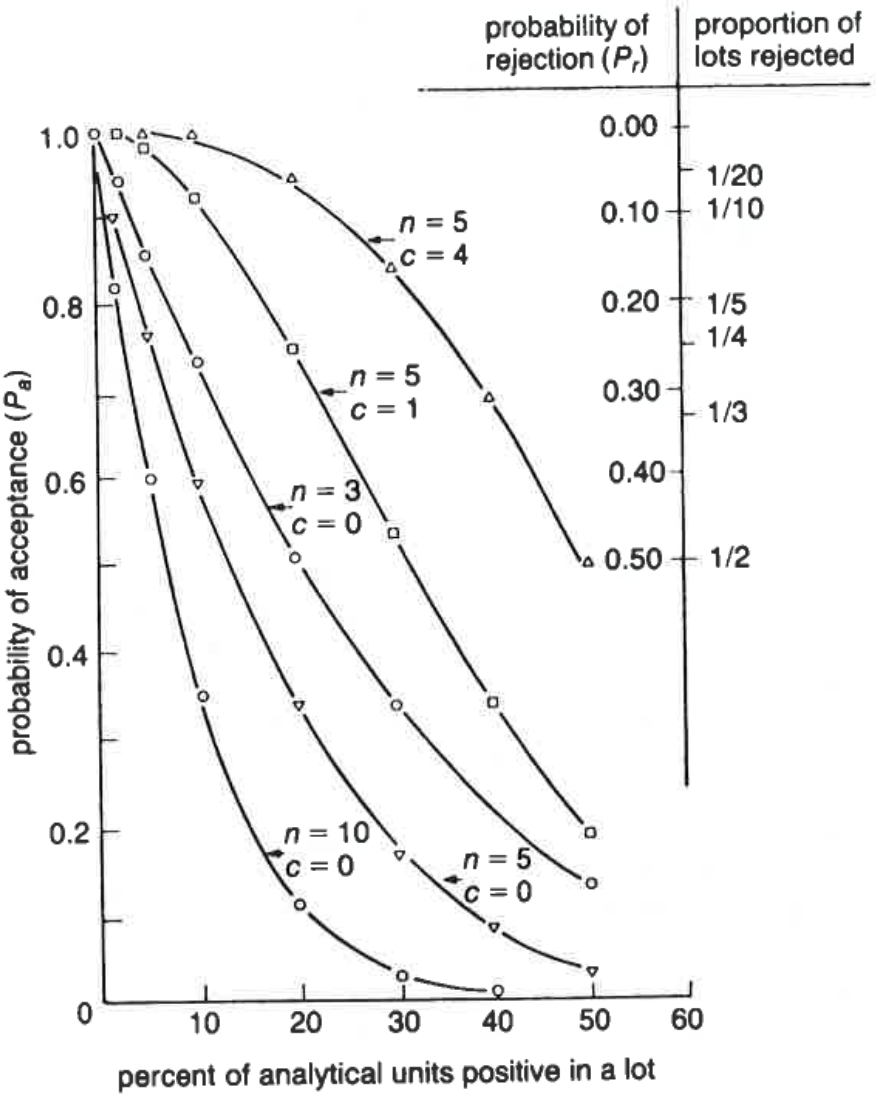


Figure 10–2 Operating characteristic curves for sampling plans with small numbers of analytical units.

contaminated. If these units were poultry carcasses weighing about 1 kg, this level of contamination would correspond to counts on the order of one *Salmonella* cell per 4 kg of food; while with beef carcasses, the figure would be on the order of one per 400 kg. This kind of situation is very different from the examination of analytical units on the order of 25 g (e.g., of egg). The plan $n = 60$, $c = 0$ rejects about one-third of lots in which 0.7% of analytical units are defective, which (these being 25 g analytical units) corresponds to a minimum level of contamination of about 1 cell per 3 kg. That is to say, the plan $n = 60$, $c = 0$, applied even to 25 g analytical units (e.g., of egg), actually provides less protection than the plan $n = 5$, $c = 1$ applied to whole-carcass samples of poultry of effective weight about 1 kg; even the latter plan would reject a substantial proportion of current production of poultry carcasses.

In such circumstances, the immediate application of the more stringent sampling plans to poultry, and still more to larger carcasses, seems at present unrealistic. The application of even so loose a plan as $n = 5$, $c = 1$ would immediately require a substantial improvement on current levels of contamination; and that improvement could then be continued

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by proceeding to plans of successively greater stringency. It is important to understand the difference between the sampling plans for some raw meats and those for other foods as regards *Salmonella*, and to note the effect of the size of the analytical unit on stringency of examination, as mentioned at the beginning of this chapter. (Editor's note: This assessment reflects the status of *Salmonella* in raw meat and poultry through the final revision of ICMSF Book 2 (ICMSF, 1986). Significant improvements in control of *Salmonella* have since been made by some processors through improved on-farm controls and procedures used for slaughtering and chilling. Information in this and previous sections should be considered when selecting a sampling plan for various food-pathogen combinations.)

10.7 DISCREPANCIES BETWEEN ORIGINAL AND RETEST RESULTS

The following is adapted from a newsletter prepared by Flowers & Curiale (1993). The text discusses two very common debates that arise in microbiological testing, namely:

1. One laboratory detects a pathogen in a lot and another laboratory does not.
2. Retesting a positive lot fails to confirm the initial result.

When a pathogen is detected in a food product by a two-class attributes plan, there is often a desire to retest retained samples or resample the lot in question to verify the finding. This is particularly true when there are economic and/or public health consequences associated with a positive result.

Often, retesting does not confirm the original positive result even when a much greater portion of product is analyzed in the retest. There may be a desire to believe the original result to be in error, perhaps due to contamination during sampling or analysis. Although laboratory or sampling error are plausible explanations, other explanations also should be considered such as nonrandom or heterogeneous distribution, low prevalence of contamination, and pathogen die-off between the original and repeat tests.

The discrepancy between original and retest results requires an understanding of how microorganisms may be distributed within a food product and the difference between prevalence and concentration of contamination. Prevalence refers to the frequency at which multiple samples from a given product test positive. Concentration refers to the number of cells present in a given amount of product. Consider the examples in Figure 10–3. Both lots, A and B, have the same prevalence but different concentrations. If lots A and B were each divided into 100 one-kg samples and each sample was tested individually with a method capable of detecting one cell kg^{-1}, both lots would likely result in 6 positives per 100 samples tested. However, lot B actually contains a 100-fold higher concentration, because each positive sample contains clumps of approximately 100 cells.

It is not uncommon in practice for microbial contamination to exist in clumps as represented in lot B. The number of cells per clump will vary with the nature of the product, source of contamination, and stability of the microbial contaminant in the product. Secondly, the distribution of the microorganisms in the product must be considered. If distribution is homogeneous, there is an equal opportunity for contamination to occur at any stage and time of the operation and any individual sample unit would be as likely to detect contamination as any other. If, on the other hand, contamination is limited to a certain segment of time during processing, then the defect would not be homogeneously distributed throughout the lot. If heterogeneous, then random sampling may not detect the organism.

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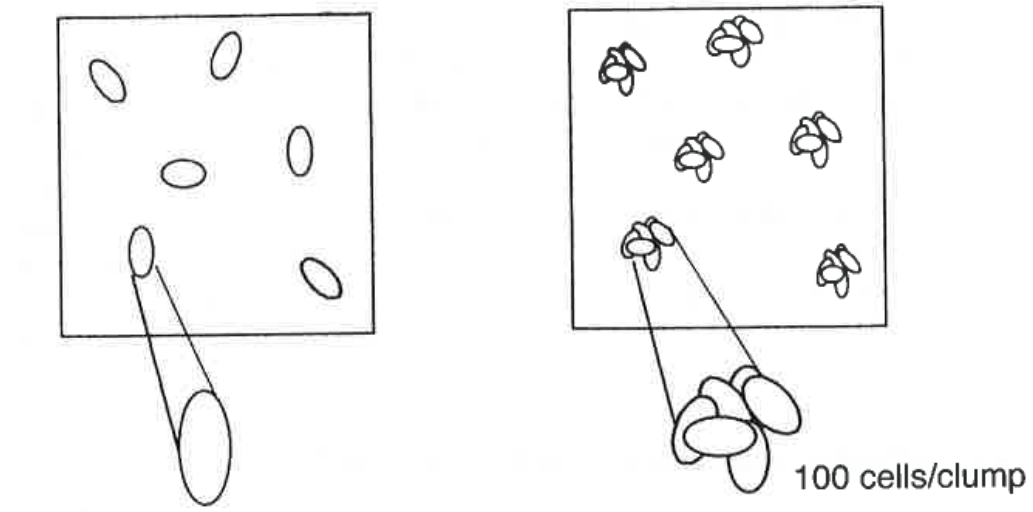


Figure 10–3 Illustration of two lots of product that upon analysis, may yield the same prevalence, but have 100-fold different concentrations.

Microbiologists commonly refer to random distribution of microorganisms as homogeneous and nonrandom distribution as heterogeneous. However, this is a misconception, as random refers to an irregular distribution that is still to be found if the material is "well homogenized." Figures 10–4a–c illustrate three types of nonrandom distribution in three lots of 20 consecutively produced boxes. In Lot A, the distribution is homogeneous but perfectly regular. Lots B and C represent heterogeneous distributions. In Lot B, contamination was greatest in the first sample, followed by decreasing levels of contamination in boxes 2, 3, and 4. This type of distribution of organisms commonly occurs when product is produced on contaminated equipment. The product flushes contamination out of the system upon start of production. Lot C is similar to B, but results from contamination being introduced into the system at some time during production. This type of contamination can originate from a variety of sources and causes, e.g., equipment failure followed by substitution of an unclean unit, contamination from line workers or maintenance personnel, contamination introduced from the process environment via aerosol created by cleaning in an adjacent area or contamination from outside the product stream falling into the product.

10.7.1 Nonrandom Distribution

Most microbiological sampling plans for lot acceptance testing assume contaminants are randomly distributed throughout a lot. In practice, microorganisms are often not randomly distributed, except in mixed liquid samples drawn from a container. Depending on when and how the contamination has occurred, the distribution of contaminants will vary considerably as to location and concentration within a batch. Consider the following examples:

Example 1: Assume, due to a sanitation failure, *Salmonella* has become established in a niche within one of 10 filler heads of a filling machine. During start-up, the first prod-

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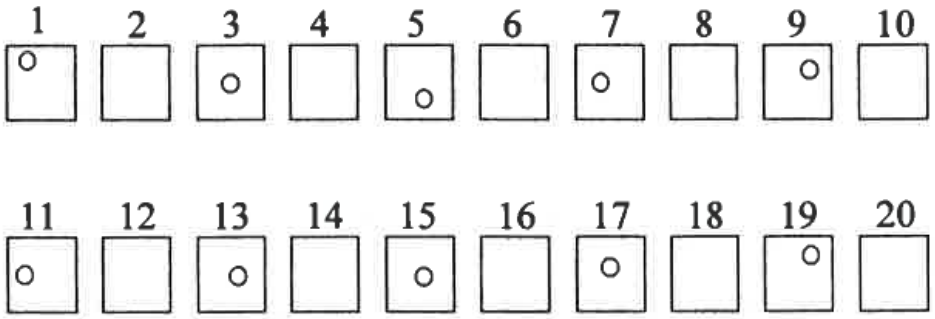


Figure 10–4a Illustration of regular (nonrandom) distribution throughout a production lot of 20 boxes (Lot A).

uct through this filler head will tend to flush out the contamination so the first product through that filler head will be more highly contaminated. Initially, one in every 10 containers would have *Salmonella*. If samples were collected every few minutes and a quantitative determination performed, the concentration of *Salmonella* in the product from the one filler head would decrease as illustrated as in Lot B of Figure 10–4b.

Analysis of random samples from the lot could by chance detect a positive sample. However, if a retest does not include one or more samples collected from the same filler head very early in production, the initial positive result will not be confirmed even if many samples are analyzed.

Example 2: A combination of condensation and product dust at the top of bucket elevators conveying dry product can provide sufficient moisture and nutrient to allow growth of *Salmonella* and, if introduced, a growth niche can occur during periods of no production. As production resumes, dry product conveyed near the top of the conveyor creates a drier environment. The residue can dry and slough off in clumps into the product stream. In this example, contamination is limited to a particular bucket, with product before or after the event being unaffected. As the product is subsequently transported, perhaps by air or screw conveyor, the clump breaks up and is diluted downstream. The effect is that *Salmonella* within the clump are distributed throughout the product stream much like a comet in space, with the highest level of contamination where the clump entered the product stream, followed by a dilution tail until the stream of product is not contaminated. This is illustrated as Lot C in Figure 10–4c.

In the above "comet-like" contamination, the first and last packages will be negative, but somewhere within the production lot is a series of contaminated packages of product.

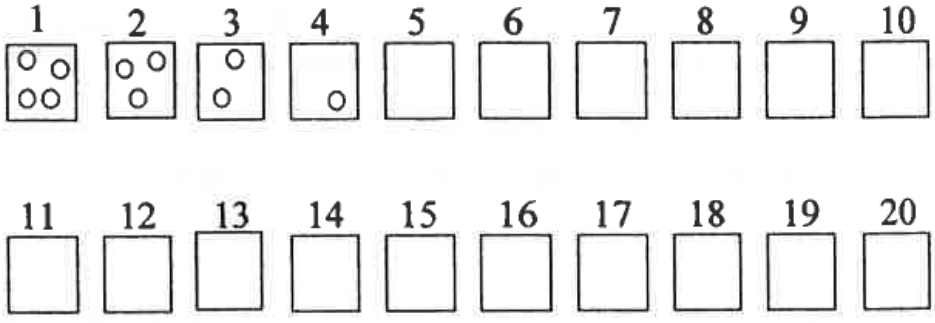


Figure 10–4b Illustration of heterogeneous (nonrandom) distribution throughout a production lot of 20 boxes (Lot B).

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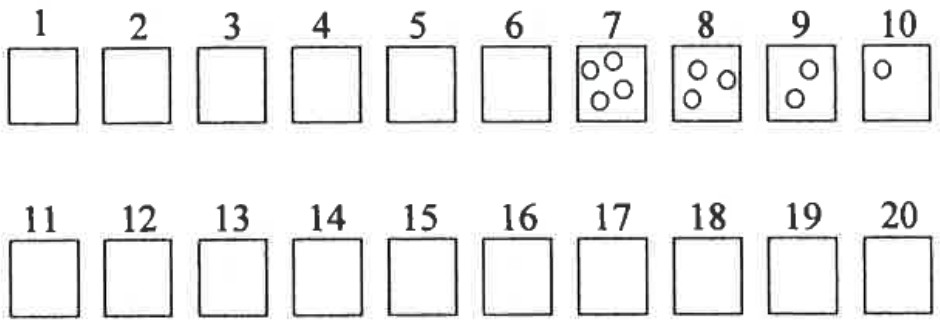


Figure 10–4c Illustration of heterogeneous (nonrandom) distribution throughout a production lot of 20 boxes (Lot C).

Again, depending on the sampling plan, this contamination may not be detected and if a positive sample is detected, extensive retesting may not confirm the positive result unless samples are collected from the affected portion of the lot.

In practice, "comet-like" contamination can occur with a wide variety of processed foods. For example, one commercial experience involved a dry-blended product that tested positive for *Salmonella* using a sampling plan of $n = 30$. The analytical units (25 g) were composited into two 375 g samples for analysis, for a total of 750 g. All the dry ingredients had been received with certificates indicating they had been sampled and tested negative for *Salmonella*. Since the blending operation was completely dry, there appeared to be little chance for contamination during mixing and packaging. Thus, the ingredients initially were suspected as the source of contamination and the remaining material was extensively resampled.

Eight pallets from an original shipment of 20 remained of a particularly suspect ingredient. Every 25 kg bag was sampled (375 g) and analyzed. Most bags from one pallet of this ingredient and a few bags from another tested positive for *Salmonella*; all others tested negative. Because the bags were sequentially numbered during filling, it was possible to determine that the initial contamination occurred during the middle of the day and decreased to below the detection limit within a couple of hours. Furthermore, one pallet that had been used contained bag numbers within the range of those found positive. The source was further confirmed when the *Salmonella* serotype isolated from the blended product matched the isolate from the ingredient. Obviously, had all three contaminated pallets been used, extensive resampling of the remaining pallets would have yielded negative results and the source of contamination would not have been determined.

Commercial experience with two-class attributes sampling plans for *Salmonella, L. monocytogenes,* and other infectious agents clearly demonstrates that, heterogeneous distribution of contamination can be a reason for discrepancies between initial test results and extensive retesting.

10.7.2 Low Prevalence of Contamination in a Lot or Batch

Microbiological criteria specify the quantity of product to be analyzed. Whether a pathogen such as *Salmonella* is detected also depends on its prevalence throughout the lot, its concentration in the analytical unit, and the sensitivity of the analytical method. When present at a high prevalence and concentration sufficient for detection by the test method, the initial analysis and subsequent retests will be positive. When present at a

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lower prevalence, not every test portion will contain the pathogen. Detection then depends on the probability of including a contaminated (i.e., positive) sample in the analysis. To confirm an initial positive result, a second positive sample must be selected. The probability of selecting a second positive sample in a row is much lower than the probability of selecting the first positive sample. Confirming an initial positive result when a pathogen is of low prevalence can be difficult, as described in the following.

In this example, a thoroughly blended product in a 500-L kettle contains one *Salmonella* cell per 3,750 g. If a two-class sampling plan is used that involves analysis of 375 g sample units, there is one chance in ten that each 375 g test sample will contain one *Salmonella* cell. If the first sample tests positive, then the second 375 g sample will likely test negative. The probability that two samples will test positive in a row is (1 in 10) × (1 in 10) = 1 in 100. Thus, there is only one chance in 100 that two consecutive samples will yield a positive result. Given this situation, a retest used to confirm an initial positive result will almost always increase the risk of accepting a contaminated lot. This is because both the initial and retest samples have equal probability of being positive when contamination is homogeneous. The probability that both will yield a positive result is the square of the probability of a single portion testing positive (Table 10–3). The lower the prevalence of contamination, the more difficult it will be to confirm. Confirmation will depend upon chance or testing until the prevalence of contamination is established. A very low prevalence of contamination is virtually impossible to confirm by resampling.

10.7.3 Low Prevalence of Contamination across Many Batches

Occasionally, test results indicate that contamination is occurring frequently, but at a very low level. In this example, a 500-L kettle is used to produce 20 lots per month of a homogeneous product and the lots are contaminated with one *Salmonella* cell per 3,750 g. Using a sampling plan involving $n = 15$ (25 g analytical units) and a composite of 375 g, only two lots will test positive. If sufficient retesting is conducted to establish that the prevalence of contamination in each of the two positive batches is one cell in 3,750 g, then it could be concluded that there was one chance in 100 that two positive lots would have been found. Since this is a low probability, one may suspect that the negative lots also may have been contaminated, but at low prevalence. A thorough analysis of several negative lots would determine whether the contamination is widespread. In any event, product histories should be charted and reviewed for sporadic instances of positive test results that indicate low level, widespread contamination.

Table 10–3 Probability of Detecting an Initial Positive and Then Finding a Second Positive When the Lot Is Retested

*Probability of initial positive**	*Probability of both initial and retest positive*
1 in 2	1 in 4
1 in 5	1 in 25
1 in 10	1 in 100
1 in 20	1 in 400
1 in 50	1 in 2,500
1 in 100	1 in 10,000

*Probability of a positive = lot size in g × no. cells/size of test sample in g

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10.7.4 Change in the Concentration of Contamination

In a contaminated ingredient, food, or food processing environment, the number of viable cells may increase, decrease, or remain the same with the passage of time. If a pathogen is multiplying, the concentration of contamination (cells g^{-1}) will increase and become easier to detect. However, if death is occurring, the probability of detection will decrease. An example of a survival curve for *Salmonella* introduced into a dry product is shown in Figure 10–5. During the first few days, the number of viable cells decreases rapidly. However, *Salmonella* can be detected because the concentration remains above the detection limit. A traditional *Salmonella* test requires about five days for a positive result. During this time, the concentration of viable cells has decreased to below the detection limit. If a retest is requested, there is an increased probability that the retest will not confirm the initial positive result. Rates for the initial rapid decrease and the subsequent slower decrease (Figure 10–5) vary by product, pathogen, and storage condition. Extensive experience with preparing inoculated samples for laboratory performance testing, evaluation of new methods, and challenge testing indicates that transferring pathogens such as *Salmonella* from a high moisture growth condition (e.g., broth cultures) into dry product results in a survival curve similar to that shown in Figure 10–5. A very similar curve would be expected for *Salmonella* that has multiplied in a plant environment and is then introduced into a dry food (e.g., the condensation/bucket elevator example). If product is sampled and tested the first few days after production, levels may be considerably higher than later when the concentration of survivors is lower. but more stable. Depending on the sensitivity of the method and the level of surviving cells after numbers stabilize, retesting may or may not confirm an original positive result.

10.8 SUMMARY

There are many possible explanations why a two-class attributes sampling plan may yield a positive result that is not confirmed by retesting. In practice, all of the phenom-

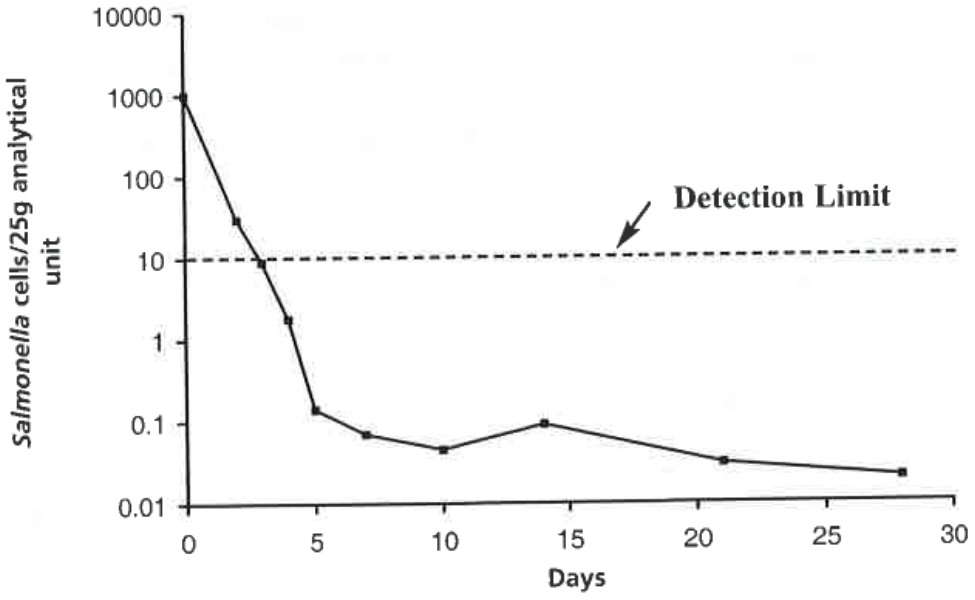


Figure 10–5 An example of a survival curve for *Salmonella* under dry conditions.

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ena described above can be involved. Contamination may be heterogeneously and nonrandomly distributed, at low prevalence, and unstable in the product. Disregarding an initial positive result because it is not confirmed by retesting should not be done unless there is clear reason to suspect laboratory error or contamination during sampling. Since it is very often difficult or impossible to confirm that results are erroneous due to laboratory or sampling error, it is extremely important that the laboratory performing qualitative tests has an effective quality assurance program to prevent laboratory error and to recognize contamination, should it occur.

10.9 REFERENCES

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Chapter 12

Sampling, Sample Handling, and Sample Analysis

12.1 Introduction
12.2 Collection of Sample Units
12.3 Intermediate Storage and Transportation
12.4 Reception of Samples
12.5 Sample Analysis
12.6 Recovery of Injured Cells
12.7 Errors Associated with Methods and Performance of Laboratories
12.8 References

12.1 INTRODUCTION

Many different types of samples are collected and submitted to the laboratory for analysis. Some are sample units from lots or consignments of foods or ingredients for lot acceptance determination. Others may be for investigational purposes to assess control of the environment, investigate the source of a problem, or validate a process. Some may have legal implications relative to a lawsuit or for regulatory compliance. This chapter briefly discusses some of the major factors that should be considered when collecting sample units, shipping them to a laboratory, preparing them for analysis, and analyzing them.

A sample unit is a small portion of the lot in which the distribution of both numbers and types of microorganisms should be representative of the whole lot. This requirement is only completely fulfilled when the food and processing conditions result in a homogeneous distribution of microorganisms. This only occurs in well-agitated fluid products. In general, the distribution of microorganisms is presumed to be random.

When cells are evenly distributed within the solid or liquid food, i.e., with a variance (σ) squared equal to the mean (μ) ($\sigma^2 = \mu$), the distribution approximates to a Poisson distribution. More frequently, cells occur in clumps or aggregates and their spatial distribution is erratic, with the squared variance larger than the mean ($\sigma^2 > \mu$), representing, for example, a negative binomial distribution (Jarvis, 1989).

The presence and distribution of pathogens, indicators, and/or spoilage microorganisms in finished products depend on many factors, including the level and type of the initial microflora of raw materials, the structure of those raw materials, their quantities and ratio in recipes, and the processing conditions. Formulating and processing the food will also present certain microorganisms with the opportunity to multiply as discrete colonies in certain of the parts of the food (Brocklehurst *et al.*, 1995; Hills *et al.*, 1997), while others will be inhibited or even die. Post-process contamination, particularly when sporadic, may lead to the random presence of very low levels of pathogens.

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Representative samples can be collected from liquids, such as milk, in bulk if the product is thoroughly mixed before sampling. While adequate mixing can be achieved in small volumes, the efficiency of mixing decreases as the bulk volume increases. In the case of dry foods, the difficulty in obtaining representative samples increases as the size of particles increases, e.g., from fine powders, such as milk powder or flour, to coarser particles, such as cereal grains, up to large particles or pieces, such as nuts or raisins. Similar difficulties occur with meat, from ground (minced) meat, to trimmed pieces, large cuts, and whole carcasses, and with multi-component foods, such as prepared dishes with several ingredients.

Similar limitations apply to samples from the processing line. Sampling where residues accumulate increases the probability of detecting deviations. For environmental samples, which should play an important role as an early warning of the ingress of, and colonization by, pathogens, the situation is even more complex. Factors including the complexity of the environment, the presence of niches where multiplication may occur, interactions with competitive microorganisms, and changing environmental conditions, such as relative humidity, temperature, exposure to disinfectants, and so on, have an important impact on the distribution of pathogens such as salmonellae and *Listeria monocytogenes*. Another important factor is the small quantity of sample often available. This reinforces the need to have a well-designed sampling plan and well-trained personnel to collect the samples.

The distribution of microorganisms is also important when an analytical unit is withdrawn from the sample unit. Homogenization minimizes the heterogeneous distribution of microorganisms in the analytical sample. As demonstrated by Kilsby & Pugh (1981) using meat, the degree of homogenization of microorganisms increases with more finely chopped meat. The variance of counts decreased from 0.334 to 0.075 for whole pieces to minced and finely chopped meat, respectively, and the likelihood of detecting salmonellae also increased.

Using the most appropriate analytical standard methods or validated alternative methods is prerequisite to obtaining reliable results. Several essential practical aspects, such as the need to adapt methods to certain food matrices, to take account of the possible presence of injured microorganisms, and to apply good laboratory practices, etc., have an important impact on the successful detection or enumeration of the microorganisms sought.

The aim of this chapter is to review the steps from sample collection to the analysis of the analytical unit, and to highlight potential problems that could affect the final result.

12.2 COLLECTION OF SAMPLE UNITS

The purpose of sampling a food is to collect a representative sample to obtain information on its microbiological status. Samples are collected throughout the whole food chain, serving different purposes depending on the information required. The needs of different parties, such as regulatory authorities, food manufacturers, or researchers, may be quite different. The reasons for sampling in the food chain, whether at the farm, at the processing facility, in a warehouse, at retail, or at the consumer's home, need to be considered. This facilitates selection of samples, correct interpretation of results, and drawing valid conclusions.

Sampling may be done for investigative purposes, to obtain information and increase the knowledge of the occurrence of microorganisms in food products, or to assess their behavior during processing and storage. Regulatory authorities sample at predetermined

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