has not been established through the application of Codex principles can be challenged when its application leads to a trade barrier.

The information in this book should make it evident that microbiological testing of product is of limited value for determining the safety of a lot of food, particularly when the prevalence and concentration of a pathogen are low. This limitation was stressed by the Codex Alimentarius Commission through the following statement: "Mandatory microbiological criteria shall apply to those products and/or points of the food chain where no other more effective tools are available, and where they are expected to improve the degree of protection offered to the consumer" (CAC, 1997a, p. 28).

This chapter will discuss the principles for establishing microbiological criteria for determining lot acceptance and leave for later chapters the selection of sampling plans. A microbiological criterion is not complete without a sampling plan that is appropriate for the risk and severity of the hazard.

5.2 PURPOSES AND APPLICATION OF MICROBIOLOGICAL CRITERIA FOR FOODS

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

- the safety of a food;
- adherence to Good Hygienic Practices (GHP);
- the utility (suitability) of a food or ingredient for a particular purpose;
- the shelf-life of certain perishable foods; and/or
- the acceptability of a food or ingredient from another country or region where the conditions of production are unknown or uncertain.

In the absence of an FSO, microbiological criteria may be used to formulate design requirements and to indicate the required microbiological status of raw materials, ingredients, and end products at any stage of the food chain as appropriate. Microbiological criteria may be relevant to the examination of foods, including raw materials and ingredients, of unknown or uncertain origin or when other means of verifying the efficacy of GHP and Hazard Analysis Critical Control Point (HACCP)-based systems are not available. Generally, microbiological criteria may be applied to define the distinction between acceptable and unacceptable raw materials, ingredients, products, or lots of food by regulatory authorities and/or food business operators. Microbiological criteria may also be used to determine whether processes are consistent with the General Principles of Food Hygiene (CAC, 1997a).

Three basic principles should be applied when establishing microbiological criteria. Microbiological criteria should:

- accomplish what they purport to do (e.g., prevent or reduce foodborne disease);
- be technically attainable through the application of GHP and HACCP; and
- be administratively feasible (NRC, 1964; NRC, 1985).

5.3 DEFINITION OF MICROBIOLOGICAL CRITERION

A microbiological criterion defines the acceptability of a product or a food lot, based on the absence or presence or number of microorganisms, including parasites, and/or quantity of their toxins/metabolites, per unit(s) of mass, volume, area, or lot.

5.4 TYPES OF MICROBIOLOGICAL CRITERIA

Microbiological criteria for use in lot acceptance determinations fall into three categories (although Codex recognizes only one category; see also Chapter 4).

- Microbiological Standard—a mandatory criterion that is incorporated into a law or ordinance.
- Microbiological Guideline—an advisory criterion used to inform food operators and others of the microbial content that can be expected in a food when best practices are applied.
- Microbiological Specification—part of a purchasing agreement between a buyer and a supplier of a food; such criteria may be mandatory or advisory according to use.

5.4.1 Microbiological Standards

Microbiological standards are used to determine the acceptability of a food with regard to a regulation or policy. They are established by control authorities and define the microbiological content that foods must meet to be in compliance with a regulation or policy. Foods not meeting a standard are in noncompliance and would be subject to removal from the market. Standards may be established for a wide variety of reasons, but are best applied when risk is sufficiently high and compliance is essential for public health protection. Microbiological standards should be established following the principles outlined by Codex (CAC, 1997b) and as further elaborated in this book. Ideally, standards should be developed following establishment of an FSO for a hazard in a food.

5.4.2 Microbiological Guidelines

Microbiological guidelines may be established by a regulatory authority, industry trade association, or a company to indicate what is expected for the microbial content of a food when best practices are applied. Food operators use microbiological guidelines as a basis to design their control systems. Guidelines are advisory in nature and may not lead to rejection of a food. Microbiological guidelines may be a preferred means to inform and direct food operators within a segment of the industry to improve its practices, particularly when insufficient data prevent the establishment of a microbiological standard. A microbiological guideline along with guidance material on what constitutes best practices may be adequate to bring about the changes necessary to improve food safety and quality. This may negate the need to establish a standard.

5.4.3 Microbiological Specifications

Buyers of food may be in industry or government (e.g., schools, institutions, military). Buyers establish purchase specifications to reduce the likelihood of accepting an ingredient or food that may be unacceptable in terms of safety or quality. Microbiological specifications define the microbiological limits for an ingredient, for example, so that when it is used, the final product will meet all requirements for safety and quality. It is common practice for buyers along the food chain to establish microbiological specifications for the materials they purchase. In most cases, the specifications are advisory and the materials are sampled only on an as needed basis. In other cases (e.g., sensitive ingredients), each incoming lot may be tested.

5.5 APPLICATION OF MICROBIOLOGICAL CRITERIA

5.5.1 Application by Regulatory Authorities

Mandatory microbiological criteria shall apply to those products and/or points of the food chain where no other more effective tools are available and where they are expected to improve the degree of consumer protection. Where these are appropriate, they should be product-specific and only applied at the point of the food chain as specified in the regulation.

In situations of noncompliance with microbiological criteria, the regulatory control actions may lead to sorting, reprocessing, rejection, or destruction of product, and/or further investigation to determine the appropriate actions to be taken. The appropriate action(s) will depend on the assessment of risk to the consumer, the point in the food chain, the product, and its intended use.

Governments establish microbiological standards only when they are deemed appropriate to ensure the safety of the foods for which they have regulatory responsibility. Thus, food control authorities serve as risk managers and, through the risk analysis process, may ultimately decide that a microbiological criterion is necessary for a food at one or more points in the food chain. Ideally, the standards will be based upon a tolerable level of risk and FSO for the hazard of concern. Microbiological standards may be used by both industry and government as the basis for assessing the acceptability of a lot or consignment of food. Microbiological standards should be applied equally to domestic and imported foods. Lots that do not meet the microbiological standard would be considered unacceptable and subject to removal from the market or rejection at port-of-entry.

5.5.2 Application by a Food Business Operator

In addition to checking compliance with regulatory provisions (see sections 5.4.1 and 5.5.1), microbiological criteria may be applied by food business operators to formulate design requirements and to examine products as one of the measures to verify and/or validate the efficacy of elements of their GHP and HACCP plans (see Chapters 3 and 4). Such criteria will be specific for the product and the stage in a process or in the food chain at which they apply. They may be stricter than the criteria used for regulatory purposes and should, as such, not be used for legal action.

Any buyer of food can enter into an agreement with a supplier to ensure that food being purchased will meet mutually agreed upon criteria. Food operators frequently establish purchase specifications for food ingredients and other materials that will ensure compliance with microbiological standards and as a means to ensure product quality. The factors outlined in Exhibit 4–2 influence whether a buyer will sample ingredients or foods upon receipt. For example, an audit of an operation may suggest that a supplier can not consistently meet purchase specifications. This may lead the buyer to sample incoming lots of purchased material until a follow-up audit indicates improvement. Lots that fail to meet the established specifications may be rejected.

5.5.2.1 Application in GHP

Microbiological criteria can be used to check certain aspects of GHP. An example would be verifying the acceptability of water if not supplied by a source tested by others. Also, food operators can establish microbial limits that are to be met when the cleaning

and disinfecting routines are properly performed. These criteria often consist of aerobic counts or indicator organisms and reflect experience of what is attainable with the equipment, materials, and conditions that exist for the operation. Another option to assess GHP is to sample product at selected times and steps in an operation and analyze for aerobic count or other indicators. Evidence of an increase in the microbial concentration on/in a product may be due to microorganisms acquired from contact with equipment during processing. In-process criteria should be based on knowledge of the conditions that influence microbial content during processing.

5.5.2.2 Application in HACCP

Microbiological criteria are not normally suitable for monitoring critical limits, as defined in the publication *Hazard Analysis and Critical Control Point System and Guidelines for its Application* (CAC, 1997c). Monitoring procedures must be able to detect loss of control at a critical control point (CCP). Monitoring should provide this information in time for corrective actions to be taken to regain control before there is a need to reject the product. Consequently, on-line measurements of physical and chemical parameters are often preferred to microbiological testing because results are often available more rapidly and at the production site. Moreover, the establishment of critical limits may require other considerations than those described in this document.

5.6 PRINCIPLES FOR THE ESTABLISHMENT OF MICROBIOLOGICAL CRITERIA

Principles for the establishment of microbiological criteria have been developed by Codex (CAC, 1997b). The principles, initially developed through World Health Organization (WHO)/Food and Agriculture Organization (FAO) consultations (Christian, 1983), have continued to evolve through a number of revisions, each with input from the International Commission on Microbiological Specifications for Foods (ICMSF). Thus, it is appropriate that this chapter be based on the principles outlined in the Codex document. This chapter is intended to give guidance for the establishment and application of microbiological criteria for foods at any point in the food chain from primary production to final consumption.

The safety of foods is principally assured by control at the source, product design and process control, and the application of Good Hygienic Practices during production, processing (including labeling), handling, distribution, storage, sale, preparation, and use, in conjunction with the application of the HACCP system. This preventive approach offers more control than microbiological testing because the effectiveness of microbiological examination to assess the safety of foods is limited. This view was emphasized by the U.S. Food and Drug Administration (FDA) in its discussion of the control measures considered necessary to achieve a required level of protection. This statement is in reference to all forms of product testing (e.g., chemical, physical, microbiological).

> End product testing, which measures outcome, cannot generally be relied upon exclusively to provide an adequate level of protection because it only tests for a specific risk or group of risks on a particular day. The results of end product sampling may or may not be representative of the actual, continuing risk, depending upon product uniformity, the amount of sampling, and other factors. Processing controls coupled with adequate verification by a regulatory authority provide an essential assurance that food will not present unacceptable risks.

> Processing controls can assure that the level of protection is met in many circumstances where end-product testing alone realistically cannot (Schultz, 1997, p. 30597).

Microbiological criteria should be established according to the following principles and be based on scientific analysis and advice and, where sufficient data are available, a risk assessment appropriate to the foodstuff and its use. Microbiological criteria should be developed in a transparent fashion and meet the requirements of fair trade. They should be reviewed periodically for relevance with respect to emerging pathogens, changing technologies, and new understandings of science.

A microbiological criterion should be established and applied only where there is a definite need and where its application is practical. Such need is demonstrated, for example, by epidemiologic evidence that the food under consideration may represent a public health risk and that a criterion is meaningful for consumer protection, or as the result of a risk assessment. A criterion should be established only when it is the best, or only, means of ensuring a food will be safe or will not spoil. Application of the criterion should be practical and technically attainable by applying GHP and HACCP. The criterion should be capable of accomplishing the intended purpose (i.e., reduce foodborne disease or spoilage).

To fulfill the purpose of a microbiological criterion, consideration should be given to:

- evidence of actual or potential hazards to health;
- microbiological status of the raw materials;
- effect of processing on the microbiological status of the food;
- likelihood and consequences of microbial contamination and/or growth during subsequent handling, storage, and use;
- intended use of the food;
- category(s) of consumers concerned;
- cost-benefit ratio associated with the application of the criterion; and
- need to inform personnel along entire food chain.

The number and size of analytical units per lot tested should be as stated in the sampling plan and should not be modified. Unacceptable lots should not be subjected to repeated testing to bring the lot into compliance.

The intended use is an important consideration. Food safety is: "assurance that food will not cause harm to the consumer when it is prepared and/or eaten according to its intended use" (FAO, 1997). Thus, a raw agricultural commodity that is intended to be thoroughly heated before consumption could contain *Salmonella* and with adequate processing, not cause harm. A stringent sampling plan for *Salmonella* in such a food would normally have no value. Proper labeling with adequate instructions for preparation and use would be more effective.

Consideration of the intended use should also include who is going to prepare the product (e.g., professional caterer or consumer). Even more important is for which group of consumers is the food intended. Babies, ill people, the elderly, and immunosuppressed individuals are more vulnerable, for example, than normal healthy adults; thus, greater care is essential for the production or preparation of food for these consumers. This should be reflected in the stringency of microbiological criteria and accompanying sampling plans.

Consideration of the cost-benefit ratio should assess whether establishing and enforcing a criterion would be an effective means of using the available resources. In addition,

the criteria should be administratively feasible by the control authority. These considerations fall under the activity of choosing an adequate risk management option within the risk management concept, where other options are considered as well.

5.7 COMPONENTS OF MICROBIOLOGICAL CRITERIA FOR FOODS

A microbiological criterion consists of:

- a statement of the microorganisms of concern and/or their toxins/metabolites and the reason for that concern (see section 5.7.1);
- microbiological limits considered appropriate to the food at the specified point(s) of the food chain;
- the number of analytical units that should conform to these limits (see section 5.7.2);
- a sampling plan defining the number of field samples to be taken, the method of sampling and handling, and the size of the analytical unit (see section 5.7.3); and
- the analytical methods of detection and/or quantification (see section 5.7.4).

A microbiological criterion should also state:

- the food to which the criterion applies;
- the point(s) in the food chain where the criterion applies; and
- any actions to be taken when the criterion is not met.

When applying a microbiological criterion to assess products, it is essential, in order to make the best use of money and personnel, that only appropriate tests be applied to those foods and at those points in the food chain that offer maximum benefit in providing consumers with foods that are safe and suitable for consumption.

5.7.1 Microorganisms, Parasites, and Their Toxins/Metabolites of Importance in a Particular Food

Microorganisms of concern and/or their toxins/metabolites include:

- bacteria, viruses, yeasts, molds, and algae;
- parasitic protozoa and helminths; and
- microbial toxins/metabolites.

The microorganisms included in a criterion should be widely accepted as relevant—as pathogens, as indicator organisms, or as spoilage organisms—to the particular food and technology. Organisms whose significance in the specified food is doubtful should not be included in a criterion.

The mere finding, with a presence-absence test, of certain microorganisms known to cause foodborne illness (e.g., *Clostridium perfringens*, *Staphylococcus aureus,* and *Vibrio parahaemolyticus*) does not necessarily indicate a threat to public health.

Where pathogens can be detected directly and reliably, consideration should be given to testing for them in preference to testing for indicator organisms. If a test for an indicator organism is applied, there should be a clear statement whether the test is used to indicate unsatisfactory hygienic practices or a health hazard.

5.7.2 Microbiological Limits

A microbiological limit is the frequency or maximum concentration of a microorganism, microbial toxin, or metabolite that can be used to differentiate acceptable from unac-

ceptable lots of food. Limits are established as a basis to assess the safety or quality of a food. Microbiological limits should be appropriate for the food and be applicable at one or more points in the food chain. Limits for specific hazards in food should be compatible with any FSOs that may have been established.

Limits used in criteria should be based on microbiological data appropriate to the food and should be applicable to a variety of similar products. The process of establishing limits for use as standards should include collecting and analyzing data from a variety of operations to determine what can be expected for foods produced under acceptable conditions of GHP and HACCP. The data can be used to establish limits that can be met by all who operate under acceptable conditions. Alternatively, the limit may be made more stringent if improvement is deemed necessary in a certain segment of industry to reduce the likelihood of a hazard. This assumes operators can adapt by making certain practical modifications. If, however, the technology does not exist or is not affordable, then the more stringent limit will fail and the desired improvement will not be achieved. The process of establishing microbiological criteria, and other acceptance criteria, should be transparent and allow input from all interested parties.

Microbiological limits can be related only to the time and place of sampling and not to the presumed number of microorganisms at an earlier or a later stage. Because GHP aims at producing foods with microbiological characteristics significantly better than those required by public health considerations, a numerical limit in a guideline may be more stringent than in a standard or an end-product specification.

In the establishment of microbiological limits, any changes in the microflora likely to occur during storage and distribution (e.g., decrease or increase in the numbers) should be taken into account. Microbiological limits should take into consideration the risk associated with the microorganisms and the conditions under which the food is expected to be handled and consumed. These considerations are discussed in Chapter 8. Microbiological limits should also take account of the likelihood of uneven distribution of microorganisms in the food (see Chapters 6 and 7) and the inherent variability of the analytical procedure (see Chapter 12).

Whether foods are acceptable or not is defined in a criterion by the:

- microbiological limit(s);
- number of samples examined;
- size of the analytical unit; and
- number of units that should conform to the limits.

If a criterion requires the absence of a particular microorganism, the size and number of analytical units (as well as the number of analytical sample units) should be indicated. It should be borne in mind that no feasible sampling plan can ensure the absence of a particular organism in the entire contents of the lot.

The microbial populations in many foods processed under GHP and HACCP are generally not explicitly expressed because they are as low as is reasonably achievable. In some cases, the levels cannot be measured due to the technical problems involved. For instance, a lot of canned food that has received a "bot cook" will most probably not contain surviving spores of *C. botulinum* in 10^{10} or 10^{11} g of product. Likewise, enteric pathogens are not likely to be found in many kilograms of pasteurized products. Setting limits for pathogens in processed foods in which a validated kill step is included during processing should not be an arbitrary activity, but should be done only if there is a need to detect contaminated product.

Limits for use in purchase specifications are best established from data collected during normal production when the operation is under control. It is not uncommon for a company to establish for its own use criteria more stringent than required to ensure compliance with customer and regulatory requirements.

5.7.2.1 Indicators

Indicators are frequently used to examine foods or ingredients. Indicators can be very useful, but their selection and application must be done with care and thorough understanding of how accurately to interpret the analytical results. An important use of indicators is to verify process control and identify opportunities for process improvements. Most considerations used to set a microbiological criterion are applicable for indicators; however, indicators are not used solely for pathogenic concerns. Microorganisms, their cellular components, or their metabolic products used as indicators may indicate:

- the possible presence of a pathogen or toxin (*Staphylococcus aureus* for potential enterotoxin and/or excessive human handling in cooked crab meat).
- the possibility that faulty practices occurred during production, processing, storage, and/or distribution (coliforms in pasteurized milk).
- the suitability of a food or ingredient for a desired purpose (*E. coli* in nuts for ice cream).
- an estimate of the shelf-life of perishable foods during the expected conditions of handling and storage (yeast in yogurt).
- the possibility of changes in the food through microbial activity that would result in the food becoming more hazardous (acetophiles in spices for acidic sauces).
- the effectiveness of cleaning and disinfection (ATP (adenosine triphosphate) for residual soil).

Indicator organisms and agents can be divided into indicators of potential (a) human contamination, (b) fecal contamination, (c) survival of a pathogen or spoilage organism, and (d) post-processing contamination.

Examples of microbial indicators that can be analyzed quantitatively or qualitatively include aerobic bacteria, coliforms, *Enterobacteriaceae*, *Escherichia coli*, yeasts, molds, proteolytic bacteria, and thermophilic bacteria. Examples of cellular components that can be used as indicators include ATP, ribonucleic acid (RNA), endotoxins (e.g., limulus lysate test for cellular polysaccharides), and various enzymes (e.g., thermonuclease). Examples of metabolic products used as indicators include hydrogen sulfide (early putrefaction), carbon dioxide (spoilage by *Zygosaccharomyces bailii*), lactic acid (in certain meat products, including ham), ethanol (in fruit juice), diacetyl (in fruit juice or beer), and ergosterol (indicates mold in grain).

Exhibit 5–2 lists some of the characteristics of an ideal indicator organism. It must be recognized that indicators often represent a compromise that is less than the ideal of being able to test for the microorganism(s) or toxin(s) of concern. They offer considerable advantages, however, that will ensure their continued use. Indicators should be selected that provide the best information with the least amount of compromise.

In practice, indicators seldom, if ever, prove the presence or the absence of a target microorganism; they merely indicate the possibility. For purposes of process control, the absence of, or low numbers of, an indicator microorganism can be useful as a sign that a process is under control, and therefore, there will be a lower likelihood of occurrence of an unacceptable target microorganism. However, a pathogen (e.g., salmonellae) may be

Exhibit 5–2 Factors To Consider When Selecting an Indicator for a Specific Purpose

- Presence indicates potential for spoilage, faulty practice, or faulty process
- Easily detected and/or quantified
- Survival or stability (including inactivation kinetics) similar to or greater than the hazard or spoilage microorganism
- Growth capabilities (requirements) similar or faster than the hazard or spoilage microorganism
- Identifiable characteristics of indicator are stable
- Methods are rapid, inexpensive, reliable, sensitive, validated, and verified with positive control
- Quantitative results have a correlation between indicator concentration and level of hazard or spoilage microorganism
- Results are applicable to process control
- Analyst health is not at risk
- Analytical method does not jeopardize the environment and is suitable for in-plant use

present independent of an indicator. This is more likely to occur if the pathogen can establish itself in a processing environment and multiply. *E. coli* and coliforms are not reliable indicators for the presence of salmonellae where this can occur.

Indicators can be very useful, but their selection and application must be done with care and thorough understanding of how to interpret the analytical results correctly.

5.7.3 Sampling Plans, Sampling Method, and Handling of Samples Prior to Analysis

Sampling plans include the sampling procedure and the decision criteria to be applied to a lot, based on examination of a prescribed number of sample units and subsequent analytical units of a stated size by defined methods. A well-designed sampling plan defines the probability of detecting microorganisms in a lot, but it should be borne in mind that no sampling plan can ensure the absence of a particular organism. Sampling plans should be administratively and economically feasible.

In particular, the choice of sampling plans should take into account:

- risks to public health associated with the hazard (severity and likelihood of occurrence of the hazard);
- the susceptibility of the target group of consumers (very young or old, immunocompromised, etc.);
- the heterogeneity of distribution of microorganisms where variable sampling plans are employed;
- the randomness of sampling;
- the acceptable quality level (i.e., percentage of nonconforming or defective sample units tolerated); and
- the desired statistical probability of accepting or rejecting a nonconforming lot.

The information needed for the first two points could be obtained through a risk assessment; however, good epidemiologic data often suffice. For many applications, two or three class attribute sampling plans may prove useful. For a detailed discussion of establishing

sampling plans, see Chapters 6, 7, and 8. In general, the greater the risk, the more stringent (e.g., greater number of samples) should be the sampling plan.

The statistical performance characteristics or operating characteristic curve should be provided in the sampling plan. Performance characteristics provide specific information to estimate the probability of accepting a nonconforming lot. The sampling method should be defined in the sampling plan. The time between taking the field samples and analysis should be as short as reasonably possible and, during transport to the laboratory, the conditions (e.g., temperature) should not allow increase or decrease of the numbers of the target organism. By controlling these conditions, the results should reflect, within the limitations given by the sampling plan, the microbiological conditions of the lot.

5.7.4 Microbiological Methods

Whenever possible, only methods for which the reliability (accuracy, reproducibility, inter- and intra-laboratory variation) has been statistically established by comparative or collaborative studies in several laboratories should be used. Moreover, preference should be given to methods that have been validated for the commodity concerned, preferably in relation to reference methods elaborated by international organizations. While methods should be the most sensitive and reproducible for the purpose, methods to be used for in-plant testing might often sacrifice to some degree sensitivity and reproducibility in the interest of speed and simplicity. They should, however, have been proven to give a sufficiently reliable estimate of the information needed.

Methods used to determine the suitability for consumption of highly perishable foods (i.e., foods with a short shelf-life) should be chosen so that results are available before the foods are consumed or exceed their shelf-life.

The microbiological methods specified should be reasonable with regard to complexity, availability of media, equipment, etc., ease of interpretation, time required, and costs. Additional information on methods and their reliability is provided in Chapter 12.

5.7.5 Reporting

The test report shall give the information needed for complete identification of the sample, the sampling plan, the test method, and, if appropriate, the interpretation of the results.

5.7.6 Disposal of Unacceptable Lots

When a lot fails to meet a microbiological criterion, the lot must be disposed of in an acceptable manner. If the lot has failed a microbiological standard, the food is not in compliance with an existing regulation and would be subject to current regulatory policy. A lot failing a guideline may be acceptable depending on the circumstances. If the lot has failed a specification, depending on risk to consumers, its intended use, potential product failure due to spoilage, or other effects, the food may be rejected by the buyer. In each case, however, the cause for failure to meet the criterion should be determined and corrective actions implemented.

The option selected for disposal of a rejected lot should be influenced by the risk to consumers, the type of food, applicable regulatory policy, and the food's original intended

use. Options for disposal may include sorting, reprocessing, and destruction. In certain circumstances, the food might be used as an ingredient when a subsequent process will eliminate a hazard. The option selected must be in compliance with existing regulations.

5.8 EXAMPLES OF MICROBIOLOGICAL CRITERIA

Table 5–1 provides an example of microbiological criteria that might result from application of the principles described in this chapter. The example is derived from an earlier recommendation from ICSMF (ICMSF, 1986). The criteria include indicators [i.e., APC (aerobic plate count), coliforms], methods of analysis, number of samples to be collected, number of samples that must conform to the criteria, and the microbiological limits per g. This brief summary must be supplemented with additional information such as a statement of why these criteria were selected and are considered necessary; the step(s) in the food chain where the criteria are to be applied; method of sample collection, handling, and preparation for analysis; the analytical unit (in this example, the unit for the *Salmonella* analysis is 25 g); whether the analytical units can be composited for analysis (in this example, the five 25 g analytical units could be combined into a single 125 g composite for analysis); and disposition of lots that do not meet the criteria. The criteria can be made more stringent if the eggs are intended for a sensitive population (e.g., hospitals, institutions for the elderly).

A second example of a microbiological criterion addresses the concern for *S. aureus* enterotoxin in cooked crustaceans. Cooked crustaceans are often hand-peeled, and *S. aureus* may be transferred from workers' hands to the cooked product during peeling. Subsequent temperature abuse could result in growth of *S. aureus*, particularly since a

Table 5–1 Example of Microbiological Criteria for Pasteurized Liquid, Frozen, and Dried Egg Products

Microorganism or group	*Analytical Method*	*No. Samples (n)*	*c*	*Limit per g*	
				m	*M*
APC	ISO 4833	5	2	5×10^4	10^6
Coliforms	ISO 4831	5	2	10^1	10^3
Salmonella spp.	ISO 6579	5*	0	0 [negative/125]	—
		$10^{\dagger}$	0	0 [negative/250]	—
		$20^{\dagger\dagger}$	0	0 [negative/500]	—

*,†,††The number of samples is to be chosen in accordance with whether the expected conditions of storage and use of the product will cause *)a reduction, †)no change, or ††)an increase in the number of *Salmonella* spp. between when the eggs are sampled and consumed.

c: the maximum allowable number of defective sample units (i.e., two-class plans for *Salmonella*) or marginally acceptable sample units (i.e., three-class plans for APC and coliforms). When more than this number are found in the sample, the lot is rejected.

m: a microbiological limit which, in a two-class plan, separates good quality from defective quality or, in a three-class plan, separates good quality from marginally acceptable quality. Values equal to *m*, or below, represent an acceptable product and values above it are either marginally acceptable or unacceptable. This value also could be seen as the value considered by those establishing the criteria to be acceptable and attainable through application of GHP and/or HACCP.

M: a microbiological limit which, in a three-class plan, separates marginally acceptable quality from defective quality. Values above *M* are unacceptable.

competitive flora may be lacking in the cooked, peeled product. For these reasons, cooked, peeled shrimp and other crustaceans have been associated with staphylococcal foodborne illness. Microbiological testing can be used to assess the safety of this food category (e.g., at port-of-entry). Considering the high levels of *S. aureus* associated with risk and the relatively small number of samples required for assessment, application of a microbiological criterion is a practical option to evaluate the acceptability of these products with respect to the likely presence of staphylococcal enterotoxin. The European Union, for example, has adopted a three-class sampling plan with $n = 5$, $c = 2$, $m = 100$ cfu g^{-1}, and $M = 1{,}000$ cfu g^{-1} for *S. aureus* in cooked crustaceans.

Chapters 6, 7, and 8 discuss the factors that must be considered when establishing microbiological criteria and provide further discussion of n, c, m, and M, and choice of sampling plan. In addition, a decision tree is provided in Figure 8–1 to help decide when microbiological criteria can be effective in relation to other control options.

5.9 REFERENCES

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Chapter 6

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
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6.10 What Is a Lot?
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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.

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.

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

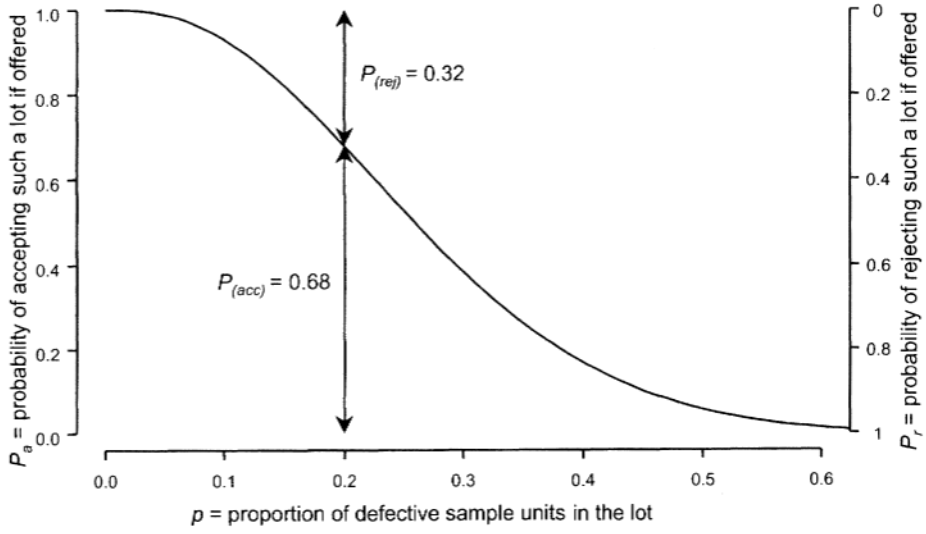


Figure 6–1 The operating characteristic curve for a sampling plan with $n = 10$, $c = 2$, i.e., the probability of accepting lots, in relation to the proportion defective among the sample units in the lot being examined. If, for example, the lot comprises 20% ($p = 0.2$) defective sample units, the lot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

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).

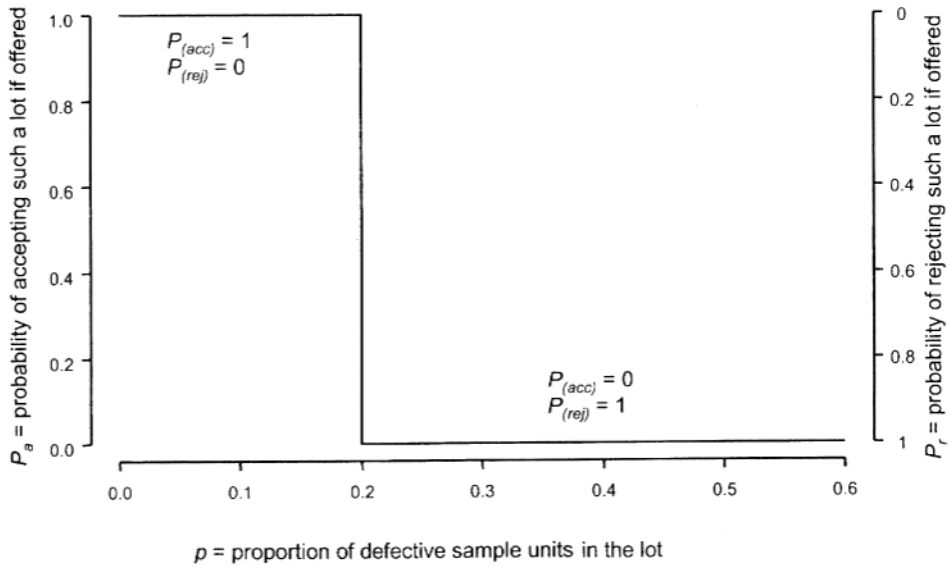


Figure 6.2 The operating characteristic curve (OC) for the idealized situation of complete discrimination between lots with a proportion of defective sample units below 20% and lots with such a proportion above 20%.

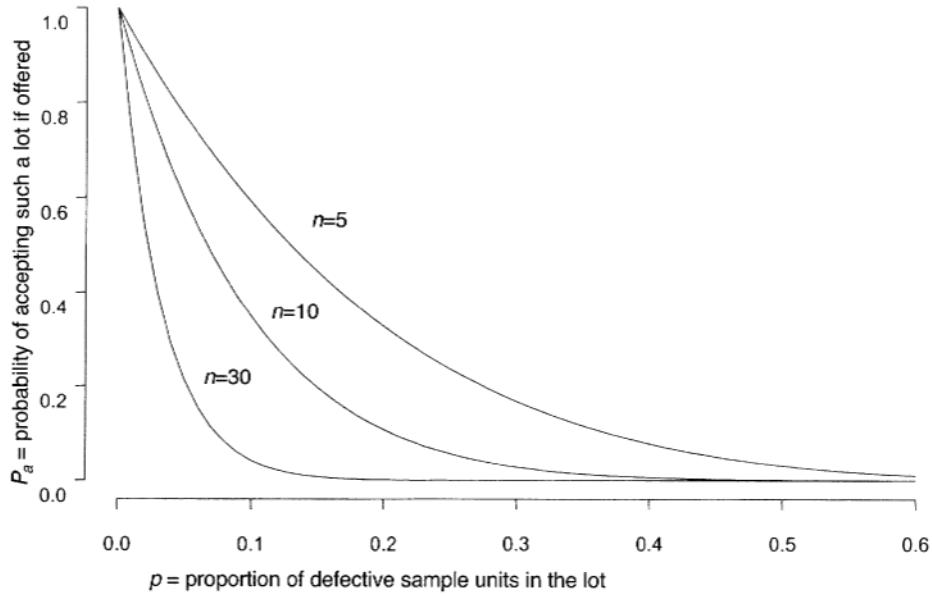


Figure 6–3 Operating characteristic curves for sampling plans with $n = 5$, $n = 10$, $n = 30$ and $c = 0$. With increasing numbers of sample units examined (n), the slope of the OC curve becomes steeper.

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

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

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.

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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Chapter 7

Sampling Plans

7.1 Introduction
7.2 Attributes Plans
7.3 Variables Plans
7.4 Comparison of Sampling Plans
7.5 References

7.1 INTRODUCTION

This text is concerned primarily with plans that may be applied to lots of food presented for acceptance at ports or other points of entry. Often, little or no information is available to the receiving agency about the method by which the food was processed or the record of previous performance by the same processor. Under these circumstances, attributes plans are appropriate. Variables sampling plans (see section 7.3), which depend upon the nature of the frequency distribution of microorganisms within lots of foods, are suitable only if this distribution is known. This limits severely their usefulness in port-of-entry sampling, but they may be particularly helpful to food producers monitoring their own production.

7.2 ATTRIBUTES PLANS

7.2.1 Two-Class Attributes Plans

A simple way to decide whether to accept or reject a food lot may be based on some microbiological test performed on several (n) sample units. This will usually be a test for the presence (positive result) or absence (negative result) of an 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.

As explained in Chapter 6, the decision-making process is defined by two numbers. The first is represented by the letter n, and defines the number of sample units required for testing. The second number, denoted c, is the maximum allowable number of sample units yielding unsatisfactory test results, for example, the presence of the organism, or a count above the defined concentration, denoted by m, which in a two-class plan separates good quality from defective quality (Figure 7–1a). Thus, in a presence/absence decision on a lot, the sampling plan $n = 10$, $c = 2$ means that 10 sample units are taken and a test made on each; if 2 or fewer show the presence of the organism, the lot is accepted (with respect to this characteristic); but if 3 or more of the 10 show the presence of the organism, the lot is rejected, although not necessarily destroyed (see Chapter 6, section 6.9, for an explanation of rejection).

Only under circumstances where the lot is intimately mixed can data obtained from the application of a two-class plan based on presence/absence of a microorganism be used to give an approximate guide to the likely concentration of that microbe within the lot. Such

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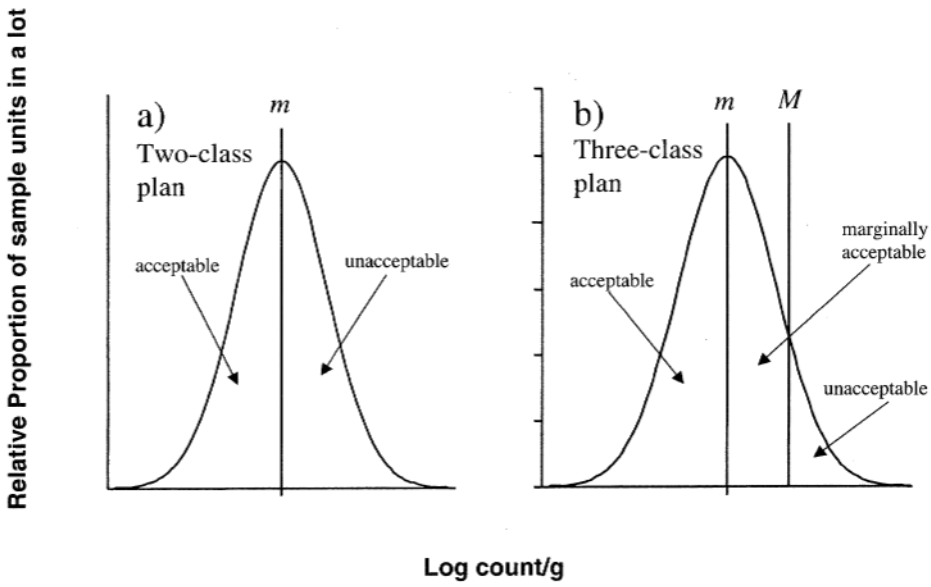


Figure 7–1 Two- and three-class attributes plans.

techniques should only be applied when the analyst is confident that there is a uniform distribution of the microorganisms of concern.

The performance of the sampling plan depends upon n and c (see Chapter 6, section 6.8). The larger the value of n at a given value of c, the better is the discrimination between lots of acceptable and unacceptable quality. Thus, compared with $n = 10$, $c = 2$, the plan $n = 15$, $c = 2$ is more discriminating, while the plan $n = 5$, $c = 2$ is more lenient. On the other hand, for given sample size n, if c is decreased, the better the food quality must be to have the same chance of being passed. Conversely, if c is increased, the plan becomes more lenient and will more often pass food lots of a given quality whenever offered for test (i.e., there is a higher probability of acceptance, P_a). Probabilities of acceptance for a set of plans are given in Tables 7–1 and 7–2. Figure 7–2 displays the operating characteristic (OC) curves for a few of these plans to illustrate the characteristics of various two-class attributes plans.

The importance of the sample size, n, is also stressed when we take a different approach to assess lot quality. Suppose a sampling plan was applied not only to decide between acceptance and rejection of the lot, but also to give an estimate of the proportion of defectives in the lot. Such estimates should be reported in terms of confidence intervals, giving a range of values and supported by the actual sampling results. The width of these ranges, i.e., the precision of the estimates, is greatly influenced by n. For illustration purposes, lower and upper limits of 95% confidence intervals are given in Table 7–3 for some combinations of sample sizes, n, and actual numbers of positive sample units, k.

Another aspect that might be considered is the influence of the size of the lot. A sample size of, say, $n = 30$ sample units might be taken at random from a lot of any size (or consignment if appropriate—see Chapter 6, section 6.10 and Chapter 17, section 17.5.3.2) for use in a two-class plan with $c = 0$. If the lot contains a very large number

Table 7–1 Two-Class Plans ($c = 0$): Probabilities of Acceptance (P_a) of Lots Containing Indicated Proportions of Acceptable and Defective Sample Units

Composition of lot		*Number of sample units tested from the population (n)*							
% acceptable (100 − p)	*% defective (p)*	*3*	*5*	*10*	*15*	*20*	*30*	*60*	*100*
98	2	0.94	0.90	0.82	0.74	0.67	0.55	0.30	0.13
95	5	0.86	0.77	0.60	0.46	0.36	0.21	0.05	0.01
90	10	0.73	0.59	0.35	0.21	0.12	0.04	<	<
80	20	0.51	0.33	0.11	0.04	0.01	<		
70	30	0.34	0.17	0.03	<	<			
60	40	0.22	0.08	0.01					
50	50	0.13	0.03	<					
40	60	0.06	0.01						
30	70	0.03	<						
20	80	0.01							
10	90	<*							

*< means $P_a < 0.005$.

of sample units, one obtains an OC curve such as that shown in Figure 7–2c. According to that OC curve, a lot with one defective unit out of 40 (proportion defective, $p = 0.025$) will be accepted about half the time ($P_a = 0.5$) when using the plan $n = 30$, $c = 0$. Calculation of this probability is based on the binomial distribution model. If the lot contains a smaller number of sample units, the probabilities of acceptance P_a for various values of p should, in principle, be calculated using a different distribution model, because the 30 sample units might now constitute an appreciable fraction of the whole lot. However, acceptance probabilities would be only slightly smaller than those shown in Figure 7–2c. This effect only becomes important when one-quarter to one-half of the lot is taken as a sample, a circumstance that rarely occurs in bacteriological analysis of lots of food.

Suppose that instead of $n = 30$, $c = 0$, we use $n = 5$, $c = 0$. The latter plan is undiscriminating, but the associated probabilities of acceptance are, again, nearly independent of the consignment size.

In summary, a sampling plan of the form described gives nearly the same degree of protection against acceptance of unacceptable lots as against rejection of acceptable ones, regardless of the size of the lot. The attributes schemes given in this book do not increase stringency as lot size increases. The sampling plan is, therefore, independent of the size of the lot, provided the lot is large in comparison to sample size. If the important criterion is the actual number of defective units, rather than the proportion of the lot that is defective, then the sampling plans given are not appropriate.

7.2.2 Three-Class Attributes Plans

Three-class attributes plans (see Bray, Lyon, & Burr, 1973) were devised for situations where the quality of the product can be divided into three attribute classes, depending upon the concentration of microorganisms within the sample units. As in two-class plans based on quantitative analytical results, counts above a concentration m, which in a three-

Table 7–2 Two-Class Plans (Selected *c* Values): Probabilities of Acceptance (P_a) of Lots Containing Indicated Proportions of Acceptable and Defective Sample Units

Composition of lot												
% acceptable	*% defective*	*n = 5*			*n = 10*			*n = 15*			*n = 20*		
(100 − p)	*(p)*	*c = 3*	*c = 2*	*c = 1*	*c = 3*	*c = 2*	*c = 1*	*c = 4*	*c = 2*	*c = 1*	*c = 9*	*c = 4*	*c = 1*
98	2	1.00	1.00	1.00	1.00	1.00	0.98	1.00	1.00	0.96	1.00	1.00	0.94
95	5	1.00	1.00	0.98	1.00	0.99	0.91	1.00	0.96	0.83	1.00	1.00	0.74
90	10	1.00	0.99	0.92	0.99	0.93	0.74	0.99	0.82	0.55	1.00	0.96	0.39
80	20	0.99	0.94	0.74	0.88	0.68	0.38	0.84	0.40	0.17	1.00	0.63	0.07
70	30	0.97	0.84	0.53	0.65	0.38	0.15	0.52	0.13	0.04	0.95	0.24	0.01
60	40	0.91	0.68	0.34	0.38	0.17	0.05	0.22	0.03	0.01	0.76	0.05	<
50	50	0.81	0.50	0.19	0.17	0.05	0.01	0.06	<	<	0.41	0.01	<
40	60	0.66	0.32	0.09	0.05	0.01	<	0.01			0.13	<	
30	70	0.47	0.16	0.03	0.01	<		<			0.02		
20	80	0.26	0.06	0.01	<						<		
10	90	0.08	0.01	<									
5	95	0.02	<*										

n = number of sample units tested from the population
*< means $P_a < 0.005$.

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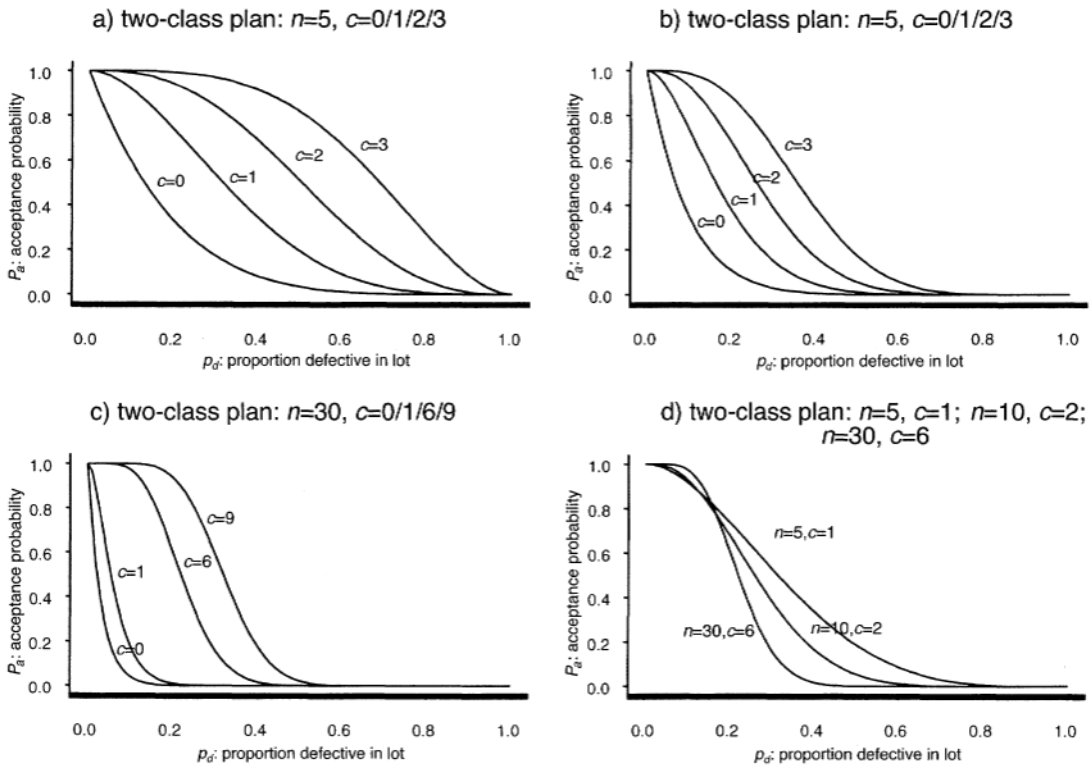


Figure 7–2 Operating characteristic curves for different sample sizes (n) and different criteria of acceptance (c) for two-class plans.

class plan separates good quality from marginally acceptable quality, are undesirable, but some can be accepted. However, a count above a second concentration M for any sample unit is unacceptable, and if any count for the n sample units from a lot exceeds M, that lot is rejected (Figure 7–1b). This concept is based on the idea that analytical results for sample units drawn from a lot are of a quantitative nature. In this case, quantities of microorganisms in sample units can be described in terms of frequency distributions that can be characterized by some measures of location and spread.

Figure 7–3 illustrates the effect of various frequency distributions of microbial content within a lot on the location of m and M for three-class plans. Curve 1 represents an entirely satisfactory lot, with low numbers of bacteria generally, and thus a low average count with little variation and no counts exceeding m. Curve 2 represents a lot with a similar average count, but with a much wider variation, so that a small proportion of sample units would have counts exceeding m, though none exceed M. If the proportion in the range m to M were small, the situation would be acceptable; if this proportion were larger, it might still be acceptable, but it would serve as a warning call to the producer, as tending toward the situation shown in curve 3. Curve 3 represents a lot with a higher average count and larger variation, such that a small proportion of sample units exceeds M and would result in immediate rejection, while a substantial proportion falls in the range m to M, which itself could also suffice to justify rejection (see Chapter 6, section 6.9, for

Table 7–3 Lower and Upper Limits of 95% Confidence Intervals for the Estimated Proportion Defective Based on *k* Positive Results When *n* Sample Units Are Analyzed

n	*k*	*Lower limit*	*Upper limit*
5	0	0.000	0.451
5	1	0.005	0.716
5	2	0.053	0.853
5	3	0.147	0.947
10	0	0.000	0.259
10	1	0.003	0.445
10	2	0.025	0.556
10	3	0.067	0.652
15	0	0.000	0.181
15	1	0.002	0.319
20	0	0.000	0.139
20	1	0.001	0.249

explanation of the term *rejection*). Curve 4 represents a lot of even greater unacceptability, requiring rejection.

Hence the definition of a three-class sampling plan incorporates two limits, *m* and *M*, *M* being higher than *m*, which distinguish three classes of sampling results, the number of sample units to be drawn from the lot *n*, and the maximum number of sample units *c* that are allowed to fall into the region between *m* and *M*. The maximum number that may exceed *M* is always set to 0 in the plans in this book.

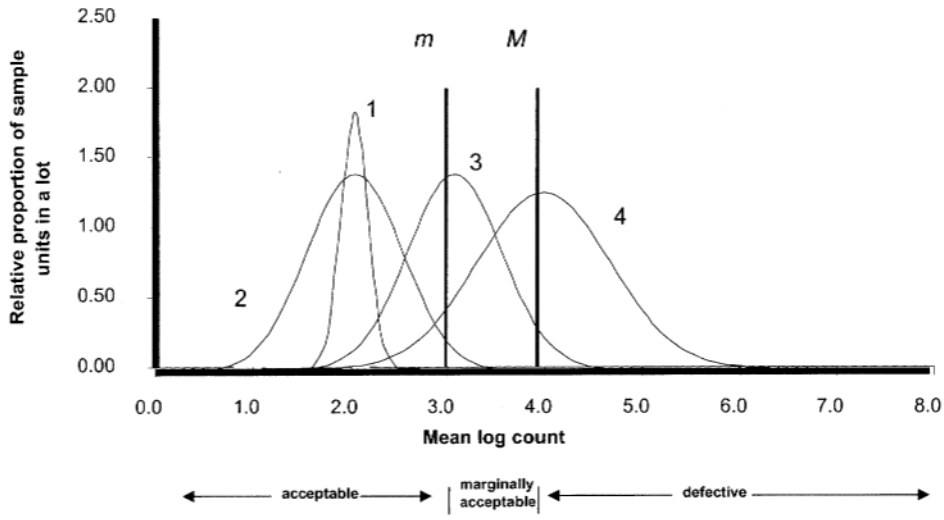


Figure 7–3 Three-class attributes plans.
m = count above a defined concentration, separating good quality from marginally acceptable quality.
M = count above a second defined concentration, separating marginally acceptable quality from unacceptable quality.

Accordingly, in the three-class plans there are again only two numbers, n and c, from which it is possible to find the probability of acceptance, P_a, for a food lot of given microbiological quality. To describe the lot quality, we consider all sample units that could be drawn from the lot, which must yield counts in three classes: 0 to m, m to M, and above M. Since the proportions in the lot for the three classes must total 1, one need only specify two of them in describing lot quality. We might call these proportions the proportion *defective*, i.e., above M (p_d), and the proportion *marginally acceptable*, i.e., m to M (p_m). The proportion *acceptable*, equal to or less than m, must be 100% minus the sum of p_d and p_m. By appropriate calculations, we can find the probability of acceptance, P_a, for a given lot quality for any specified sampling plan. For example, for the plan $n = 10$, $c = 2$, P_a will be 0.21 for a lot distribution for which 20% of the sample counts are marginally acceptable ($p_m = 20\%$) and 10% defective ($p_d = 10\%$). That is, on the basis of the particular values decided upon for m and M, only about 21 lots out of 100 of that quality will be accepted, because they have no defective counts and two or fewer marginally acceptable counts out of the 10 samples chosen from the lot. The other lots will all be rejected.

Probabilities associated with a collection of three-class plans are shown in Table 7–4 for various lot qualities. The scheme depends for acceptance or rejection not only on the proportion of defective material (p_d), but also on the proportion of marginally acceptable product (p_m). For example, a lot with 20% marginal quality and 10% defective quality with a sampling plan based on $n = 10$, $c = 2$ will, on average, be accepted 21% of the times it is put forward for testing. Using the identical plan $n = 10$, $c = 2$, a lot containing 0% defective material, but 40% marginal quality has a lower chance of acceptance (17%).

Table 7–4 gives only some examples of acceptance probabilities for selected combinations of proportions p_m and p_d. To gain an impression of the overall behavior of a three-class attributes plan, the complete operating characteristic function should be referred to.

Compared with two-class plans, the OC functions of three-class plans are more complex and more difficult to visualize since their values depend on combinations of two proportions, p_m and p_d, and not only on one. Because of these dependencies and the variety of lot qualities that can occur, for a three-class sampling plan, the OC function should be plotted as an OC surface, either in a three-dimensional graph, or as a contour map in the two-dimensional (p_m, p_d) area with contour lines for selected acceptance probabilities. Such OC function maps are shown in Figure 7–4 for three-class sampling plans with $n = 5$ and different acceptance numbers $c = 0$, $c = 1$, $c = 2$, and $c = 3$.

All lots with combinations of p_m and p_d lying on the same contour line in such a graph have the same probability of acceptance that is indicated at the end of the line. If, for instance, the three-class plan $n = 5$, $c = 1$ is applied, all kinds of lots with (p_m, p_d) combinations on the outermost line are accepted with a probability of only 0.1, or 10% of the times such a lot is examined. Thus, the three-class attributes scheme is affected to some extent by the frequency distribution of microorganisms within the batch, but the advantages of the scheme are its simplicity and general applicability, which make it appropriate to port-of-entry sampling.

However, there is a need to elaborate sound methods to set the values of m and M. These should be related to actual concentrations of microorganisms and the frequency distributions of analytical results. There are statistically based techniques for achieving this, although assumptions must be made. However, one example, based upon assumptions that can readily be checked and have been found (historically) to be reasonable,

Table 7–4 Three-Class Plans: Probabilities of Acceptance (P_a) of Lots Containing Indicated Proportions for Selected Numbers of Sample Units and *c* Values (p_d = percent defective, p_m = percent marginal)*

	p_m								
p_d	*10*	*20*	*30*	*40*	*50*	*60*	*70*	*80*	*90*
$n = 5, c = 3$									
50	0.03*	0.03	0.02	0.01	<†				
40	0.08	0.07	0.06	0.04	0.02	<			
30	0.17	0.16	0.15	0.12	0.07	0.03	<		
20	0.33	0.32	0.31	0.27	0.20	0.12	0.04	<	
10	0.59	0.58	0.56	0.52	0.43	0.32	0.18	0.06	<
5	0.77	0.77	0.75	0.69	0.60	0.47	0.31	0.14	0.02
0	1.00	0.99	0.97	0.91	0.81	0.66	0.47	0.26	0.08
$n = 5, c = 2$									
50	0.03	0.02	0.01	<					
40	0.08	0.06	0.04	0.02	<				
30	0.16	0.14	0.11	0.06	0.02	<			
20	0.32	0.29	0.24	0.16	0.09	0.03	0.01	<	
10	0.58	0.55	0.47	0.36	0.23	0.12	0.05	0.01	<
5	0.77	0.72	0.63	0.50	0.35	0.20	0.09	0.02	<
0	0.99	0.94	0.84	0.68	0.50	0.32	0.16	0.06	0.01
$n = 5, c = 1$									
50	0.02	0.01							
40	0.06	0.04	0.01	<					
30	0.14	0.09	0.05	0.02	<				
20	0.29	0.21	0.13	0.06	0.02	0.01	<		
10	0.53	0.41	0.27	0.16	0.07	0.03	0.01	<	
5	0.70	0.55	0.38	0.23	0.12	0.05	0.01	<	
0	0.92	0.74	0.53	0.34	0.19	0.09	0.03	0.0l	
$n = 10, c = 3$									
40	0.01								
30	0.03	0.02	0.01	<					
20	0.10	0.08	0.05	0.02	<				
10	0.34	0.29	0.20	0.10	0.03	0.01	<		
5	0.59	0.51	0.36	0.20	0.08	0.02	<		
0	0.99	0.88	0.65	0.38	0.17	0.05	0.01	<	
$n = 10, c = 2$									
30	0.02	0.01	<						
20	0.09	0.06	0.02	0.01	<				
10	0.32	0.21	0.10	0.04	0.01	<			
5	0.55	0.39	0.20	0.08	0.02	<			
0	0.93	0.68	0.38	0.17	0.05	0.01	<		
$n = 10, c = 1$									
30	0.02	<							
20	0.07	0.03	0.01	<					
10	0.24	0.11	0.04	0.01	<				
5	0.43	0.21	0.08	0.02	<				
0	0.74	0.38	0.15	0.05	0.01	<			

*Each of these blocks of numbers, relating P_a to p_m and p_d represents a three-dimensional relation called an OC surface, corresponding to the two-dimensional OC curve.

†< means $P_a < 0.005$.

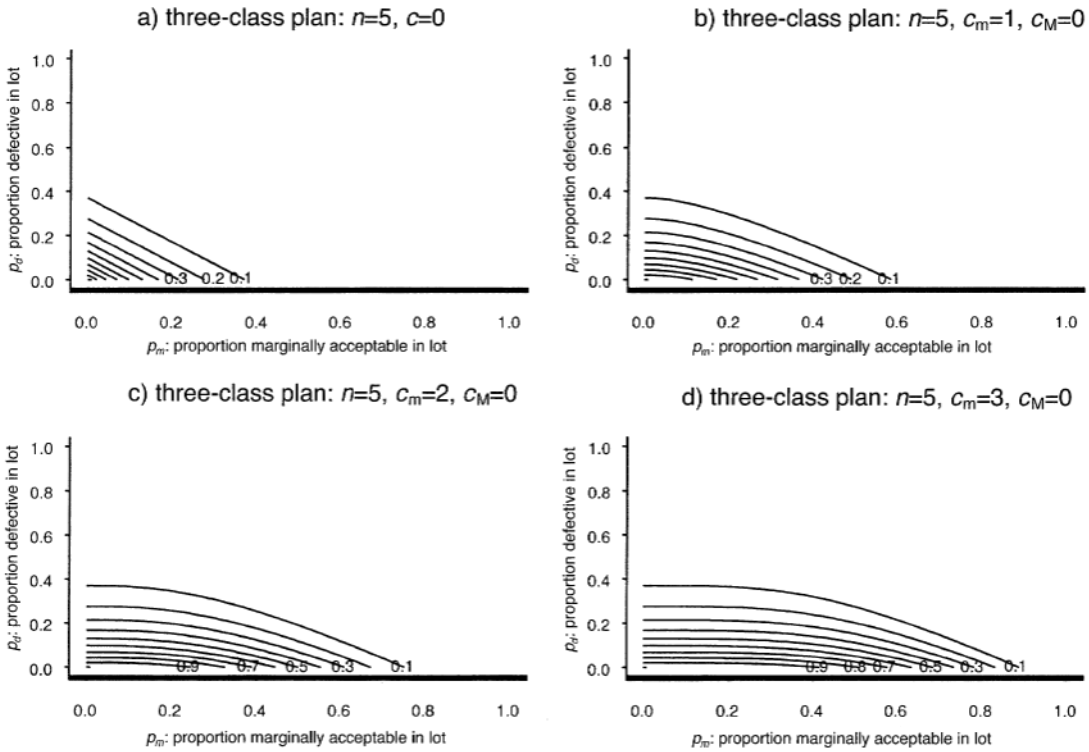


Figure 7–4 Contour maps of operating characteristic functions of three-class attributes plans for sample size $n = 5$ and different criteria of acceptance (c).
*Numbers within graphs (e.g., 0.1, 0.2, 0.3 in graph (a)) represent probability of acceptance.

is found in Dahms & Hildebrandt (1998), which is explained in more detail in section 7.4.4.

7.3 VARIABLES PLANS

Where the underlying distribution of microorganisms within the lot is known, or can be assumed, there is the option to use variables sampling plans. When properly applied, such plans can prove more useful under some conditions than the attributes plans for a particular purpose. Variables plans make full use of the microbial counts, rather than ascribing the counts to categories or ranges.

7.3.1 Identification

To define a variables plan, the underlying frequency distribution for the microorganisms of concern within the sample units in a lot must be known. There are many types of frequency distributions, and they differ in complexity. Some of the simpler distributions are symmetrical in shape and can be described by their mean and some measure of the distribution about that mean. The normal, or Gaussian, distribution is such an example. The normal distribution is defined by its mean value (which is also the median) for the

range of concentrations found, and a measure σ, the standard deviation, which defines the possibility of finding any other concentration in a unit. More complex distributions may not be symmetrical, so that the mean is unlikely to equal the median.

It is not the purpose of this book to describe the options available to define frequency distributions. What is essential to apply a variables plan is that the frequency distribution is known. To achieve this, data may be required to support the application of a particular distribution. The more complex the frequency distribution (i.e., more parameters are needed to define it), the more data are needed to gain confidence that it is an appropriate distribution to apply. An example of a distribution often observed for microorganisms in foods, the Log-Normal distribution, is used below (section 7.3.3). It is worth noting that the scale has been adjusted to obtain a normal distribution. This adjustment results in a symmetrical, two parameter (mean and standard deviation) distribution, achieved by using log(concentration) instead of concentration.

It is important to remember that any measurement of the parameters for a distribution is based upon a sample and is therefore an estimate of those parameters. These measured parameters must accommodate the uncertainty implicit in the measurement. The smaller the sample, the larger the likely error could be. The example given in section 7.3.3 illustrates this principle by allowing for sample size in its decision matrix.

7.3.2 Prescribing Confidence in Decisions

Making critical microbiological decisions about the safety or quality of a lot of food involves three steps. The first is to define the acceptable limits for the lot, the second is to specify the confidence with which we wish to identify acceptable and unacceptable lots, and the third is to choose the appropriate sampling plan. The following is an example of the way in which a variables plan may be designed. In this case, the decision rule is based upon an assumption that the underlying distribution of contaminants in the lot is log-normal (i.e., the log of the concentrations is normally distributed). While this assumption is often correct, in practice, its justification needs to be clear and recorded. Assuming a log-normal distribution, sampling plans based on the characteristics of this distribution can be used to develop acceptance sampling plans.

7.3.3 Operation

It is necessary to obtain and handle samples and sample units in the same way as for attributes plans. The log-transformation of the concentration measurements is used to compute the sample mean ($\bar{x}$) and standard deviation (s). These two values are then used to make the decision whether to accept or reject the lot. The lot is rejected if $\bar{x} + k_1 s > V$, where V is a log-concentration related to safety/quality limits. The value k_1 is obtained by reference to appropriate tables and is chosen to define the stringency of the plan for a given number of sample units, n.

Selection of k_1

Table 7–5 contains a range of k_1 values for sample unit numbers between 3–10. To choose k_1, it is necessary to decide on the maximum proportion p_d of the units in the lot that can be accepted with a concentration above the limit value, V. Having selected p_d, the desired probability P can be chosen, where P is the probability of rejecting a lot that contains at least a proportion p_d above V.

Table 7–5 k_1 Values Calculated Using the Non-Central *t*-Distribution–Safety/Quality Specification (Reject if $\bar{x} + k_1 s > V^*$)

Probability (p) of rejection	*Proportion (p_d) exceeding V*	*Number of sample units n*							
		3	*4*	*5*	*6*	*7*	*8*	*9*	*10*
0.95	0.05	7.7	5.1	4.2	3.7	3.4	3.2	3.0	2.9
	0.1	6.2	4.2	3.4	3.0	2.8	2.6	2.4	2.4
	0.3	3.3	2.3	1.9	1.6	1.5	1.4	1.3	1.3
0.90	0.1	4.3	3.2	2.7	2.5	2.3	2.2	2.1	2.1
	0.25	2.6	2.0	1.7	1.5	1.4	1.4	1.3	1.3

*$\bar{x}$ = sample mean; V = log-concentration related to safety/quality limits.

For example, if five sample units are analyzed per lot, then the k_1 value can be chosen from Table 7–5. If a lot in which 10% of sample units exceeded V is to be rejected with a probability of 0.95, then the k_1 value 3.4 would be used.

In practice, the two values p_d and P will be selected along with the value V. The scheme then allows n to be selected over the range 3–10. The larger n becomes, the lower the chance of rejection of an acceptable lot.

Selection of the limit value, V

The limit value V is selected by the microbiologist as the safety or quality limit, expressed as log-concentration. This value is likely to be numerically very similar to the value M used in the three-class attributes plans (section 7.2.2).

Table 7–6 gives the results for the aerobic plate count (APC) analyses of five sample units obtained from a lot of poultry. An appropriate variables sampling plan might be $P = 0.90$, $p_d = 0.25$, with a limit value of $V = 7$. The k_1 value, obtained from Table 7–5, is 1.7. Applying the formula $\bar{x} + k_1 s$ gives $5.039 + (1.7 \times 0.378)$, which equals 5.682. This value is less than the limit value of 7, and the lot is therefore accepted.

The use of variables plan for good manufacturing practice

Food producers often find it advantageous to specify a good manufacturing practice (GMP) standard. It may be possible to apply the variables plan under these circumstances, applying the formula outlined previously. The criterion is to accept the lot if $\bar{x} + k_2 s < v$. The k_2 value for the GMP plan is obtained from Table 7–7. The values P and p_d are selected as before and the appropriate k_2 value is obtained. The limit value, v, will be very similar numerically to the limit value, m, used in the three-class attribute plan.

For a more extensive treatment of this topic, see Kilsby (1982), Kilsby *et al.* (1979), and Malcolm (1984).

Table 7–6 An Example of Aerobic Plate Counts for a Sample of Poultry ($n = 5$)

APC	*$\log_{10}$ (APC)*	*mean log ($\bar{x}$)*	*standard deviation (s)*
40,000	4.602		
69,000	4.839		
81,000	4.909	5.039	0.378
200,000	5.301		
350,000	5.544		

Table 7–7 k_2 Values Calculated Using the Non-Central *t*-Distribution–GMP Limit (Accept if $\bar{x} + k_2 s < v^{\dagger}$)

Probability (P) of acceptance	*Proportion (p_d) exceeding v*	*Number of sample units n*							
		3	*4*	*5*	*6*	*7*	*8*	*9*	*10*
0.90	0.05	0.84	0.92	0.98	1.03	1.07	1.10	1.12	1.15
	0.10	0.53	0.62	0.68	0.72	0.75	0.78	0.81	0.83
	0.20	0.11	0.21	0.27	0.32	0.35	0.38	0.41	0.43
	0.30	0.26*	0.13*	0.05*	0.01	0.04	0.07	0.10	0.12
	0.40	0.65*	0.46*	0.36*	0.30*	0.25*	0.21*	0.17*	0.16*
	0.50	1.09*	0.82*	0.69*	0.60*	0.54*	0.50*	0.47*	0.44*
0.75	0.01	1.87	1.90	1.92	1.94	1.96	1.98	2.00	2.01
	0.05	1.25	1.28	1.31	1.33	1.34	1.36	1.37	1.38
	0.10	0.91	0.94	0.97	0.99	1.01	1.02	1.03	1.04
	0.25	0.31	0.35	0.38	0.41	0.42	0.44	0.45	0.46
	0.50	0.47*	0.38*	0.33*	0.30*	0.27*	0.25*	0.24*	0.22*

*Negative values
$^{\dagger}\bar{x}$ = sample mean; v = log-concentration limit value

7.4 COMPARISON OF SAMPLING PLANS

7.4.1 General Remarks

The decision for, or the design of, a suitable sampling plan depends on the given purpose, i.e., on the sampling material, the type of microbiological result being assessed, and on available prior information on production processes and frequency distributions of sampling results in lots. In the following paragraphs, some statistical aspects of the choice of a sampling plan will be discussed comparing two-class with three-class attributes plans and three-class attributes plans with variables plans.

Only when the result of a microbiological analysis is given as a count, or in another quantitative manner, is there a choice between types of sampling plans. For mere qualitative results (presence-absence tests), only two-class plans are applicable.

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. Is a typical distribution/shape expected to occur? Is the production process known and well documented? Especially for the design of variables sampling plans, some knowledge and data concerning the production processes and variations in distributions that may occur between lots is required. Because variables plans are based on the assumption that log-transformed sampling results follow a normal distribution, they should only be used when this assumption can be justified. For such situations, the performance of attributes and variables plans can be compared.

The following considerations are restricted to such a situation. Sampling plans will be compared by means of their OC function, which is calculated and plotted for various scenarios. Because lot distributions are assumed to be of the normal type (after log-transformation), lot quality is described by the mean concentration of microbes for all units in the lot, μ, and the standard deviation, σ, as a measure of variation. Therefore, acceptance probabilities are calculated for lots with varying μ and σ. With σ held fixed, the OC curve

of a sampling plan can be plotted as a function of varying mean concentrations μ for all three types of sampling plans.

7.4.2 Determining the Concentration of Microorganisms Controlled by Attributes Sampling Plans

As outlined in Chapter 3, to compare the equivalence of different control measures, it is necessary to be able to relate their performance in terms of achieving an FSO. In other words, the performance of each control measure needs to be expressed as the resultant frequency or concentration of a microbiological hazard in a food. This is essential if the use of microbiological criteria is to be validated as a control measure to achieve a given FSO through controlling the initial level of a hazard (see Chapter 3, Section 3.5.1, Example 5).

The method for relating the performance of attributes plans to concentration is to use the frequency distribution of analytical results in sample units to establish the proportion of defective samples, as proposed by Hildebrandt *et al.* (1995). A normal distribution for the log concentration of microbes is assumed, and the area under the normal density function below m is used to define the value for the proportion acceptable. The area between m and M defines the value for proportion marginally acceptable (p_m), and the area above M (or m for a two-class plan) defines the value for proportion defective (p_d). The mathematics involved in calculating the three proportions for a given mean log concentration are detailed in Legan *et al.* (2001).

OC curves expressed in terms of mean concentration are developed by fixing the standard deviation σ, and then increasing the mean of a normal distribution through a range of values. Figure 7–5 illustrates this for a two-class plan with $n = 5$, $c = 0$, $m = 1.0$ log cfu g^{-1}. A distribution with $\sigma = 0.8$ and three different means is shown in Figure 7–5a. The σ value of 0.8 is chosen based on published concentrations of mesophilic *Clostridium* spores in raw pork, beef, and chicken (Greenberg *et al.*, 1966) and similar observations in other food materials. All parts of the distribution above m in each position are defective. The proportion defective in the distribution in each position (or in a position defined by any other mean) is plotted against mean log count to show how the proportion defective increases with mean log count (Figure 7–5b). Finally, the operating characteristic curve for the specified plan is used to determine the probability of acceptance from the proportion defective at each mean log count. This probability of acceptance is plotted against mean concentration (Figure 7–5c).

The protection each sampling plan gives can then be expressed in terms of the mean concentration of microorganisms associated with a defined probability of accepting the sampled material. In Chapter 8, this approach will be used to compare the concentration of microorganisms controlled under different cases.

7.4.3 Comparison of Two-Class and Three-Class Attributes Plans

Table 7–8 compares the operating characteristics of the two types of attributes plans recommended in this text, on the basis of equal sample sizes n, acceptance numbers c, and lot qualities. To facilitate comparison, lot quality is measured as the proportion of the lot worse than level m and, correspondingly, the same value of c is taken for the two-class plan as the c of marginally acceptable quality for the three-class plan.

The two-class plans do not distinguish values between m and M from those above M. If not more than c sample units give results above m, the lot is acceptable, regardless of

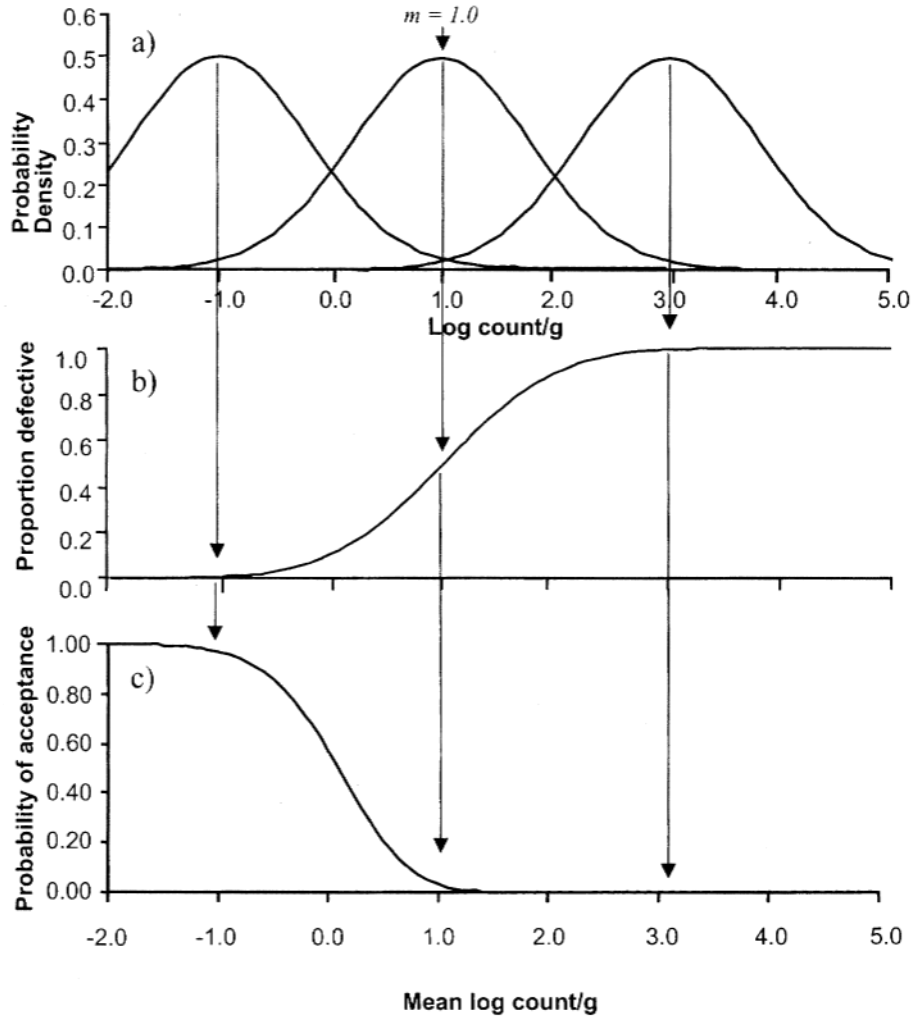


Figure 7–5 OC curves expressed in terms of mean concentration.

how far individual results exceed m. But the corresponding three-class plan does make a distinction, by including an extra subdivision of lot quality since the limit M separates marginally acceptable from defective quality.

By comparing OC surfaces for three-class plans with a fixed number of sample units n, but with varying values for c (Figure 7–4), it becomes obvious that the surface heights change mainly in the p_m-direction, i.e., for varying proportions of marginally acceptable quality in the lot. The reason is that the number of sample units that is allowed to exceed M remains constant at 0. In fact a three-class plan might be interpreted as a mixture of two two-class plans: a two-class plan (n, c) referring to the limit m, and a two-class plan $(n, 0)$ referring to the limit M. In extreme situations, one of these two-class plans can be dominating the decision process. Generally, however, the actual performance of a three-class plan depends on the variety of combinations of p_m and p_d that are likely to occur in practice.

Table 7–8 Probabilities of Acceptance (P_a) for Two- and Three-Class Attributes Sampling Plans When the Lot Examined Contains 5%, 20%, or 50% of Sample Units > *m*

Sampling plan		5% > *m*			20% > *m*				50% > *m*			
		Two-class	Three-class plan		Two-class	Three-class plan			Two-class	Three-class plan		
n	*c*	plan	0% > *M**	5% > *M*	plan	0% > *M*	10% > *M*	20% > *M*	plan	0% > *M*	25% > *M*	50% > *M*
5	0	0.77	0.77	0.77	0.33	0.33	0.33	0.33	0.03	0.03	0.03	0.03
5	1	0.98	0.98	0.77	0.74	0.74	0.53	0.33	0.19	0.19	0.11	0.03
5	2	1.00	1.00	0.77	0.94	0.90	0.58	0.33	0.50	0.50	0.19	0.03
5	3	1.00	1.00	0.77	0.99	0.99	0.59	0.33	0.81	0.81	0.23	0.03
10	0	0.60	0.60	0.60	0.11	0.11	0.11	0.11	<	<	<	<
10	1	0.91	0.91	0.60	0.38	0.38	0.24	0.11	0.01	0.01	0.01	<
10	2	0.99	0.99	0.60	0.68	0.68	0.32	0.11	0.05	0.05	0.02	<
10	3	1.00	1.00	0.60	0.88	0.88	0.34	0.11	0.17	0.17	0.03	<
15	0	0.46	0.46	0.46	0.04	0.04	0.04	0.04	<	<	<	<
20	0	0.36	0.36	0.36	0.01	0.01	0.01	0.01	<	<	<	<
60	0	0.05	0.05	0.05	<	<	<	<	<	<	<	<

*Note that any % > *M* is included in the % > *m*; e.g., for the case where 20% > *m* and 10% > *M*, it follows that 10% is between *m* and *M*.
< means $P_a < 0.005$.

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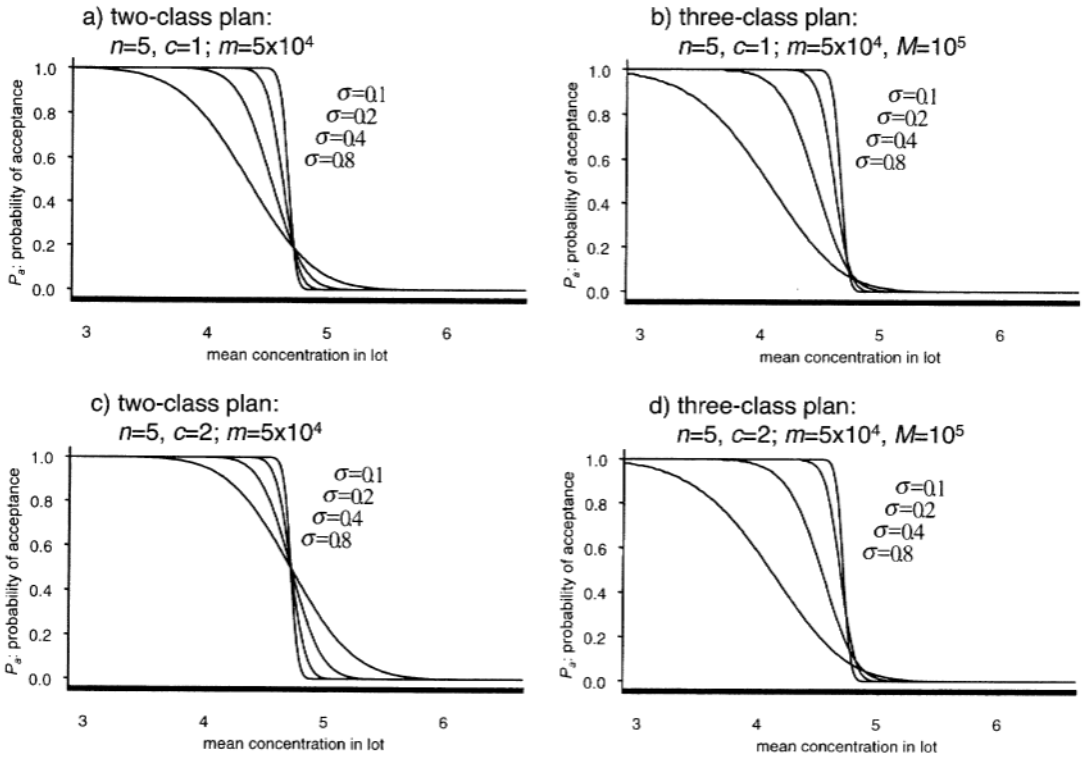


Figure 7–6 Comparison of two- and three-class attributes plan with different criteria of acceptance (c) when distribution of log-transformed analytical results is assumed to be normal.

Hildebrandt *et al.* (1995) studied the performance aspects of two-class and three-class sampling plans in the case that log-normal lot distributions can be assumed by comparing the two-class plan $n = 5$, $c = 1$; $m = 5 \times 10^4$ cfu ml^{-1} with the three-class plan $n = 5$, $c = 1$; $m = 5 \times 10^4$, $M = 10^5$ cfu ml^{-1}.

Four different types of lots characterized by lot standard deviations of $\sigma = 0.1$, $\sigma = 0.2$, $\sigma = 0.4$, and $\sigma = 0.8$ were considered to study the impact of the standard deviation of the lot in relation to the distance between m and M on the operation of the three-class sampling plan. In Figure 7–6(a), the OC curves for the two-class plan are shown; Figure 7–6(b) contains the OC curves for the three-class plan. The OC function values are calculated by deriving the proportions of marginal and defective quality, p_m and p_d, resulting from the various possible combinations of μ and σ with given microbiological limits $m = 5 \times 10^4$ and $M = 10^5$.

As can be seen for lot distributions with a low standard deviation ($\sigma = 0.1$) in relation to the distance between m and M, there is hardly any difference in performance between the two-class and the three-class sampling plans. As the within-lot standard deviation is increased in relation to the distance between m and M, the OC difference becomes larger, showing reduced acceptance probabilities when the three-class plan is applied.

If the number of acceptable units in the sample, c, is changed from $c = 1$ to $c = 2$, the effect on the operation of two-class plans is much more obvious than the effect on three-class plans (Figures 7–6(c) and 7–6(d)), especially for lots with higher standard devia-

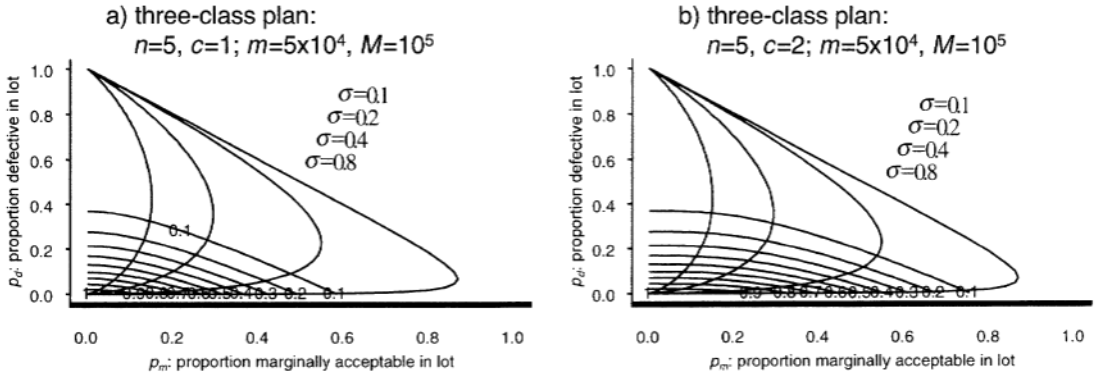


Figure 7–7 Contour maps for three-class attributes plan and possible combinations of proportion marginally acceptable (p_m) and defective (p_d) in lot, for various standard deviations (σ) when distribution of analytical results is assumed to be normal.

tion. This example demonstrates the dominance of the $c_M = 0$ rule (the null-tolerance concerning M, incorporated in three-class plans when lots are examined that are characterized by high standard deviation in relation to the distance between m and M).

A plot of the possible combinations of p_m and p_d in a given sampling situation together with the contour lines of the three-class plan serves to summarize these points (Figure 7–7). When lots are homogenous (standard deviation is low in relation to the distance between m and M, or m and M are different in relation to lot standard deviations likely to occur), the three-class plan (n, c) will operate like a simple two-class plan with (n, c) accordingly. When lots are heterogeneous (standard deviation is high in relation to the distance between m and M, or the distance from m to M is narrow in relation to lot standard deviations likely to occur), the three-class plan will operate like a two-class plan with (n, $c_M = 0$). Hence the performance of three-class plans depends not only on the combination of n and c, but on m and M and their distance in relation to lot heterogeneity.

7.4.4 Construction of Three-Class Plans Using Previous Information

The considerations in the preceding section started with given microbiological limits m and M without questioning the reasons for their selection. Often, however, these limits are described as the maximum level of target organisms m acceptable under conditions of good manufacturing practice (GMP) and the limit of target organisms M, that, if exceeded, is considered unacceptable, i.e., defective.

This reference to GMP conditions and to empirical studies describing frequency distributions for quantitative analytical results that are achievable under these conditions implies that the design of sampling plans could be based on knowledge about production technologies leading to values for m and M that take into account the maximum mean concentration of contaminants, as well as the maximum extent of heterogeneity, under conditions of GMP.

As an example, guidance for defining the microbiological limits with regard to GMP conditions has been given by Dahms & Hildebrandt (1998). They addressed the problem that there might be an unduly high probability of rejecting a lot still meeting GMP conditions if the distance between m and M is too narrow in relation to the acceptable heterogeneity. Especially with regard to nonpathogenic microorganisms such as total bacte-

rial count or indicators of hygiene that represent no health risk for the consumer, it is questionable whether to reject a lot meeting GMP requirements solely because of a single sampling result exceeding M. In this context, the difference $(M - m)$ should be chosen such that lots characterized by marginal concentrations of microorganisms and by acceptable or even unavoidable heterogeneity run only a minor, and known, risk of rejection due to a single sample lying above M.

Based on the assumption that sampling results (after log-transformation) follow a normal distribution, an *indifferent lot* for the two-class sampling plan $n = 5$, $c = 2$ is first considered. The term indifferent lot indicates that there is an equal probability of 0.5 to accept the lot or to reject it. The definition of this two-class sampling plan implies that an indifferent lot is characterized by a mean concentration of microorganisms just at the limit m: $\mu = m$, i.e., just at the maximum acceptable mean concentration under conditions of GMP. As soon as lot mean concentrations exceed this limit, the probability of rejecting the lot will be greater than the probability of accepting it. Therefore, the hypothesis tested with this two-class plan is one concerning the mean concentration of contaminants of the lot being examined.

Application of a three-class plan to the same lot with $n = 5$, $c = 2$, i.e., the introduction of a second limit M and the requirement $c_M = 0$, can lead to a reduction of acceptance probabilities. However, whether the resulting difference between using the two-class and the three-class plans is relevant or not depends on the distance between m and M in relation to lot heterogeneity (section 7.4.3). With regard to these relationships, it is proposed to define the additional risk of rejecting an indifferent lot with a given, acceptable, heterogeneity (standard deviation) as a first step, i.e., to define the required reduction of acceptance probability for a lot with marginal mean concentration of contaminants $\mu = m$ and marginal spread σ. As a second step, a value for the upper limit M should be chosen that meets this requirement.

For the situation in which previous information indicates that log-transformed sampling results follow a normal distribution with a known standard deviation σ that is achievable under GMP conditions, Dahms & Hildebrandt (1998) derived a formula to calculate the required distance between m and M:

$$M - m = u_{1-p_d^*}\sigma$$

Here $u_{1-p_d^*}$ is the $(1-p_d^*)$-quantile of the standard normal distribution, with p_d^* being the marginal acceptable proportion of defectives exceeding M. This value can be calculated as:

$$p_d^* = \frac{5}{8} - \sqrt{\frac{25}{64} - \frac{4}{5} \cdot a}$$

for $n = 5$, $c = 2$ and the additional risk to reject an indifferent lot given as a. Table 7–9 lists some values for the distance between m and M for different combinations of a and σ.

These considerations illustrate how to select M, once m has been set. This procedure is mainly oriented toward the design of sampling plans for nonpathogenic microorganisms such as indicators of hygiene. However, the relationship between M and m could be used in a similar way for pathogens. One would simply start by first setting M with regard to safety, and choosing m accordingly.

Following a procedure like this, a three-class sampling plan can be constructed to meet defined requirements concerning its stringency in comparison with the equivalent two-

Table 7–9 Distances between m and M for Given Additional Risk a To Reject Indifferent Lots with Acceptable Heterogeneity (Standard Deviation) σ for a Three-Class Sampling Plan with $n = 5$, $c = 2$

			$M - m$		
a	p_d^*	$u_{1-p_d^*}$	$\sigma = 0.2$	$\sigma = 0.4$	$\sigma = 0.8$
0.01 (1%)	0.006	2.51	0.502	1.004	2.008
0.05 (5%)	0.033	1.84	0.368	0.736	1.472
0.10 (10%)	0.068	1.49	0.298	0.596	1.192

class plan. A characteristic of the three-class plan is the statement of two hypotheses that are implicitly tested when this sampling plan is applied—one concerning the marginal mean concentration of contaminants of an acceptable lot as m, and the other concerning the marginal spread that is acceptable by fixing the distance between m and M.

7.4.5 Comparison of Three-Class Attributes and Variables Plans

The three-class plan is a simple plan to apply. It relies upon ascribing a concentration measurement to one of three broad concentration bands. To achieve this simplicity, the plan sacrifices discrimination. For example, if m is 1,000 and M is 10,000, then the three-class attributes plan assigns the same level of concern to 1,001 and 9,999 contaminants in a sample unit. Also, it assigns a totally different level of concern to 999 and 1,000 contaminants. The variables plan described above has the advantage that it has high discrimination between individual concentration measurements. On the other hand, the variables plan is mathematically more complex to operate and to understand, and its performance depends upon the validity of the assumptions made about the frequency distribution.

Taking the three-class plan $n = 5$, $c = 2$; $m = 5 \times 10^4$, $M = 10^5$ as an example, the operation characteristic of this attributes sampling plan can be compared with that of a variables sampling plan with $n = 5$, $V = 10^5 = M$, $p_d = 0.05$, and $P = 0.95$. Figure 7–8 shows the OC curves for the variables sampling plans when the lot standard deviation σ is assumed to be known in comparison with the OC curves for the three-class sampling plan already discussed under section 7.4.3. It should be noted that the decision rule of the variables plan is unable to respond if the actual standard deviation is larger than the assumed σ, whereas the decision rule of the three-class plan remains the same, responding to higher measured concentrations. In this comparison, we can see that for homogenous lots, both plans have a high stringency in discriminating between lots of acceptable and unacceptable quality. However, whereas the three-class plan discriminates at the marginal lot mean concentration m, the variables plan discriminates at mean concentrations nearer to $V = M$, as this is the starting point to define the marginal mean contamination $V - k \times \sigma$ that is relevant for this type of plan.

With increasing lot standard deviation, the slopes of both OC curves become less steep, this effect being stronger for the three-class plan. The variables plan remains more stringent, i.e., acceptance probabilities for lots of acceptable quality remain quite high, whereas they fall more rapidly than those of the three-class plan as soon as lot quality changes from acceptable to unacceptable.

Closer operation comparisons of these types of sampling plans can be achieved if previous information is used in the design of the three-class plan, especially in choosing the appropriate distance between m and M. However, it is important to realize that these two

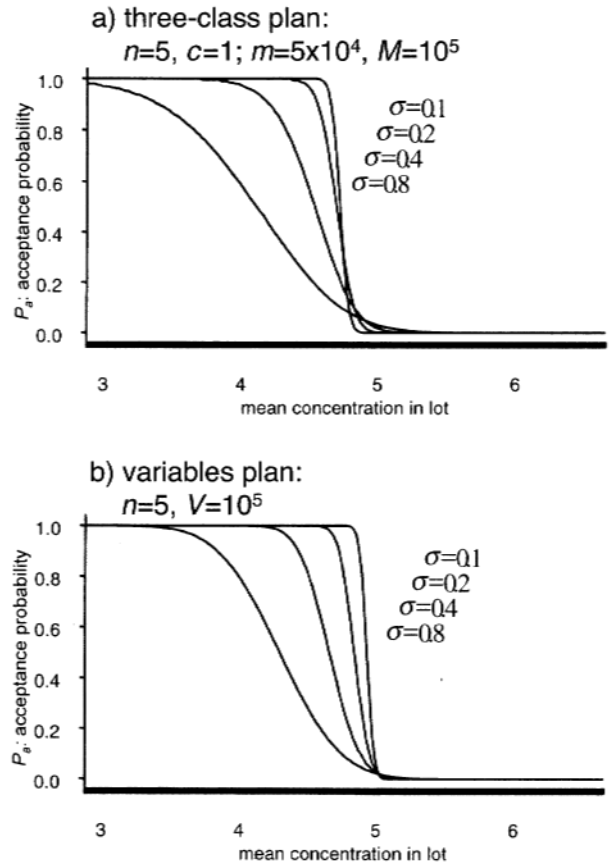


Figure 7–8 Comparison of three-class attributes plan and variables plan with standard deviation of concentrations in lot assumed to be known.

approaches are fundamentally different. Because the bases for decision making are fundamentally different, the two approaches should not be expected to be generally equivalent. The attributes schemes were designed for situations in which no assumptions about underlying distributions could be made (e.g., port-of-entry), and they will perform well under those circumstances against their set parameters. When it is possible to have high confidence in underlying frequency distributions (e.g., in food manufacturing units), alternative approaches may allow for different sets of assumptions and more cost-effective sampling plans related to these assumptions. Therefore, choice of sampling plan should depend upon knowledge of the lot and its intended use.

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Chapter 8

Selection of Cases and Attributes Plans

8.1 Introduction
8.2 Microbial Criteria: Utility, Indicator, and Pathogens
8.3 Factors Affecting the Risk Associated with Pathogens
8.4 Categorizing Microbial Hazards According to Risk
8.5 Definition of Cases
8.6 Deciding between Two-Class and Three-Class Attributes Sampling Plans
8.7 Determining Values for *m* and *M*
8.8 Specific Knowledge about the Lot
8.9 What Is a Satisfactory Probability of Acceptance?
8.10 Selecting *n* and *c*
8.11 Sampling Plan Performance of the Cases
8.12 References

8.1 INTRODUCTION

While the overall philosophy of this book is to control microbial hazards through raw material selection, Good Hygienic Practices (GHP) and Hazard Analysis Critical Control Point (HACCP), and not to rely on microbiological testing, there are occasions when testing might be considered. If it is concluded that testing is appropriate, this chapter provides guidance on the choice of sampling plan and discusses their limitations. The recommended sampling plans are based on statistical considerations in Chapters 6 and 7, the severity of the hazard, and the potential for change in risk (decrease, no change, or increase) before a food is consumed. The International Commission on Microbiological Specifications for Foods (ICMSF) recommended 15 cases that reflect different levels of risk (ICMSF, 1974, 1986). The greater the risk, the higher the case number, the more stringent the sampling plan (see section 8.5 and Table 8–1).

The principles for establishing microbiological criteria were described in Chapter 5. Microbiological criteria can be used to assess:

- the safety of food;
- adherence to GHP;
- the utility (suitability) of a food or ingredient for a particular purpose;
- the keeping quality (shelf-life) of certain perishable foods; and/or
- the acceptability of a food or ingredient from another country or region where the conditions of production are unknown or uncertain.

Chapter 5, however, did not describe how to choose a sampling plan, an essential component of all microbiological criteria. Due to the importance and complexity of choosing a sampling plan, Chapter 6 discussed the concept of probability and factors to consider

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when collecting representative samples from a lot or consignment of food. In Chapter 7, two basic sampling plans (two-class and three-class attributes plans) were described. The choice of sampling plan should take into account a number of factors, including the risk to public health associated with each hazard and susceptibility of the target group of consumers. This chapter incorporates information from the previous chapters and provides a scheme that can be used in deciding on a sampling plan that is based on risk.

The stringency of sampling plans for foods should be based on the hazard to the consumer from pathogenic microorganisms and their toxins or toxic metabolites, or on the potential for quality deterioration to an unacceptable state. Plans should also take account of the types of microorganisms present and their numbers. Some microorganisms merely spoil a food, others can cause illness, and still others are taken to indicate the likelihood of contamination by pathogens. Some pathogens cause mild illnesses that seldom spread; others cause mild illnesses that spread rapidly; and yet others cause severe illness. The degree of foodborne hazard is often increased if the organism has grown to high numbers in the food and, conversely, is usually reduced if the number is reduced. In some cases, the food merely acts as a vehicle for transmission of the infectious microorganism. Treatment in the normal course of distribution, storage, and preparation for consumption may decrease, leave unchanged, or increase numbers of microorganisms, while labile toxins would decompose and stable toxins remain.

The choice of sampling plan for microbiological criteria should first reflect the purpose. Is the microbiological criterion intended to assess the general quality and acceptability of a food (i.e., utility) or is it intended to assess microbiological safety either indirectly (i.e., indicator) or directly (i.e., pathogen, toxin, toxic metabolite)?

8.2 MICROBIAL CRITERIA: UTILITY, INDICATOR, AND PATHOGENS

8.2.1 Utility Tests

Some microbiological tests provide information regarding general contamination, incipient spoilage, or reduced shelf-life. Evidence should support the use of a utility test for the intended purpose. For example, evidence should support the use of a total aerobic count as a measure of incipient spoilage. Such tests may be useful indicators of product quality. However, utility tests are not related to health hazards, but rather to economic and aesthetic considerations; therefore, the level of concern is low. Utility tests are included in cases 1–3 (see Table 8–3) and satisfied by relatively lenient sampling plans. They may involve direct microscopic counts, yeast and mold counts, aerobic plate counts, or specialized tests, such as for cold tolerant organisms or for species causing a particular type of spoilage (e.g., lactobacilli in mayonnaise or thermophilic spore-formers in sugar).

8.2.2 Indicator Tests

Microorganisms that are not normally harmful but may indicate the presence of pathogenic microorganisms may be used as indirect indicators of a health hazard. For example, for dried egg products, Enterobacteriaceae can be used as indicators of the presence of salmonellae. In these products, any practically applicable sampling plan cannot detect the low level of salmonellae. It is important to recognize that relationships between pathogen and indicators are not universal and are influenced by the product and process.

Care must be taken when selecting indicator organisms. For instance, "coliform counts" have been widely used as universal indicators of hygiene, but in many products, psychrotrophic Enterobacteriaceae will inevitably be present, and the apparently high coliform counts do not necessarily indicate hygienic failure or consumer risk. Microorganisms naturally present in the product may also interfere with the analysis and result in meaningless counts. For instance, aeromonads are often detected by coliform count procedures, rendering counts of coliforms meaningless for many seafood products where aeromonads are common members of the microflora.

Indicator organisms may be useful in other situations, e.g., when assessing efficiency of cleaning and disinfection or in investigational sampling. A food plant laboratory may prefer not to test for a specific pathogen (salmonellae or *Listeria monocytogenes*) because culturing those organisms in the laboratory could increase the risk of the organism being introduced in the food processing environment. Therefore, a generic test, e.g. for *Listeria*-like organisms, may be considered safer because the moist, cold conditions known to favor the dominance of *L. monocytogenes* will also favor generic, nonpathogenic strains of *Listeria*.

An inspector at a port-of-entry may know very little about the history of a consignment of food, e.g., whether a heat process adequate to kill relevant microorganisms was used in processing, whether the food was contaminated after processing, or whether the consignment was temperature-abused during shipment. Tests for relevant microorganisms can indicate whether certain foods have been underprocessed. For instance, high numbers of mesophilic spore-forming bacteria in low-acid, shelf-stable canned foods indicate probable underprocessing when it is certain the container is not leaking. The presence of Enterobacteriaceae or coliforms in some properly pasteurized foods indicates recontamination after heat processing. *Escherichia coli* in water indicates recent fecal contamination, and *Staphylococcus aureus* in cooked foods can indicate contamination from the human skin or nose. Because of the uncertain relationship between indicators and specific pathogens, the level of concern is moderate and it is inappropriate to apply sampling plans with high stringency for indicator microorganisms.

8.2.3 Pathogen Tests

There are occasions when testing for a pathogen may help to ensure food safety. Using the decision tree in Figure 8–1 will assist in deciding whether testing will be useful. Tests for specific pathogens can be applied:

- in routine sampling when experience indicates that testing is an effective means of consumer protection;
- for verification of GHP/HACCP systems when a suitable indicator microorganism is not available; and
- in investigational sampling when the epidemiology of a foodborne disease outbreak points to a particular lot of food as the cause of illness, or when there are other circumstances creating suspicion of the presence of a pathogen or toxic metabolite.

8.3 FACTORS AFFECTING THE RISK ASSOCIATED WITH PATHOGENS

Microbiological criteria and sampling plans should reflect the severity of the disease and be appropriate for the food. Certain well-known food-pathogen combinations have

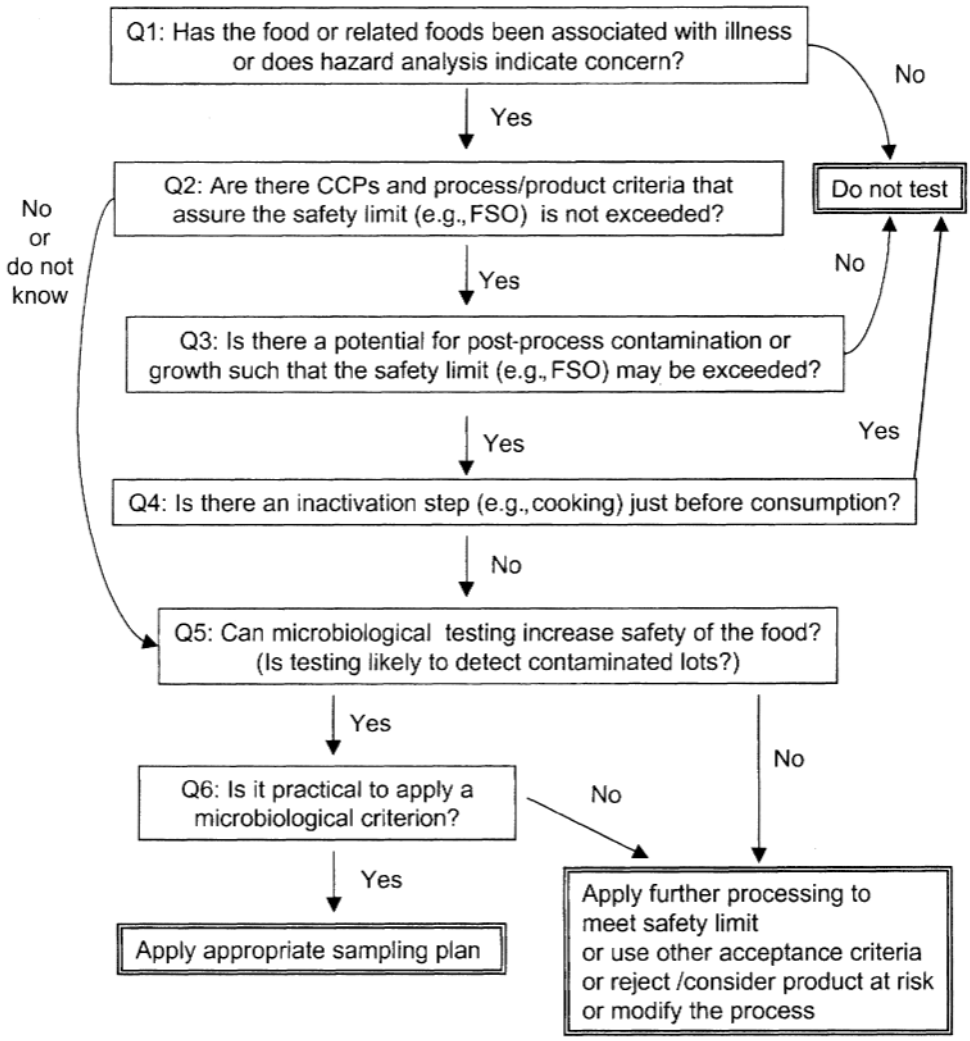


Figure 8–1 Decision tree to help decide when sampling and testing for foodborne pathogens can be used as lot acceptance criteria.

become recognized. Some understanding of the conditions that determine whether a food is likely to contain pathogens or their toxic metabolites is necessary. Frequently, there are strong regional and cultural influences on these associations.

8.3.1 Epidemiologic Considerations

Water and some types of seafood have been shown to be common vehicles in outbreaks of typhoid, cholera, and hepatitis A infection. Meat and poultry are commonly identified as vehicles in outbreaks of salmonellosis. Ham and cream-filled pastries are frequently implicated in outbreaks of staphylococcal foodborne illness. Outbreaks of gastroenteritis

caused by *Vibrio parahaemolyticus* are usually associated with seafood. Cooked meat and cooked poultry or stews and gravy that have been subjected to time-temperature abuse after cooking are the usual vehicles in outbreaks of enteritis caused by *Clostridium perfringens*. Botulism is a rare disease usually associated with the ingestion of inadequately processed home-preserved foods, particularly cured pork products, fermented fish, fish eggs or marine mammals, and low-acid foods, including vegetables. Histamine poisoning, which is rarely a serious disease, is typically associated with scombroid fish species. Raw milk is commonly identified as the vehicle of campylobacteriosis, brucellosis, salmonellosis, and more recently enterohemorrhagic *E. coli* infection. In addition, cheese made from raw milk has been the cause of brucellosis, staphylococcal intoxication, bloody diarrhea, and hemolytic uremic syndrome caused by enterohemorrhagic *E. coli*.

The association between gastroenteritis caused by *Bacillus cereus* and temperature-abused cooked rice is well established. Undercooked ground beef is a vehicle of enterohemorrhagic *E. coli* O157:H7 infections, although recent outbreaks have been associated with fresh produce, fermented meat products, contaminated water, and unpasteurized dairy products. Produce such as raspberries has been associated with outbreaks of cyclosporiasis, whereas contaminated water has been the principal vehicle of cryptosporidiosis.

8.3.2 Ecological Features

The primary sources of foodborne microbial pathogens include a variety of animal, human, and environmental reservoirs. After contamination of food, behavior of the pathogens is influenced by the food composition, the presence of other microflora, and the environmental conditions of the food.

Many of the pathogens that affect humans are widely distributed in the agricultural environment, for example, *Salmonella* spp., *Campylobacter* spp., *L. monocytogenes*, *Yersinia enterocolitica*, pathogenic *E. coli*, *C. perfringens,* and *S. aureus*. Although foodborne disease has long been primarily associated with animal products (e.g., meat, seafood, and dairy products), in recent years many large outbreaks have been traced to produce, including lettuce, sprouts, and raspberries (Beuchat, 1996). Humans are also a reservoir of certain foodborne pathogens, some of which may persist for weeks or months in the carrier state, for example, *S. typhi*, *Shigella* spp., and hepatitis A and Small Round Structured Viruses (SRSV) such as Norwalk-like viruses (ACMSF, 1995).

Particular products present greater risk than others due to possible contamination during production and harvest, their intrinsic properties that affect microbial growth and survival, traditional preparation and handling practices specific to that food, and, often, the absence of a critical control point that will eliminate the hazard. For example, foods consumed raw, such as oysters, present high risks to susceptible consumers since they may be contaminated with SRSVs or *V. vulnificus* at harvest. Ready-to-eat foods may be recontaminated with *L. monocytogenes* that may grow during subsequent refrigeration unless there is a competitive anti-listerial microflora in the product.

Local customs and standards of community hygiene, especially those related to food, water supply, and sanitation, are important determinants of the extent and variety of foodborne illnesses. Effectiveness of prevailing standards for safeguarding water supplies, milk supplies, and shellfish harvesting areas warrants consideration. Control of food processing; detecting, recalling, or condemning contaminated foods; vermin control; public health supervision of food service establishments; and appropriate use of

refrigeration in processing plants, food service establishments, and homes all play a role in reducing the incidence of foodborne illness and influence the selection of sampling plans for particular commodities from particular sources.

Dietary customs specific to a region also influence the foodborne hazards. For example, the Japanese custom of eating raw fish has contributed to the relatively high incidence of *V. parahaemolyticus* infection in that country. Similarly, the various fermented traditional marine foods consumed among the Inuits in Alaska and Canada are likely to contribute to incidences of type E botulism in these regions.

Members of the *Aeromonas hydrophila* group occur in raw fish, raw meats, and other foods. Although high counts of *A. hydrophila* can occur in patients with various types of diarrhea, its role as a cause of diarrhea remains in dispute. *Plesiomonas shigelloides* can be isolated from water and raw aquatic products. High numbers of *P. shigelloides* have occasionally been demonstrated in patients with diarrhea, but its role in foodborne or waterborne illness remains in dispute.

8.3.3 Clinical Features

Certain foodborne microorganisms are inherently associated with severe illnesses in humans. *C. botulinum* types A, B, E, and F, for instance, can produce toxins that cause neurological illness in healthy people, even when very small amounts are ingested. If not effectively treated with antitoxins and provided with respiratory support, the case fatality rate may exceed 50%. Virulence properties of *S. typhi*, *S. dysenteriae* I, *V. cholerae*, certain strains of *S. typhimurium*, *E. coli* O157:H7, and *C. perfringens* type C enable these pathogens to cause severe disease, even death. Cholera often presents a medical emergency in the developing world because 50–70% of dehydrated cholera patients who are malnourished die unless they are appropriately treated by oral or intravenous fluid and electrolyte replacement. *L. monocytogenes*, causing listeriosis, mainly affects susceptible, typically immunocompromised, populations; however, among these patients, the mortality can be as high as 25%. People with underlying chronic disease, in particular males with a history of high alcohol consumption, are prone to infection by *V. vulnificus* that is associated with iron overload in the patient.

Initially, pathogenic *E. coli* were considered to be strains of specific O serogroups causing diarrhea, mainly in infants, and referred to as the "classical" enteropathogenic *E. coli*. However, several other types of *E. coli* have been recognized and are concerns of today's food industry. Enterotoxigenic *E. coli* is a major cause of infantile diarrhea in developing countries and a leading cause of traveler's diarrhea. Enteroinvasive *E. coli* closely resemble *Shigella* in pathogenicity and antigenicity. Enterohemorrhagic *E. coli* (EHEC), such as *E. coli* O157:H7, were first identified as pathogens in 1982 and produce Shiga-like toxins. Food-associated outbreaks attributed to *E. coli* O157:H7 have been well documented and are of great concern because the infective dose is low (<100 cells) and the illness is severe, sometimes leading to kidney failure and death, especially in young children and the elderly.

Low doses (10–100 cells) of *S. dysenteriae* can cause shigellosis. The infective dose of other *Shigella* spp., *V. cholerae*, and some salmonellae may also be low in highly susceptible individuals, such as infants, and malnourished and immunocompromised persons. Also, the severity of enteritis caused by salmonellae, *Shigella* spp., and *E. coli* is greater (and probably the infective dose lower) for the very young, the aged, immunocompromised and persons with concomitant diseases than for healthy young adults. In these groups of

people, even the usually moderate gastroenteritis caused by *V. parahaemolyticus*, staphylococcal enterotoxin, *B. cereus*, or *C. perfringens* sometimes become severe.

The relatively rare instances where beta-hemolytic streptococci are foodborne may lead to tonsillitis complicated by severe sequelae of glomerulonephritis and arthritis, while cardiovascular disabilities (such as rheumatic fever) may follow. Convalescence after some other foodborne illnesses (particularly typhoid and paratyphoid fevers, brucellosis, and viral hepatitis) may be lengthy.

Many foodborne illnesses are associated with chronic secondary sequelae that can linger long after the acute effects of enteric infections. Examples include Guillain-Barré syndrome, a rapidly ascending paralysis that can lead to death, which is in part associated with antecedent *Campylobacter jejuni/coli* infections; reactive arthritis, which follows enteric infections caused by salmonellae, *Shigella* spp., *Y. enterocolitica,* and thermophilic campylobacters; hemolytic uremic syndrome, which is associated with *E. coli* O157:H7 infection; depression from chronic diarrhea caused by *Toxoplasma*; and septic arthritis following salmonellosis.

8.3.4 Diagnostic Considerations

Physicians' experience and laboratory procedures play a crucial role in diagnosing foodborne illness. For example, few physicians are likely to have previously encountered botulism; hence misdiagnoses may occur even when symptoms are typical or occasionally when the symptoms are very mild or appear similar to other illnesses. Laboratory isolation of specific pathogens is the only way certain enteric foodborne diseases can be diagnosed because the clinical syndromes of many of these diseases may be similar, e.g., bloody diarrhea may be symptomatic of bacillary or amoebic dysenteries and Shiga-like toxin-producing *E. coli*.

When a previously unrecognized foodborne disease is reported by a laboratory, awareness often increases and other incidents are revealed. The recognition of foodborne campylobacteriosis and EHEC O157:H7 infection illustrates this point.

In public health and food laboratories, completely satisfactory procedures are not yet available for the routine isolation or detection of several foodborne pathogens from foods (such as *Shigella* spp., *C. jejuni*, *Y. enterocolitica*, EHEC non-O157, *Cyclospora*, *Cryptosporidium*, and foodborne viruses). Laboratory methodology, therefore, limits the precision with which their presence can be measured.

8.4 CATEGORIZING MICROBIAL HAZARDS ACCORDING TO RISK

In this book, the term *hazard* is limited to microbiological concerns associated with foodborne illness. These are bacterial pathogens and their associated toxins or toxic metabolites, viruses, parasites, and toxigenic fungi. The risks associated with microbial hazards vary greatly, ranging from quite mild symptoms of short duration to very severe, life-threatening illnesses. When deciding on the level of concern, health hazards generally fall into three categories.

8.4.1 Moderate Hazards

Moderate hazards are rarely life threatening, do not result in sequelae, are normally of short duration, and cause symptoms that are usually self-limiting, but can result in severe

discomfort. Some microorganisms can be both severe hazards for specific populations and mild hazards for the general population. For example, *L. monocytogenes* can cause abortion in pregnant women and life-threatening disease among immunocompromised people, whereas flu-like symptoms of diarrhea of short duration occur in the general population.

8.4.2 Serious Hazards, Incapacitating But Not Life Threatening

These hazards result in disease of moderate duration and do not normally cause sequelae. Some pathogens, such as *C. jejuni* and other thermophilic campylobacters, occur most commonly in the lower, moderate category of hazard, but some strains of *C. jejuni* cause severe illness, e.g., Guillain-Barré Syndrome in susceptible persons. The majority of strains cause only mild diarrhea of moderate duration.

8.4.3 Severe Hazards, Life Threatening

These microbial hazards can result in substantial chronic sequelae or the effects can be of long duration. They can affect either the general population or be specific to populations at high risk. Factors influencing the development of illness in high-risk populations include specific host susceptibility to infection such as listeriosis in pregnant women, cultural practices such as consumption of potentially hazardous foods unique to specific subpopulations (e.g., *Burkholderia cocovenenans* associated with coconut tempeh), and geographic influences such as fumonisin intoxication associated with regions in which moldy maize is consumed.

The major microbial pathogens and toxins associated with foods in relation to their impact on public health, their frequency of involvement in disease, the types of foods that have served as vehicles, and significant factors contributing to disease are listed in Appendix 8–A. This table is not intended to be all-inclusive and no attempt has been made to arrange these pathogens and toxins according to frequency with which they cause outbreaks or cases of foodborne illness; this varies with locality. Table 1–1 (Chapter 1) indicates whether microbiological testing of foods (e.g., at port-of-entry) or other control measures has been instrumental in controlling the hazard and ensuring food safety.

8.5 DEFINITION OF CASES

The foregoing information can be used to establish sampling plans that consider the risk associated with a hazard. Thus, the choice of sampling plan must consider:

- the significance of the test result in relation to the type and severity of disease, indicator of a microbial hazard, or its commercial utility, and
- the conditions under which the food is expected to be handled and consumed after sampling.

Table 8–1 classifies 15 different cases of sampling plans on a two-dimensional grid taking into account these factors. In the table, the stringency of the sampling plan increases with the type and degree of hazard: from a situation of no health hazard but of utility only, through a low indirect health hazard (as implied by the presence of indicator organisms), to direct health risks related to diseases of moderate or severe implication. The stringency of the sampling plan also changes according to the conditions under which the food is expected to be handled. Hazards may remain unchanged, be reduced by

Table 8–1 Sampling Plan Stringency (Case) in Relation to Degree of Risk and Conditions of Use

	Conditions in which food is expected to be handled and consumed after sampling, in the usual course of events		
Type of hazard	*Reduce risk*	*Cause no change in risk*	*May increase risk*
Utility (e.g., general contamination, reduced shelf-life, spoilage)	Case 1	Case 2	Case 3
Indicator; low, indirect hazard	Case 4	Case 5	Case 6
Moderate hazard, not usually life threatening, usually no sequelae, normally of short duration, symptoms are self-limiting, can be severe discomfort	Case 7	Case 8	Case 9
Serious hazard, incapacitating but not usually life threatening, sequelae rare, moderate duration	Case 10	Case 11	Case 12
Severe hazard for (a) the general population or (b) restricted populations, causing life-threatening or substantial chronic sequelae or illness of long duration	Case 13	Case 14	Case 15

cooking, or increase because of subsequent growth of microorganisms. The most lenient plan is case 1. Stringency increases from left to right and from top to bottom of the table, so that the sampling plan for case 15 is the most stringent.

8.5.1 Choosing the Case

The choice of case to apply depends on whether the hazard may increase, not change, or decrease between when a food is sampled (e.g., at port-of-entry) and when the food is consumed. Thus, the value of microbiological testing as a method of consumer protection depends on knowledge of the type of food to be sampled. For example, it is helpful generally to understand a food's normal method of production/harvesting, processing, composition, packaging, and the conditions to which it would normally be exposed during storage and preparation. In addition, some understanding of pathogen-food interactions and the intended consumer is needed. Information of this nature is needed before an examiner can choose an appropriate case. The following illustrates such considerations.

In general, foods that have received an adequate heat treatment during a manufacturing process are generally safer than those that have not. Risk increases when heat-treated foods (i) become contaminated after processing, (ii) are exposed to conditions that permit multiplication of pathogens, and (iii) are not recooked shortly before consumption.

If a food is expected to be fully cooked before consumption and because cooking reduces the hazard, one would choose case 4, 7, 10, or 13, depending on the degree of the hazard. Raw poultry, fresh dry pasta, cake mix, and dried soup mix with beans are examples of foods in this category.

If conditions of anticipated use would not permit a change in the number of relevant bacteria (e.g., frozen storage), the appropriate case would be 5, 8, 11, or 14, depending

on the type of hazard. Ice cream is an example of a food that would be classified in one of these cases, because it is ordinarily maintained and consumed frozen.

If the food is ordinarily subjected to conditions that permit growth or an increase in the hazard, thereby increasing risk, the case would be 6, 9, 12, or 15, depending on the type of the hazard (Table 8–1). For example, one of these cases would apply to dried milk since contaminating pathogens may multiply after reconstitution.

Preservation Conditions

The preservation conditions (e.g., salt concentration, a_w, pH, temperature) of the food should be considered in relation to the growth requirements of the relevant microorganism(s). Foods with a brine concentration of approximately 10% may support the growth of staphylococci, but not salmonellae. Salmonellae, however, may survive for an extended period of time on dried meats. Hence, such products (if not refrigerated) might be classified in case 6 for staphylococci and case 11 for *Salmonella* spp.

Fresh meat supports the growth of various pathogens, whereas dried meat with a brine concentration at ≥16% in the water phase does not. Hence, if fresh meat is stored at temperatures allowing multiplication, the risk would increase corresponding to case 6, 9, 12, or 15, whereas for dried beef there would be no change in risk, so case 5, 8, 11, or 14 would apply.

Storage Temperature

Temperature is especially important. Microbial numbers, and the associated risks, generally increase at 10–20 °C and even more rapidly at warmer temperatures. In contrast, refrigeration to below 10 °C tends to control most hazards, because many pathogens do not multiply or do so more slowly at low temperatures. For example, for ham kept below 6 °C (at which temperature staphylococci do not produce toxin), case 8 rather than case 9 would apply. For foods in which psychrotrophic pathogens, such as *L. monocytogenes*, *Y. enterocolitica*, and nonproteolytic *C. botulinum* can multiply, the potential for growth will depend on decreasing the storage temperature to closer to 0 °C.

Competitive Flora

Growth of pathogens can sometimes be prevented by competition from other microorganisms. While salmonellae grow in most foods of appropriate pH, a_w, and temperature, growth of staphylococci is often restricted by the associated spoilage flora. Fresh raw meats and bacon are not normally associated with staphylococcal food poisoning because they also carry large numbers of competing microorganisms that suppress the growth of *S. aureus*. The hazard of enterotoxin formation usually arises in foods that have been processed in some way to reduce the microbial population, and then the food is contaminated with staphylococci (e.g., cooked ham contaminated after cooking).

Eating Customs

Custom also affects hazard and the choice of case. For example, *V. parahaemolyticus* grows readily on raw fish unless it is refrigerated. It is a major cause of foodborne illness in Japan, where raw fish is commonly consumed, but in other countries, though widely distributed, *V. parahaemolyticus* is a much less common cause of illness because fish is cooked before consumption. Hence, for Japan, case 8 or 9 would be appropriate for this pathogen, whereas in another country with different dietary customs, case 7 would be suitable.

Reconstituted Dried Foods

Foods that are pasteurized before distribution (e.g., powdered eggs, dried milk) are sometimes eaten without cooking when distributed in relief areas. If a food is intended for consumers with unusually high susceptibility to foodborne illnesses, the hazard will be increased (Table 8–2).

Type of Hazard

Certain microbiological hazards can increase (e.g., salmonellae, *L. monocytogenes*) if the above conditions of temperature and food composition are favorable. Many hazards such as toxins and toxic metabolites tend to be quite resistant to environmental conditions, including normal cooking, and remain stable. Other hazards, such as viruses and parasites cannot increase, but may decline in concentration, depending on the conditions to which they are exposed.

Susceptibility of the Intended Consumer

If a food is intended for consumers with unusually high susceptibility to foodborne disease, risk will be increased. Examples of special foods intended for high-risk consumer groups are described in Table 8–2.

Storage and Preparation for Serving

Only the usual conditions to which the food is expected to be exposed between when the lot is sampled and when the food is consumed should be considered. For example, a frozen food will ordinarily be kept frozen until it is cooked or reheated for serving. If a

Table 8–2 Special Foods for Consumer Groups with Increased Susceptibility

Food class	*Reason for a more stringent sampling plan*
Baby food	High susceptibility of the consumer population to enteric pathogens; severe response to infections and toxins; increased risk of fatality
Dietetic food	Infection is a severe risk for diabetics
Foods for hospitals	Patients may be prone to infection and to serious sequelae after enteric disease because of stresses from other disabilities and from immunosuppressive treatment, and intensive care; interference with convalescence from other disease; staff and patients need to be protected because of their potential for spreading disease within the hospital
Foods for AIDS patients	Immunocompromised populations are highly susceptible to enteric pathogens
Relief foods, especially dehydrated high-protein foods	Populations needing relief foods are usually highly susceptible and prone to serious complications because of malnutrition and other stressful conditions; also increased risk for person-to-person spread of disease because of confinement of the population in crowded areas often having poor sanitary conditions; particular hazards are reconstitution with contaminated water, unhygienic handling, and poor storage conditions leading to rapid bacterial growth

food is unexpectedly abused after having been sampled and approved, the sampling plan may not provide the level of protection expected.

Method of Food Preparation

An important consideration is the method of food preparation (i.e., normally eaten raw, warmed, or cooked).

8.5.2 Examples of Choosing a Case

The following examples illustrate how knowledge about microbial ecology, and food storage and use are integrated in choosing the case.

Salmonellae are serious hazards and often occur in raw protein foods (e.g., liquid eggs), but they are destroyed by pasteurization. However, recontamination of pasteurized products with salmonellae has occurred, and subsequent drying or freezing cannot be relied upon to destroy these bacteria. If such a dried food is consumed in the dry state, there is no change in hazard (case 11); if use after reconstitution is delayed, and heating does not take place before consumption (a practice that is highly undesirable with many such products), the case would be 12. Cooking promptly after reconstitution will reduce the hazard, hence case 10 would then be appropriate.

When applying the decision tree (Figure 8–1) to a raw food (e.g., raw meat or poultry) that is to be cooked, testing for *S. aureus* would not be appropriate. If, however, the food has been cooked (e.g., cooked crustaceans or whole chickens) and the food is then handled (e.g., peeling shrimp, removing skin and bones from the chickens), then contamination with *S. aureus* is a concern and case 9 would be appropriate. In certain salted foods in which salt-tolerant *S. aureus* can grow, the competing flora are inhibited by the reduced a_w. Hence, the case for staphylococci would be similar to a pasteurized food (case 9).

Foods to which a significant amount of salt has been added also may be of increased concern. For example, the addition of salt to cheese curd or to sausage batter during the manufacture of cheddar cheese or fermented sausage will retard the growth of the indigenous flora, but not the more salt tolerant *S. aureus*. Under normal circumstances, the addition of a starter culture will control the growth of *S. aureus*; but, if the starter culture is rendered inactive by the addition of too much salt or the presence of an antibiotic, extensive *S. aureus* can occur. Considering this information, case 9 could be applied to these foods, but with caution, because the number of viable *S. aureus* will decline over time and may not reflect the likelihood of enterotoxin in the food.

B. cereus and *C. perfringens* are also moderate hazards, differing from *S. aureus* in that they produce spores that survive mild heating. Few processes will reduce the hazard provided by these bacteria, so case 8 or 9 is usually appropriate. Consideration has to be given to subsequent use of the food. For example, if a dehydrated food is eaten immediately after reconstitution, then testing would not be appropriate, compared with a food for which a delay would be expected between when the food is rehydrated and when it is consumed.

The foregoing examples illustrate and emphasize the need for some knowledge of the microbial ecology and history of a food before an examiner can choose an appropriate case or even test for a particular purpose. When choosing cases on the basis of hazard as described above, one must consider the many possible uses for the consignment of food. Some uses may be of higher risk than others, and the selection of case should reflect this possibility.

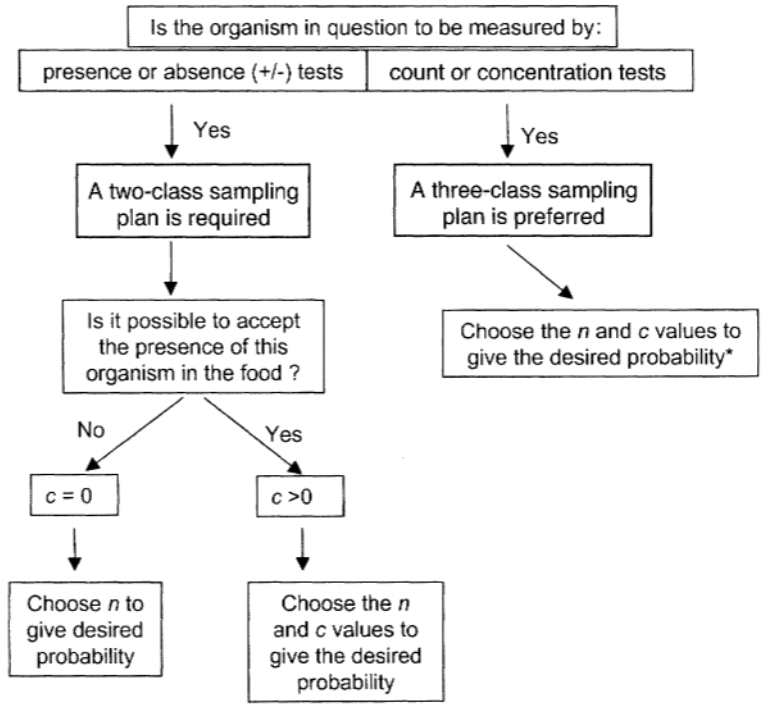
Microbial hazards are related to the presence of numbers of undesirable microorganisms or the occurrence and concentration of a toxic metabolite in a food. After choosing the category of hazard (category I, II, or III in Appendix 8–A) and the effect of subsequent conditions of handling and preparing the food on the hazard, the appropriate case is selected (Table 8–1).

8.6 DECIDING BETWEEN TWO-CLASS AND THREE-CLASS ATTRIBUTES SAMPLING PLANS

To decide whether the plan should be two-class or three-class, one must consider whether any positives (e.g., *Salmonella* or aerobic plate count levels above those reflected by GHP) can be permitted in any of the sample units. If the answer is no, a two-class plan with $c = 0$ should be used. If the answer is yes, a two- or three-class plan can apply, but if the number of microbes in a unit-volume or mass can be obtained, a three-class plan is recommended (Figure 8–2).

Three-class sampling plans may, for the following reasons, be more appropriate than two-class plans.

1. To accept a proportion of sample units yielding test values in the marginally acceptable interval (between acceptable and defective), as these plans do, is in keeping



*A variables plan may be applicable; see Chapter 7.

Figure 8–2 Deciding between two- and three-class sampling plans.

with practical experience where, even under good manufacturing conditions, a few sample units may well reveal test values beyond those normally encountered without causing any consequent problem. This situation applies especially to counts of indicator organisms.

2. Sufficient experience also defines a different level, beyond which counts indicate substantial likelihood of health or utility hazard, a level that will not be attained if control has been adequate. This, designated *M*, should remain stable unless new experience reveals an error in its initial placement. Obviously, greater stability of criteria of acceptance may be expected to promote wider adoption of sampling plans.
3. A three-class plan allows a regulatory authority to carry out a limited form of trend analysis. For example, an increasing proportion of values within the marginally acceptable (*m* to *M*) range may indicate a lack of control.

8.7 DETERMINING VALUES FOR *m* and *M*

Definitions of *m* and *M* from a statistical point of view have been given in Chapter 7. From a microbiological point of view, the level of the test organism that is acceptable and attainable in the food is traditionally defined as *m*. The value of *m* reflects implementation of GHP by an importing country or, more often, for domestic production of a food, or is the hazardous level in two-class plans. If the test organism is a pathogen for which there is no level of tolerance (i.e., for two-class plans), *m* may be zero or, more correctly, absence in all analytical units when tested by a specified method. This will reflect the detection limits of the test, e.g., < 0.04 cells g^{-1}. Hence, the value of *m* for two-class plans is usually 0. For three-class plans, *m* will usually be assigned some nonzero value.

M, used only for three-class plans, defines an unacceptable level. All lots with values exceeding *M* should be rejected (i.e., they should be withheld pending further investigation to determine whether the product can be used after further treatment or is unacceptable as food). Investigation of the processor's facilities should be undertaken when such lots are detected. In international commerce, this presupposes a system whereby the analytical results can be conveyed to appropriate officials of the country of origin.

There are several approaches to choosing the value of *M*:

1. As a utility (spoilage or shelf-life) index. Relate microbial levels to detectable spoilage (odor, flavor) or to a decrease in shelf-life to an unacceptable short period.
2. As a general hygiene indicator. Relate levels of the indicator bacteria to a clearly unacceptable condition of hygiene—whether contamination or growth, or both.
3. As a health hazard. Relate levels of pathogens to illness. In choosing the value of *M* for a pathogen, use epidemiologic and laboratory data in combination, experimental animal feeding or inoculation data, human feeding data, laboratory analyses for toxin related to levels of organisms, or other guides that indicate the level at which there is a serious concern for the hazard. For this purpose, consider the maximum amount of food likely to be eaten at one time and the susceptibility of the group of persons likely to eat the food (see also Table 8–2).

It is important to realize that *M* is defined by the hazard. The value of *m* is available and defined by GHP in three-class plans and may change with time. In Chapter 7, a procedure for establishing a relationship between *m* and *M* was outlined. However, the values for *m* and *M* do not necessarily need to have a constant relationship.

In an unusual situation, one might find a consignment composed of two widely dissimilar lots. One lot may be entirely acceptable and the other entirely unacceptable (see for example curve 1 and curve 4 in Figure 7–3); this situation would have a high proportion of units falling below m and above M, but few between m and M.

These examples illustrate how the choice of m and M, in relation to the microbial quality of the lot, influences the types of lots likely to be rejected. Moreover, they illustrate the importance to producers of limiting variation in numbers of microbes present in food, i.e., of keeping production microbiologically under control, to avoid lots of otherwise satisfactory average quality being rejected. With good or bad control, the average microbial levels are likely to be respectively low or high, and the distribution of the data around the average (expressed logarithmically) respectively narrow or broad. The spread of the distribution may be nearly as important as the average level in determining compliance or noncompliance with a particular criterion, as indicated in Figure 7–3.

The decision to accept or reject any particular lot should be based upon the laboratory results obtained and is subject to the error associated with the laboratory procedure used (see Chapter 12). It is especially important when using two-class plans with $m = 0$ and $c = 0$ that the methods employed provide accurate results, for whatever false-negative and false-positive results occur will lead to corresponding wrong decisions about lots because of the inaccurate methods.

Unfortunately, documentation of the inherent variation within a given method is rare, though experienced analytical microbiologists develop estimates that become included in their choice of criteria. Some methods, for example, most probable number methods for coliforms, have great inherent variability (see Silliker *et al.*, 1979). Similarly, it is common that familiarity of an analyst with a particular method (e.g., for *Salmonella* spp. or *S. aureus*) enables that method to be most productive for them. This is a cogent argument for widely accepted use of a standard method so that all analysts become experienced in its use: another method with different sensitivity might require change of criteria. The testing method is critical in establishing the acceptance criteria of sampling plans, hence it is not desirable to deviate from a standard method unless there is a major advantage.

All probability computations in this book (e.g., operating characteristic curves and surfaces) for sampling plans assume that the laboratory results are obtained without error.

8.8 SPECIFIC KNOWLEDGE ABOUT THE LOT

The relatively long delay between collection of samples and issue of the corresponding laboratory result may create a need for costly storage. For products with an extensive history of good quality, the need for storage can be avoided by releasing consignments as soon as samples have been taken (provided, of course, that records are adequate to trace for recall if a lot should prove to be unacceptable). If the analysis should reveal an unsatisfactory condition, future consignments could be held at the port until the test results proved satisfactory. Upon reestablishing a satisfactory record (perhaps three consecutive consignments), the food could again be released as soon as samples have been taken. These systems have been in use by many countries.

Application of a sampling plan with a comparatively small routine sample (e.g., $n = 5$), and thus with low stringency, is only appropriate to detect lots with a high proportion of defectives. When a food product is processed and shipped under uniform, adequate

controls and there is a favorable history of compliance and low risk associated with a defective lot that is not sampled, it could be argued whether acceptance sampling serves any purpose. Until such assurance has been obtained through a good history of test results and/or auditing (see Chapter 4), the only way to obtain satisfactory protection (i.e., substantial discrimination between acceptable lots and unacceptable lots) is by increasing the sample size (larger n). However, as noted previously (Chapters 6 and 7), to double the reliability may require four times as many sample units. The cost of the additional testing should be balanced against the potential gain in discriminatory power; hence a practical decision should be made. However, it should be balanced as well against the impact on the actual level of risk a wrong decision would have.

It may not be feasible to sample at random over the entire consignment. It may only be possible to sample randomly from a portion of the consignment from which the sample units can be drawn. If so, results apply only to the portion of the consignment sampled, not to the whole consignment, and it is necessary to consider whether the results can be applied to the entire consignment. For example, accessible containers may be those nearest the door of a vehicle, nearest the hatch of a ship's hold, or on the periphery of a stack in a warehouse. The sample units chosen may sometimes represent the portion of a lot or consignment that has been exposed to greater hazard, through contamination or conditions that permitted microbial growth. Convenient as it may be, this practice may also provide greater consumer protection through the selection from the portion of the lot most likely to be hazardous. But this would not be so for a perishable food that is packed too warm and cools slowly in the boxes at the center of a pallet.

Another aspect that can only be addressed by referring to specific knowledge about the lot is the distribution of the microorganism(s) that are of concern (i.e., their frequency distributions). As discussed in Chapter 7, knowledge of these distributions, especially their standard deviations, is needed to evaluate the performance of sampling plans based on quantitative criteria. The calculation of sampling plan performance, to be discussed in section 8.11, assumes a standard deviation of 0.8. Frequency distributions having standard deviations above or below 0.8 will result in sampling plans that differ in their performance.

8.9 WHAT IS A SATISFACTORY PROBABILITY OF ACCEPTANCE?

Since decisions to accept or reject lots are made on samples drawn from the lots, occasions 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) as given in Tables 7–1, 7–2, and 7–4. The producer's risk (the inverse of consumer's risk) is expressed by $1 - P_a$.

When acceptance sampling is chosen to measure whether a food safety objective (FSO)/performance criterion is met, besides the tolerable level of risk, from which the FSO is derived, an additional uncertainty is introduced, viz., the risk of incorrectly accepting a lot that does not meet the FSO/performance criterion. Such an incorrect deci-

sion may increase consumer risk. In principle, the minimally required probability to reject a lot not meeting the FSO/performance criterion (or the confidence in appropriate operation of the sampling plan) should be adapted to the level of concern. This would lead to increasing minimal rejection probabilities with higher case numbers. The stringency of a plan is measured by the probability of accepting lots in which a particular proportion of sample units is defective. A relatively lenient three-class plan ($n = 5$, $c = 3$) accepts a lot with a 5% proportion of defective units and 30% marginal units on about three occasions in four ($P_a = 0.75$). The most stringent two-class plan ($n = 60$, $c = 0$) would accept lots with the same 5% proportion of defective units on about one occasion in 20 ($P_a = 0.05$), and lots with 0.5% defectives on about 19 occasions in 20 ($P_a = 0.95$).

If it is important to determine correctly whether a 0.5% proportion of defective units is in compliance with an FSO/performance criterion, the question might be asked whether the plan can provide any worthwhile protection, even with a significant increase in the number of sample units. This demonstrates that microbiological testing will have very little value in process validation when the process is designed to produce a low proportion of defective units.

In practice, a probability of acceptance on three occasions in four ($P_a = 0.75$) means that one lot in every four will be rejected, a loss serious enough to compel a manufacturer to tighten microbial control to a level well below the limit(s) set in the test employed. Even the rejection of one lot in 20 ($P_a = 0.95$) may suffice to have this effect. Nevertheless, the protection conferred on the consumer for a particular lot is seriously limited when using small numbers of sample units such as $n = 5$; hence the recommendation is to use large values for n when a direct hazard is recognized. In these situations, following the decision tree on testing and use of microbiological criteria (Figure 8–1) will result in more reliable assurance of safety.

The sampling plan stringency must be considered in view of the associated criteria as well. Consider the example of a moderate health hazard, such as the presence of *S. aureus* in cooked, peeled shrimp. If the microbiological limit m were placed at a numerical level well below that likely to represent hazard (M), one could frequently accept lots containing a high proportion of marginally acceptable units, which would require only a lenient sampling plan. If, however, m were placed nearer the hazardous level, one would accept such lots infrequently, requiring the use of a relatively stringent sampling plan. If m were at a hazardous level, one would not accept lots containing any units exceeding that level, and two-class plans of high stringency would be required. Adjustment is possible by choosing limits known by experience to be associated with safety. When this is done, even though a high proportion of lots with substandard units will be accepted, the probability of consuming food that would cause illness is kept low.

8.10 SELECTING *n* AND *c*

The choice of n and c varies with the desired stringency (probability of rejection and power of discrimination) and hence with the cases in the grid of Table 8–1. For stringent cases, n is high and c is low; for lenient cases, n is low and c is high. As n decreases with the attributes plans proposed in this book, the chance of acceptance of unacceptable lots increases. This fact must be taken into account if the number of sample units (n) exceeds the analytical capability of a laboratory and n is reduced.

The procedure (see Chapters 6 and 7) for selecting the number of sample units, n, should be first to fix desired acceptance and rejection probabilities for lots of defined acceptable and unacceptable qualities. Then the number of sample units required for this purpose is derived. However, the choice of n is usually a compromise between what is an ideal probability of assurance of consumer safety and the workload the microbiology laboratory can handle, as well as the cost and time needed for the tests. Consider first the nature of the hazard; then decide the appropriate probabilities of acceptance and rejection for the hazard in question. Guidance on determining these probabilities requires establishing a relationship between the confidence desired and the impact a wrong decision (acceptance of a defective lot) would have on the actual risk. Determining the required number of sample units then depends on the desired confidence.

The stringency of any of the sampling plans can be increased by adjusting c and/or n. In this way, pressure can be brought to bear upon hygienic practices, the nature of purchasing specifications, the severity of processing, and the extent and nature of the quality control practiced within the food industry concerned. The desirable effect should be carefully weighed, and the decision made should be known and understood by producer, manufacturer, distributor, and control agency alike.

In summary, the final judgment on which a sampling plan is based should involve the relative weight to be placed on the above microbiological, epidemiologic, and ecologic factors, as well as the statistical probabilities of acceptance or rejection desired and economic considerations arising within the laboratory (e.g., the nature of its physical facilities, equipment, and work capacity of its personnel). Some degree of subjective judgment remains unavoidable because data adequate to allow fully objective decisions may not be available. Under these circumstances, individual judgments can vary widely and, if applied in international trade, could lead to widely differing sampling plans and inadvertent barriers to trade until the differences between trading partners are resolved.

8.11 SAMPLING PLAN PERFORMANCE OF THE CASES

The cases described in Table 8–1 have been widely adopted in sampling plans. The ICMSF sampling plans were developed based on past experience, available data, practical constraints and statistical considerations and have provided helpful guidance. It should be noted, however, that the sampling plans assigned for each case in Table 8–3 may not provide the desired stringency required to come to a reliable decision for lot acceptance or whether a performance standard or FSO has been met. The plans simply imply that the number of samples indicated for each case provides the desired level of protection; whether this assumption holds must be assessed by the user.

The techniques described in Chapter 7 can be used to calculate the stringency of the cases in terms of the probability of accepting a lot at various concentrations of organisms. The stringency of each case and recommended sampling plan has an associated level of performance. Table 8–4 shows the performance of the sampling plans for cases 4 to15. The table shows the mean concentrations that would be associated with an acceptance probability of 0.05 (i.e., a rejection probability of 0.95) for the sampling plan in each case. For cases 4 to 9, the three-class attributes sampling plans involve quantitative analysis. For cases 10 to15, the two-class attributes sampling plans involve qualitative analysis (presence/absence testing) using 25 g analytical units.

To demonstrate the relative sensitivities of cases 4 to 9, constant values were used for m (10^3 cfu g^{-1}) and M (10^4 cfu g^{-1}) and a standard deviation of 0.8 log cfu g^{-1} was

Table 8–3 Suggested Sampling Plans for Combinations of Degrees of Health Concern and Conditions of Use (i.e., the 15 "Cases")

	*Conditions in which food is expected to be handled and consumed after sampling in the usual course of events**		
Degree of concern relative to utility and health hazard	*Conditions reduce degree of concern*	*Conditions cause no change in concern*	*Conditions may increase concern*
Utility; general contamination, reduced shelf-life, incipient spoilage	Increase shelf-life Case 1 Three-class n=5, c=3	No change Case 2 Three-class n=5, c=2	Reduce shelf-life Case 3 Three-class n=5, c=1
Indicator; Low, indirect hazard	Reduce hazard Case 4 Three-class n=5, c=3	No change Case 5 Three-class n=5, c=2	Increase hazard Case 6 Three-class n=5, c=1
Moderate hazard; direct, limited spread	Case 7 Three-class n=5, c=2	Case 8 Three-class n=5, c=1	Case 9 Three-class n=10, c=1
Serious hazard; incapacitating but not usually life threatening, sequelae are rare, moderate duration	Case 10 Two-class n=5, c=0	Case 11 Two-class n=10, c=0	Case 12 Two-class n=20, c=0
Severe hazard; for (a) the general population or (b) restricted populations, causing life threatening or substantial chronic sequelae or illness of long duration	Case 13 Two-class n=15, c=0	Case 14 Two-class n=30, c=0	Case 15 Two-class n=60, c=0

*More stringent sampling plans would generally be used for sensitive foods destined for susceptible populations. (See Table 8–2)

assumed. Similarly, cases 10 to 15 are compared for a standard deviation of 0.8. In cases 10 to 15, "m=0/25 g" indicates that each 25 g analytic unit should test negative for the hazard of concern. The number of samples tested is indicated by the value of n. The calculations indicate that lots having or exceeding the mean concentrations will be rejected with a probability of at least 0.95 when the indicated sampling plan is applied. Thus, the mean concentrations define the sensitivity of each sampling plan.

The information for sampling plan performance provided in Table 8–4 enables the examiner to better understand the expected performance of a given sampling plan. For example, the most stringent sampling plan chosen for case 15 (n = 60, c = 0 with no positive samples detected (m = 0)) will, with 95% probability, reject the lot if the mean concentration of the pathogen is at least 1.9 cfu 1000 g^{-1}. If lower mean concentrations of the pathogen are present (e.g., 1 cfu 5000 g^{-1}), the sampling plan will accept the lot with a probability higher than 5%. In the sampling plan for case 8 (three-class-plan, n = 5,

Table 8–4 Cases and Sampling Plan Performance, Assuming a Standard Deviation of 0.8. Lots Having the Calculated Mean Concentrations or Greater Will be Rejected with at Least 95% Probability

	Cases, sampling plans and calculation of their performance		
Type of hazard	*Conditions reduce hazard*	*Conditions cause no change in hazard*	*Conditions may increase hazard*
Indirect	**Case 4** (three-class, *n*=5, *c*=3) e.g. *m*=1000/g, *M*=10000/g **Mean conc. = 5128/g**	**Case 5** (three-class, *n*=5, *c*=2) e.g. *m*=1000/g, *M*=10000/g **Mean conc. = 3311/g**	**Case 6** (three-class, *n*=5, *c*=1) e.g. *m*=1000/g, *M*=10000/g **Mean conc. = 1819/g**
III. Moderate	**Case 7** (three-class, *n*=5, *c*=2) e.g. *m*=1000/g, *M*=10000/g **Mean conc. = 3311/g**	**Case 8** (three-class, *n*=5, *c*=1) e.g. *m*=1000/g, *M*=10000/g **Mean conc. = 1819/g**	**Case 9** (three-class, *n*=10, *c*=1) e.g. *m*=1000/g, *M*=10000/g **Mean conc. = 575/g**
II. Serious	**Case 10** (two-class, *n*=5, *c*=0) e.g. *m*=0/25g **Mean conc. = 32/1000g** **(1cfu/32g)**	**Case 11** (two-class, *n*=10, *c*=0) e.g. *m*=0/25g **Mean conc. = 12/1000g** **(1 cfu/83g)**	**Case 12** (two-class, *n*=20, *c*=0) e.g. *m*=0/25g **Mean conc. = 5.4/1000g** **(1 cfu/185g)**
I. Severe	**Case 13** (two-class, *n*=15, *c*=0) e.g., *m*=0/25g **Mean conc. = 7.4/1000g** **(1 cfu/135g)**	**Case 14** (two-class, *n*=30, *c*=0) e.g. *m*=0/25g **Mean conc. = 3.6/1000g** **(1 cfu/278g)**	**Case 15** (two-class, *n*=60, *c*=0) e.g. *m*=0/25g **Mean conc. = 1.9/1000g** **(1 cfu/526g)**

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$c = 1$ with $m = 10^3$ and $M = 10^4$), a mean concentration of at least 1819 cfu g^{-1} is required for the plan to reject the lot with 95% probability. The basis for the calculations is described in Legan *et al.* (2001).

The poor performance of the sampling plans in detecting lots with low concentrations of pathogens demonstrates that lot acceptance testing is an unreliable approach to ensure consumer safety. It is for this and other reasons discussed throughout the text that greater emphasis should be placed on control systems, such as GHP and HACCP.

8.12 REFERENCES

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Appendix 8–A

Ranking of Foodborne Pathogens or Toxins into Hazard Groups

Severity of threat to health	*Frequency of involvement in foodborne disease*	*Examples of vehicles*	*Other factors contributing to significance*
I.A. Severe hazard for general population, life threatening or substantial chronic sequelae or long duration			
Brucella melitensis, B. abortus, B. suis (brucellosis)	Common in endemic areas	Raw milk and cheese, especially from goats and sheep	Convalescence often prolonged
Botulinal neurotoxin *(Clostridium botulinum, C. butyricum, C. barati)* (botulism)	Rare	Improperly processed canned or preserved low-acid foods: home-cured meat products; smoked fish, other marine products; foil wrapped baked potato in salad, garlic in oil	Rapid recognition and treatment essential for patient survival; substantial mortality
Enterohemorrhagic *E. coli* (e.g., *E. coli* O157:H7, O111:NM) (hemorrhagic colitis and hemolytic uremic syndrome)	Sporadic; epidemic	Undercooked ground beef, unpasteurized apple juice, vegetable sprouts, lettuce, venison, yogurt, fermented sausage, untreated and recreational water, contact with farm animals	Very severe for children and elderly, severe complications including kidney failure and death, low infectious dose, acid tolerance
Salmonella typhi, S. paratyphi A, B (*S. schotmulleri*) and C (typhoid and paratyphoid fevers)	Endemic in many parts of the world; occasionally epidemic	Untreated water, raw milk, meat products, raw shellfish	Prolonged medical care required, asymptomatic chronic carrier state commonly occurs
Shigella dysenteriae I (shigellosis)	Sporadic; endemic	Salads, untreated water	High mortality rate, especially among children, low infectious dose
Burkholderia cocovenenans	Rare	Coconut tempeh	Restricted geographic populations

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Appendix 8–A continued

Severity of threat to health	*Frequency of involvement in foodborne disease*	*Examples of vehicles*	*Other factors contributing to significance*
Vibrio cholerae O1 and O139 (cholera)	Sporadic; endemic; sometimes epidemic	Raw seafood from polluted water; untreated water	Substantial mortality among dehydrated, untreated persons; moderate symptoms with available rehydration treatment
Mycobacterium bovis (tuberculosis)	Rare	Raw (unpasteurized) milk	
Aflatoxins, produced by *Aspergillus flavus* and *A. parasiticus* (aflatoxicosis)	Common in tropical regions	Nuts and oilseeds, especially peanuts and maize, figs, milk	Most potent liver carcinogens known; acutely toxic in high doses; carcinogenic, teratogenic, and probably immunosuppressive at low levels
v CJD/BSE; Protease Resistant protein (PrP); Bovine Spongiform encephalopathy (BSE); variant Creuzfeldt-Jakob disease	Sporadic	Specified bovine offal (brain, spinal cord, intestines, tonsils, thymus, spleen, spinal cord) from adult cattle	Severe central nervous system disorder resulting in death; no treatment or cure.
I.B. Severe hazard for restricted populations, life threatening or substantial chronic sequelae or long duration			
Campylobacter jejuni serovar O:19 and other serotypes associated with GBS (Guillain-Barré Syndrome)	Sporadic	Poultry, water	Associated with Guillain-Barré Syndrome
Enteropathogenic *E. coli* (EPEC) Enterotoxigenic *E. coli* (ETEC)	Frequent in certain regions	Untreated water; food contaminated by nonpotable water or infected food handler	Symptoms are mild but can be severe in infants, major cause of infant mortality in certain regions
Clostridium perfringens type C (enteritis necroticans)	Rare	Cooked pork	High mortality in protein-deficient persons, associated with malnutrition and a diet rich in trypsin inhibitors

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Clostridium botulinum (types A and B)	Sporadic	Honey	Infant botulism
Enterobacter sakazakii	Rare	Dried milk powder in infant formula (temperature abuse of dehydrated infant formula)	Causes death in infant populations (up to 70% mortality rate among neonates)
Listeria monocytogenes	Sporadic; occasionally epidemic	Soft cheeses, paté, smoked fish, ready-to-eat food	High risk groups include immunocompromised persons and pregnant women; high mortality (ca. 25%) in high risk populations; infrequent illness in immunocompetent persons; low numbers of *L. monocytogenes* are frequently consumed in foods
Vibrio vulnificus	Sporadic	Raw oysters	High mortality (ca. 50%) among persons that have elevated levels of serum iron; liver disorder associated with high alcohol consumption
Hepatitis A virus	Common	Raw or underprocessed bivalve mollusks, salads, untreated water	Very severe for patients with liver disease, convalescence prolonged
Cryptosporidium parvum	Sporadic; endemic; occasionally epidemic	Untreated water; unpasteurized apple juice, contaminated produce, unpasteurized milk	Severe prolonged diarrhea that is life threatening in immunocompromised; prognosis is poor for AIDS patients; usually short-term diarrhea that resolves spontaneously in immunocompetent persons

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Appendix 8–A continued

Severity of threat to health	*Frequency of involvement in foodborne disease*	*Examples of vehicles*	*Other factors contributing to significance*
II. Serious hazard; incapacitating but not life threatening; sequelae infrequent; moderate duration			
Salmonella enteritidis, Salmonella typhimurium and other *Salmonella* serovars (salmonellosis)	Very common; epidemic	Eggs; poultry; dairy products; wide range of other foods	Serious for young and elderly persons; cross contamination from raw meat and poultry; eggs and poultry meat can be internally contaminated during production; some serovars of *Salmonella* are highly virulent, reactive arthritis occurs in 1–2% of cases (Reiter's syndrome)
Yersinia enterocolitica (pathogenic), *Yersinia pseudotuberculosis* (yersiniosis)	Sporadic	Milk; chitterlings; untreated water	Most infections occur in children less than 5 years of age, with symptoms of mild gastroenteritis; in older children symptoms are severe, presenting a pseudo appendicular syndrome; only certain serovars of *Y. enterocolitica* are noteworthy pathogens; sequelae; arthritis can occur in genetically predisposed persons that carry the human leucocyte antigen (HLA-B27)
Shigella flexneri, S. boydii, S. sonnei (shigellosis) (nondysentery)	Sporadic in industrialized countries; sometimes endemic in developing countries	Foods subjected to contamination by infected persons or sewage-contaminated water, such as salads; untreated water	Serious for young and elderly persons; secondary infections among contacts; sometimes low infectious dose, HUS occasionally
Listeria monocytogenes	Sporadic, rare	Foods where multiplication has occurred during storage	Low numbers of *L. monocytogenes* are often consumed on a wide variety of foods

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Hepatitis A	Common	Raw or undercooked bivalve mollusks, salads, untreated water, strawberries	Less severe if no liver disease, but convalescence prolonged
Arcobacter butzleri and *A. cryaerophila*	Sporadic	Untreated water; poultry	Appendicitis infrequently occurs
Cryptosporidium parvum	Sporadic, endemic, occasionally epidemic	Untreated water, unpasteurized apple juice, contaminated produce, unpasteurized milk	
Cyclospora cayetanensis	Sporadic; endemic; occasionally epidemic	Raspberries; lettuce; water	Severe, prolonged diarrhea
Trichothecene toxins, especially deoxynivalenol, nivalenol, and T-2 produced by *Fusarium graminearum* and related species	Not clear	Cereals, especially wheat and maize in temperate climates	Immunosuppressive, probably contributing to increased disease incidence in endemic areas
Zearalenone, produced by *Fusarium graminearum* and related species	Probably not common	Cereals, especially wheat and maize in temperate climates	Estrogenic effects, not commonly observed in humans
Fumonisins, produced by *Fusarium moniliforme* and related species	Not clear	Fungus endemic in maize, toxins present in staple diets in regions of high maize consumption	Immunosuppressive, carcinogenic to rats and probably humans, implicated in esophageal cancer
Ochratoxin A, produced by *Penicillium verrucosum, Aspergillus ochraceus,* and related species, *A. carbonarius* and perhaps related species	Not clear	Cereals and pig meats in cool temperate climates, dried fruit, wines, and coffee beans	Nephrotoxic, probably contributing to reduced life spans in parts of Europe

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Appendix 8–A continued

Severity of threat to health	*Frequency of involvement in foodborne disease*	*Examples of vehicles*	*Other factors contributing to significance*
III. Moderate, not usually life threatening; no sequelae; normally short duration; symptoms are self-limiting; can be severe discomfort			
Bacillus cereus (*B. cereus* gastroenteritis), including emetic toxin	Common	Fried and boiled rice; reconstituted cereal products; puddings, custards	Usually diarrhea and/or vomiting of short duration; death is rare
Clostridium perfringens type A (*C. perfringens*)	Common	Cooked, non-cured meats; poultry; gravy	Symptoms usually mild but are more serious in elderly or debilitated persons; death is uncommon
Escherichia coli (EPEC, ETEC)	Common in developing countries; infrequent in developed countries	Foods handled by persons carrying EPEC or ETEC; foods contaminated with nonpotable water	Usually diarrhea of short duration in general population
Staphylococcus enterotoxins (*Staphylococcus aureus*) (enterotoxicosis or food poisoning)	Common	Cooked foods handled by persons carrying *S. aureus* then temperature-abused; ham; fermented sausages; cereal-filled pastries; cheese; milk; salads; peeled crustaceans, bivalve mollusks; mushrooms	Explosive vomiting and moderate diarrhea; symptoms usually resolve without treatment within 2 days of onset; death is rare
Vibrio cholerae non O1 and non O139	Sporadic	Raw bivalve mollusks, cross-contaminated cooked crustaceans	
Vibrio parahaemolyticus (*Vibrio parahaemolyticus* gastroenteritis)	Common in Japan; epidemic	Cooked crustaceans; raw bivalve mollusks; raw marine fish	Common where seafood is consumed raw
Small Round Structured Virus (SRSV), including Norwalk virus	Common	Raw bivalve mollusks, food handled by infected persons; bakery products	Symptoms are usually mild
Biogenic amines (e.g., histamine)	Infrequent	Scombroid fish, some cheeses	Biogenic amines are probably necessary for disease, serious for persons taking MAO

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Chapter 9

Tightened, Reduced, and Investigational Sampling

9.1 Introduction
9.2 Application of Tightened Sampling and Investigational Sampling
9.3 Tightened Sampling Plans
9.4 Example of the Influence of Sampling Plan Stringency in Detecting Defective Lots
9.5 Selecting the Sampling Plan According to Purpose
9.6 Reduced Sampling
9.7 References

9.1 INTRODUCTION

Tightened sampling involves the use of more frequent sampling and/or more stringent sampling plans than normal. Reduced sampling involves less frequent sampling than normal. Investigational sampling consists of sampling to determine the cause of a problem.

Factors warranting tightened or reduced sampling may relate to the food product, the manufacturer, or the country of origin (Exhibit 9–1). In tightened sampling, the sampling plans discussed in earlier chapters are no longer applied. Tightened sampling usually requires increased sample size (n) with other adjustments to make the sampling plan more stringent. In a three-class plan with fixed m and M, stringency is increased by making c smaller or n larger. When two-class plans are involved and c is 0, the sample size n must be increased to obtain a more stringent plan, assuming m is fixed. In the case of reduced sampling, the frequency of sampling a particular food is reduced, but if a lot is sampled, the two- or three-class sampling plan originally specified is applied.

Investigational sampling is a term used to describe sampling that is related to a known or suspected problem. Investigational sampling differs from tightened sampling in that the cause of a problem is sought. Such problems can arise from failure of a lot (or a series of lots) to pass routine inspection or from some new information, such as field reports of illness or unexpected spoilage related to the product. Investigational sampling may be done to (1) confirm that a problem exists, (2) assist in describing the nature and extent of a problem, (3) provide information on possible sources of a problem, (4) help decide what to do with a product (e.g., determine whether the entire lot is unacceptable or if the contamination is limited to a certain portion), and (5) prevent the problem from reoccurring.

It is important to note the difference between investigational sampling and the type of sampling described elsewhere in this book. Attributes or variables sampling detects effects, whereas investigational sampling seeks to determine causes. The success of investigational sampling depends greatly on the expertise of the investigator, the investi-

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Exhibit 9–1 Circumstances Warranting Tightened or Reduced Sampling of Food

Warranting increased frequency of sampling and/or more stringent sampling plan	Warranting reduced frequency of sampling
The food operation	
An audit indicates the operation does not have an adequate system of controls based on GHP* and HACCP†	The operation has an effective system of control based on GHP and HACCP
Records indicate a deviation at a CCP‡ in the HACCP plan has occurred	Records indicate the operation is under control
Information indicates the operation has used an ingredient from a source that has caused problems in other similar operations	
Food from the operation has recently been implicated in illness	Food from the operation has a favorable history of safety
The food	
The composition of the food differs from other foods of the same type, and an increase in a hazard is likely to occur under expected conditions of storage and distribution	The composition of the food differs from other foods of the same type, and the potential hazards will decrease or be eliminated during expected conditions of storage and distribution
Previous tests frequently unsatisfactory	Previous tests satisfactory
Routine tests for indicators have revealed a trend toward increased contamination	Routine tests for indicators show continuing control
The food has a history of being a source of foodborne illness	Rarely involved in foodborne illness
The food has been found to be a source of a newly emerging pathogen or new type of an existing pathogen	
Circumstances suggest involvement of this type of food in a recent outbreak	
A food that traditionally has been for the general public is to be directed toward a sensitive population	Not primarily intended for sensitive populations
New type of food or new formulation with reason to be concerned about a microbiological hazard	The parameters necessary for controlling foodborne illness are well known and widely applied
Examination results from different laboratories are in conflict, and disposition of the food is in question	
Country or region of origin	
Food control systems are in question	The food control systems are known to be equivalent for control of the food or ingredients in question
Endemic or epidemic situations exist that increase concern for consumers of the food	Endemic or epidemic situations do not exist that would increase concern for consumers of the food

*GHP-Good Hygienic Practices
†HACCP-Hazard Analysis Critical Control Point
‡CCP-Critical Control Points

gator's knowledge of the product and process involved in its manufacture, and the conditions of storage, distribution, and use. The attributes and variables plans previously discussed for lot acceptance are intended for routine application to lots or consignments presented for inspection at various stages prior to sale to the public. Two- and three-class attributes plans are used for regulatory, port-of-entry, and other receiver-oriented situations, where little information is available concerning the microbiological history of the lot. Variables plans can be used when the distribution of microorganisms (aerobic plate count, pathogens, indicators) is known. Variables plans may also be applicable to in-plant quality control, where these assumptions can be properly verified. In either case, the emphasis in lot acceptance sampling plans is to do the minimum amount of work necessary to obtain the degree of security that each plan can provide.

When a potential problem has been identified (e.g., recontamination of a product with *Salmonella*), further examination into the nature and extent of the problem is frequently required (i.e., investigational sampling). Such might be the case if a lot rejected under routine inspection is in dispute, or if the cause of a problem is being sought. For example, routine acceptance sampling may have indicated occasional recontamination in a plant from an increased rejection rate of lots. A larger random sample may then be taken from the rejected lots to confirm that a problem exists and to estimate the extent and distribution of the contamination. Emphasis may then shift to the processing plant where samples could be taken from various sites in an attempt to locate the source of contamination, e.g., certain processing equipment, a particular ingredient. It is necessary to identify the source so that appropriate measures can be taken to correct the problem. In another situation, analysis of rejected lots may show that contamination is associated with certain production times (e.g., first few hours, after breaks, following equipment repair). This information can be used to focus on the circumstances that may be causing contamination and how it may be prevented.

Note that these hypothetical investigations have used sampling at various stages in a food operation. Such sampling is rarely random. Biased sampling is much more efficient in an investigation because it takes advantage of prior knowledge of the operation, visual observations, and logic that may lead the investigator to sample those locations that are the most likely sources of the problem, e.g., a conveyor. Random sampling of easily accessible (and thus easily cleaned) sections of the equipment may ignore product accumulations in hollow gears that drive conveyors, which may escape proper cleaning and disinfection. A common approach to investigational sampling used by industry is to collect in-line samples of product at selected stages of processing with a bias toward those most likely to be contaminated or indicative of the source of a problem.

Biased sampling also is useful for suspected lots of food that are in storage or have been received at a location. For example, it may be evident from pack or container appearance that a dried product may have become wet due to container leakage during transport. In this case, it may be appropriate to take samples from the areas most likely to have become wet (e.g., the top packs on a pallet). Indications that a nonrandom problem may have occurred include transit pack damage, pallet collapse, or evidence of spoilage or discoloration in the outer packs suggesting abusive holding conditions. Other approaches to sampling, such as stratified, systematic, cluster, or various combinations of these, also may be used. Generally, once the purpose of sampling has been established, then the most efficient type of sampling applicable to this purpose is chosen. Such topics are beyond the scope of this book and are statistically complex, but an excellent discussion is found in Cochran (1977).

9.2 APPLICATION OF TIGHTENED SAMPLING AND INVESTIGATIONAL SAMPLING

Tightened and investigational sampling generally are applied to situations where there is an increased level of concern or perceived risk; for example, a process deviation has occurred, performance criteria have not been met, a product has failed to meet microbiological criteria, the food is from an operation with a history of inconsistent control, the food is from a region where there has been a recent increase in illness involving the same or similar type of food, or an environmental monitoring indicator has shown that the equipment used to produce or package the product did not meet acceptable hygienic criteria. Tighter sampling also may be warranted when there is insufficient knowledge about a particularly sensitive ingredient or a food is intended for a sensitive population and, thus, there is increased concern for making a correct decision as to its acceptability. For example, shipment from a new supplier or from a country in which the hygienic and manufacturing practices are not known may warrant increased sampling. These circumstances may confront both industry and regulatory agencies, and both may employ tightened sampling and investigational sampling. However, the objectives and approaches of industry and regulatory agencies may differ. Control authorities are primarily interested in protecting the consumer. Industry will have that same goal, but also must protect the economic interests of the company. Both seek to identify acceptable from unacceptable product, but it is far more important to industry to know why a problem may have occurred and how it can be corrected than solely to protect the consumer.

Control authorities may have less information available than industry about the source and production of a particular suspect lot, and therefore may be limited to the use of random sampling and tightened sampling. Industry, with much more information at its disposal, may choose to use an investigational approach, deliberately employing biased sampling. Biased sampling of finished product is often more efficient than random sampling when contamination might be associated with a time or sequence of production, such as the first product produced after a shutdown, shift change, ingredient switch, mechanical repairs, etc.

Occasionally, control authorities also may employ tightened sampling or investigational sampling. For example, if an outbreak occurs involving a certain product produced by several producers, the control authority will have a much broader focus than the individual manufacturers and may have more epidemiologic information at its disposal to help identify the source of the problem. In this case, the control authority may apply both biased sampling and tightened sampling, using random samples to determine which manufacturer is the source of the outbreak. Industry would be much more likely to employ tightened sampling to differentiate between acceptable and unacceptable lots of ingredients and/or finished product.

Tightened and investigational sampling can also be important in the design and application of Good Hygienic Practices (GHP) and Hazard Analysis Critical Control Point (HACCP). Tightened sampling, involving many more samples than would be practical for routine testing, can be used for process validation or as part of the HACCP plan verification. Investigational sampling may be required to identify areas in a plant where GHP needs to be changed to eliminate an organism of concern (e.g., more efficient disinfection techniques).

Failure to reach the critical limit (i.e., process criterion) for a Critical Control Point may necessitate tighter sampling of a lot. Experience with the food, the likely occurrence of a hazard, and the severity of the hazard(s) will influence the decision of whether sampling the lot or another option (e.g., reprocessing, destroying the lot) should be applied.

Suspicion that a food may contain a microbiological hazard may arise from a variety of sources of information:

- a hazard has been detected in other lots from the same operation.
- an audit of an operation has revealed questionable control.
- a consumer complaint has raised suspicion about the food.
- an ingredient recently has been implicated as a source of illness.
- an unfavorable trend in an indicator of pathogen contamination has been detected.

These and other circumstances (Exhibit 9–1) may lead to tightened sampling until accumulated evidence indicates this is no longer necessary.

9.3 TIGHTENED SAMPLING PLANS

One possible means to make a sampling plan more stringent is to increase the sample size (n) that is collected and analyzed. When two-class plans are involved (i.e., $c = 0$) and m is fixed, the number of analytical units (n) must be increased to obtain a more stringent plan. Another option for two-class sampling plans is not to change n but to increase the size of the analytical unit (e.g., from 25 g to 100 g) for testing. The spatial distribution and independence of the defectives throughout the lot will influence whether this option will increase confidence of detecting a defective sample unit within the lot and, thereby, lead to rejection of the food. When three-class plans with fixed m and M are involved, acceptance criteria can be made more stringent by making c smaller or n larger.

Typically, a sampling plan in tightened sampling will involve an increase in number of samples taken (i.e., increasing n). The two-class plans shown in Table 9–1 and 9–2 give some idea of how such plans might be affected by changing n. This is illustrated in another manner in Table 9–3 in which the probability of detecting one or more positive samples in batches with very low occurrence of defectives is given. Thus, if one unit in every 1,000 were contaminated, even a sample of $n = 500$ would result in a 61% chance of accepting the lot; that is, there is only a 39% chance of finding one or more contami-

Table 9–1 Probability of Acceptance (P_a) for Two-Class Sampling Plans with $n = 10$ to $n = 300$ and $c = 0$

Composition of lot		*Number of sample units (n)*						
% acceptable	*% defective*	*10*	*20*	*30*	*50*	*100*	*200*	*300*
99	1	0.90	0.82	0.74	0.61	0.37	0.13	0.05
98	2	0.82	0.67	0.55	0.39	0.13	0.02	<
97	3	0.74	0.54	0.40	0.22	0.05	<	
96	4	0.66	0.44	0.29	0.13	0.02		
95	5	0.60	0.36	0.21	0.08	0.01		
94	6	0.54	0.29	0.16	0.05	<*		
93	7	0.48	0.23	0.11	0.03			
92	8	0.43	0.19	0.08	0.02			
91	9	0.39	0.15	0.06	0.01			
90	10	0.35	0.12	0.04	0.01			

*< means $P_a < 0.005$.

Table 9–2 Probability of Acceptance (P_a) for Two-Class Sampling Plans with $n = 300$ to $n = 5000$ and $c = 0$

Composition of lot		*Number of sample units (n)*					
% acceptable	*% defective*	*300*	*500*	*1000*	*2000*	*3000*	*5000*
99.9	0.1	0.74	0.61	0.37	0.14	0.05	0.01
99.8	0.2	0.55	0.37	0.14	0.02	<	<
99.7	0.3	0.41	0.22	0.05	<		
99.6	0.4	0.30	0.13	0.02			
99.5	0.5	0.22	0.08	0.01			
99.4	0.6	0.16	0.05	<*			
99.3	0.7	0.12	0.03				
99.2	0.8	0.09	0.02				
99.1	0.9	0.07	0.01				
99.0	1.0	0.05	0.01				

*< means $P_a < 0.005$.

nated units with $n = 500$ when the contamination rate is 0.1%. Clearly, such a probability of detection/rejection would be unacceptable for highly toxic contaminants or severe microbiological hazards.

It is unlikely that the sampling plans with n between 300 and 5,000 would be used for microbiological testing because of laboratory effort and cost. It may be possible, however, to conduct simple nonmicrobiological tests on large numbers of sample units. For example, the options for testing a batch of canned food may be to conduct a simple test such as pH measurement, visual observation of can seams, examination for vacuum or swelling, or microscopic examination to identify potentially hazardous cans. While microbiological analysis involving conventional cultural media may be very effective in identifying contamination, a large number of examinations would not be practical. A measurement of product pH may be less likely to identify a container in which growth has taken place, but large numbers of analytical units can be examined. Because contamination may occur at a relatively low rate, a simple test that allows examination of a large number of units would be more effective.

Table 9–3 The Probability of Obtaining One or More Defectives in a Sample of n Sample Units with Proportion p of the Lot Defective

Number of sample units examined per sample, n	*Probability of obtaining one or more defectives with the following proportions (p) of defective*			
	0.01	*0.001*	*0.0001*	*0.00001*
200	0.87	0.18	0.02	0
1000	1.00	0.63	0.10	0.01
2000	1.00	0.86	0.18	0.02
3000	1.00	0.95	0.26	0.03
4000	1.00	0.98	0.33	0.04
5000	1.00	0.99	0.39	0.05

A common question is how many analytical units would need to be tested to provide some level of confidence that a lot will be rejected if it is hazardous to consumers? In general, the number of samples necessary will depend on the level of defective units that can be tolerated and the desired confidence to detect a defective lot. If, for example, a two-class attributes sampling plan is applied as a *zero tolerance* plan (i.e., $c = 0$), a defective lot would be detected as soon as at least one sample unit is defective. When sampling a given lot, the probability of finding at least one defective unit depends on the actual percentage of defectives in that lot and the number of sample units drawn. Hence, to select a sampling plan and derive the number of sample units (i.e., n), two decisions are first necessary: a decision on the percentage of defective units in a lot that defines a defective lot, and a decision on the required confidence level (e.g., 95%), i.e., on the desired probability of detecting at least one defective unit, if the lot is defective.

For such two-class plans, Table 9–4 provides guidance on the number of random sample units that would be needed to provide 90, 95, and 99% confidence that at least one defective sample unit will be detected in lots with defective levels ranging from 0.1 to 50% (Cannon & Roe, 1982). For example, if a confidence level of 95% is desired that a lot does not have 2% or greater defectives, then it would be necessary to collect 149 sample units from the lot. To increase the level of confidence to 99%, it would be necessary

Table 9–4 Number of Sample Units Required for 90, 95, and 99% Confidence of Detecting at Least One Defective Sample Unit in Lots with Defective Levels Ranging from 0.1 to 50%

Desired Confidence Level (%)	*Definition of Defective Lot (%)*	*Number of Sample Units*
90	50	4
	20	11
	10	22
	5	45
	2	114
	1	230
	0.1	2302
95	50	5
	20	14
	10	29
	5	59
	2	149
	1	299
	0.1	2995
99	50	7
	20	21
	10	44
	5	90
	2	228
	1	459
	0.1	4603

to collect 228 sample units. If the percentage of defectives that should be detected is 10%, then 44 sample units would be required for the same 99% level of confidence. As the proportion of defectives falls below about 1%, the intensity of sampling and testing increases to levels that are impractical in a microbiology laboratory.

In another form of tightened sampling, certain foods may be sampled at greater than normal frequency. The sampling plans discussed in Chapters 6–8 assume that every lot is sampled, and thus, frequency of sampling is not discussed. In practice, however, microbiological criteria and sampling plans are commonly established for a wide variety of foods, but very few lots are actually sampled. The established microbiological criteria define what is considered acceptable and are often applied on an as needed basis (see Table 4–3 and Exhibit 9–1). In practice, the frequency of testing a food can range from none, or rarely, to 100% of the lots. Increasing the application of an established sampling plan beyond that which is routinely applied would be an additional form of tightened sampling.

9.4 EXAMPLE OF THE INFLUENCE OF SAMPLING PLAN STRINGENCY IN DETECTING DEFECTIVE LOTS

Sampling plans of different stringency that are applied at different steps in the food chain or in an operation may reveal differing levels of control. For example, a drink mix containing nonfat dry milk (NFDM) was prepared by mixing dry ingredients. No kill step was applied. The ingredients were found to be negative for *Salmonella* using a case 14 sampling plan (see Chapter 8). Yet, the finished product was found to be positive using the same sampling plan. The ingredients were usually of a much greater quantity than the lot size of the product, such that a lot of NFDM tested at case 14 ($n = 30 \times 25$ g) would be used in several smaller lots of drink mix, also tested at case 14. The NFDM made up about 60% of the finished product. Thus, a greater quantity of NFDM was being tested as a component of the finished product than was tested to approve its use as an ingredient. This is illustrated in the following:

- Lot size of NFDM = 100,000 kg
  Each lot of NFDM sampled at case 14 ($n = 30$).
  Thus, 30×25 g = 750 g tested per 100,000 kg.
- Lot size of drink mix = 10,000 kg
  Each lot sampled at case 14 ($n = 30$).
  Thus, 30×25 g tested = 750 g per 10,000 kg.
- Drink mix = 60% NFDM or 6,000 kg NFDM per 10,000 kg lot of drink mix
  100,000 kg of NFDM was used to make 16.67 lots of diet drink.
  Since 750 g was tested per lot of drink mix, this meant that a total of 12,502.5 g was analyzed per 16.67 lots.
  Thus, 60% NFDM × 12,502.5 g of drink mix = 7,501.6 g of NFDM analyzed as a component of the drink mix.

In this example, 750 g of NFDM was sampled and tested for acceptance as an ingredient, but a total of 7,501.6 g was sampled and tested as a component of the finished drink mix. Thus, the NFDM was being tested at about a 10-fold greater stringency as a component of the finished product than when being tested for acceptance as an ingredient. Such experience should lead to a tightened sampling of the NFDM and/or a change of suppliers.

Thus, the potential exists for low level contamination to be missed by one sampling plan, but be detected by another more stringent sampling plan applied at another step in a food operation or elsewhere in the food chain. This example demonstrates that the choice of stringency of an attributes sampling plan applied to an ingredient must take into consideration lot size, use level, and microbiological criteria applied to the finished product. Further, if microbiological records are reviewed for the purpose of problem solving, negative results for ingredients should not eliminate them as a possible source of contamination until the balance between ingredient and finished product sampling plan stringency is considered.

9.5 SELECTING THE SAMPLING PLAN ACCORDING TO PURPOSE

Before choosing a sampling plan, the objective of the sampling should be clearly defined. Is the sampling intended to differentiate between acceptable and unacceptable product or to investigate and discover the cause of a problem? The stringency of the plan and the use of biased and unbiased sampling will depend greatly on the objective of the sampling.

One of the most difficult situations in choosing a sampling plan for either investigational or acceptance sampling is when there is zero tolerance for the attribute being tested, such as *Salmonella, Listeria monocytogenes,* or *E. coli* O157:H7. No sampling plan, short of 100% analysis of the food, can ensure complete absence of the defect. Thus, the investigator is faced with a situation where a decision must be made as to what plan stringency is adequate, but still practical to perform. Plan selection is much easier when a food safety objective or other established limit exists. The plan stringency needed to detect levels of the defect can then be determined. Sometimes the established limit may be too low to be practically sampled and tested for either investigational purposes or to differentiate between acceptable and suspect product. However, the investigator can at least determine the plan stringency that would be needed, even if it is not practical.

Even when the official regulatory position is "zero tolerance," there may be prescribed sampling plans that are widely used. Such is the case with *Salmonella* in the US. The US Food and Drug Administration has established a zero tolerance policy for *Salmonella* in most processed foods, yet employs sampling plans equivalent to International Commission on Microbiological Specifications for Foods cases 13–15 (see Chapter 8), referred to as categories I, II, and III in the FDA Bacteriological Analytical Manual (FDA, 1998). Even at the highest stringency ($n = 60$), a lot containing 2% defectives would test negative 30% of the time. It is not uncommon in the US to select a plan with a higher stringency to provide some security that the defect could be detected with greater sensitivity than if the normal attribute plan were used.

When selecting a sampling plan, the population to be sampled must be determined along with the potential sources of the problem. Consider for example a situation where three slicers are used to slice three varieties of meat. After slicing, equal portions of each variety are placed into a package and sealed. If one slicer has not been cleaned properly, it could be a source of contamination, particularly for the first half hour or more of slicing. A sampling plan that does not select samples from each slicer, or alternately does not include a sufficient number of samples to be relatively certain to include product from each slicer, will fail to detect the source of the problem. Clearly, if the sample results can identify a specific slicer, then a biased sample that ensures that the defective slicer is represented will be more efficient than a random sample of a sufficient number to be relatively

certain each filler head is represented. This is especially true if the contamination may be time related, and one would like to have samples from each slicer for each time segment.

9.6 REDUCED SAMPLING

Reduced sampling involves sampling at a lesser frequency or, if circumstances warrant, not at all. When lots are sampled, however, the sampling plans described in Chapter 8 are recommended. Conditions that can lead to reduced sampling frequency may relate to the food or the source of the food (Exhibit 9–1). For example, reduced sampling is warranted when an audit of a food operation, as described in Chapter 4, leads to the conclusion that an adequate food safety management system is in place. Furthermore, foods of low risk (e.g., in which pathogens die or cannot multiply, or which have a long history of safety) could be sampled at a lesser frequency.

9.7 REFERENCES

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Chapter 10

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
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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-

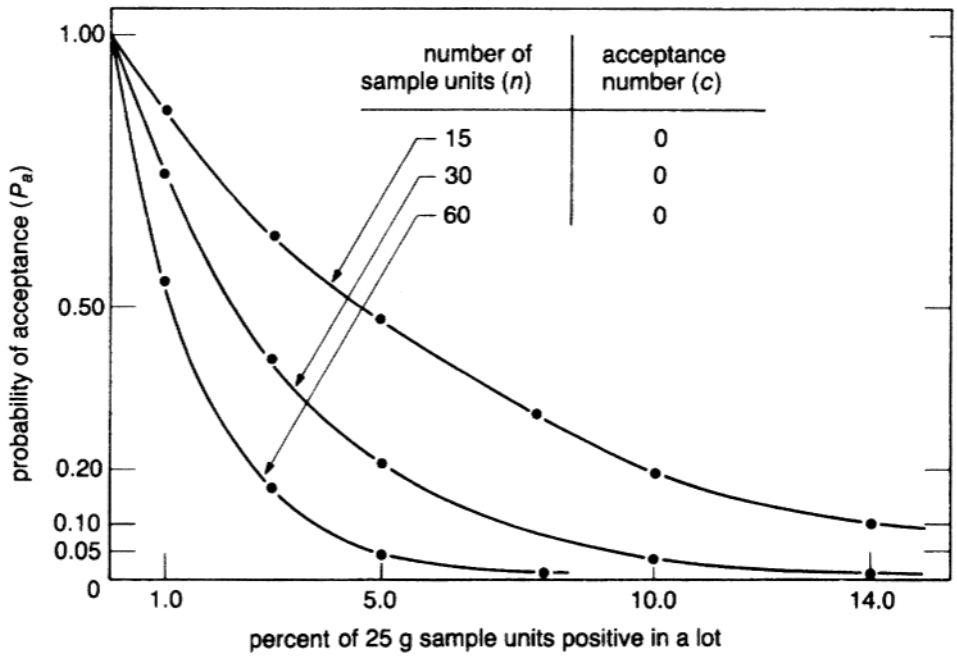


Figure 10–1 Relations between the sampling plan and the degree of security conferred—operating characteristic curves for three sampling plans proposed for *Salmonella*.

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.

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).

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.

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

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

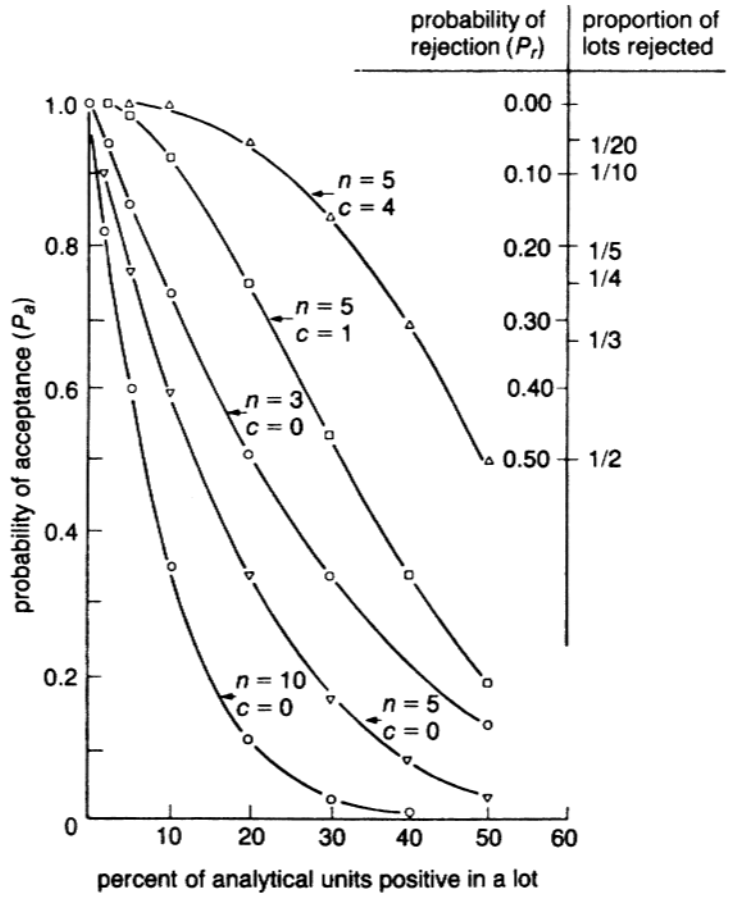


Figure 10–2 Operating characteristic curves for sampling plans with small numbers of analytical units.

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

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.

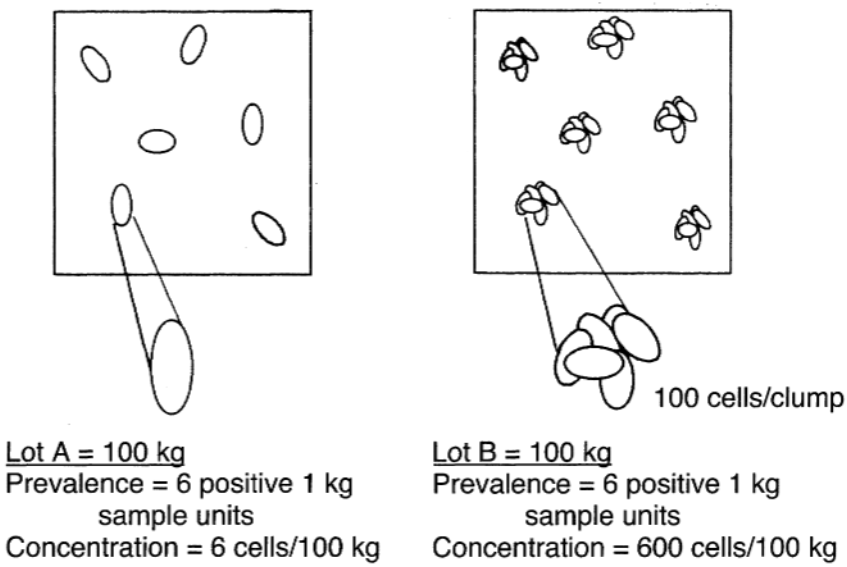


Figure 10–3 Illustration of two lots of product that upon analysis, may yield the same prevalence, but have 100-fold different concentrations.

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-

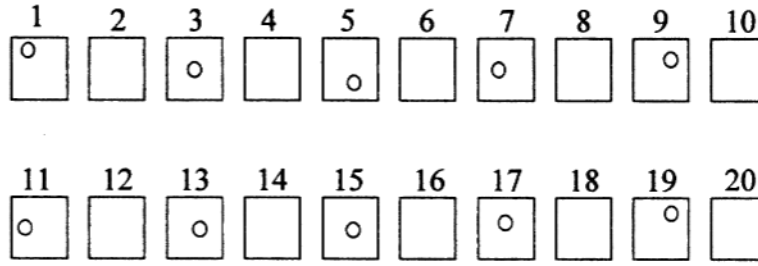


Figure 10–4a Illustration of regular (nonrandom) distribution throughout a production lot of 20 boxes (Lot A).

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.

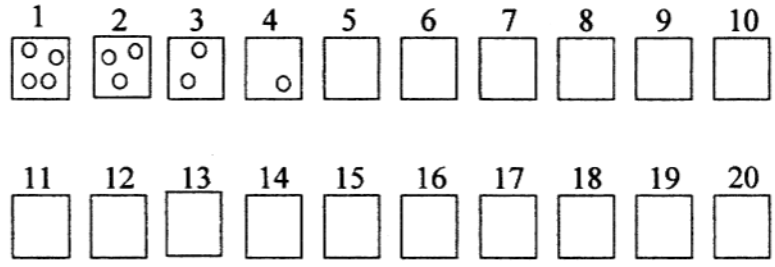


Figure 10–4b Illustration of heterogeneous (nonrandom) distribution throughout a production lot of 20 boxes (Lot B).

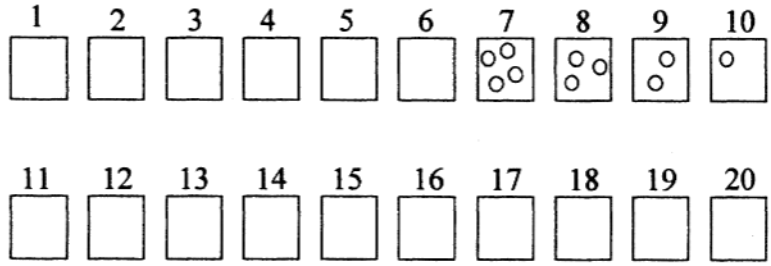


Figure 10–4c Illustration of heterogeneous (nonrandom) distribution throughout a production lot of 20 boxes (Lot C).

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

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

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-

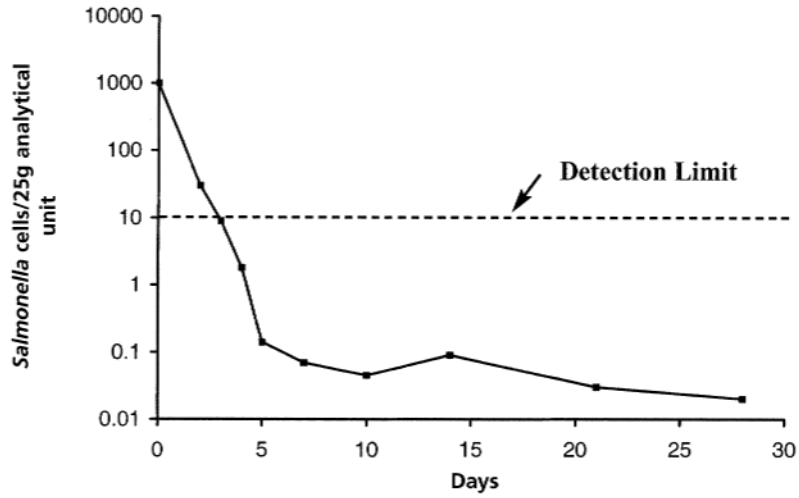


Figure 10–5 An example of a survival curve for *Salmonella* under dry conditions.

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 11

Sampling To Assess Control of the Environment

11.1 Introduction
11.2 Principles of GHP
11.3 Post-Process Contamination
11.4 Establishment and Growth of Pathogens in the Food Processing Environment
11.5 Measures To Control Pathogens in the Food Processing Environment
11.6 Sampling the Processing Environment
11.7 References

11.1 INTRODUCTION

This chapter discusses the importance of microbiological testing to assess the risk of product contamination from the environment. Preventing contamination of ready-to-eat foods is emphasized. While limited to control of pathogens, the concepts also can be applied to microbial spoilage. Routine environmental sampling most likely will be applied in certain food processing plants and less likely at other steps along the food chain.

The microbiological safety of industrially manufactured foods requires effective design and implementation of Good Hygienic Practices (GHP) and Hazard Analysis Critical Control Point (HACCP) systems. GHP includes those prerequisite conditions that are necessary for pathogen control and implementation of an effective HACCP plan.

Food microbiologists have traditionally focused on the microbial ecology of raw materials and finished products. Such knowledge has been essential for limiting the number of pathogens in raw materials and for establishing critical limits at critical control points (CCPs) in an HACCP plan. It is, however, becoming clearer that some knowledge of the ecology of the food processing environment also is essential. Reports of foodborne outbreaks due to commercially manufactured foods reveal that many can be traced to weaknesses in GHP implementation. In some cases, post-process contamination has resulted from contact with contaminated equipment, such as during chilling, holding, conveying, or packaging.

In HACCP systems, CCPs are applied to prevent, eliminate, or reduce microbial hazards to acceptable levels. For many products, CCPs typically include a kill step (cooking, sterilization), but in minimally processed foods, CCPs may involve manipulation of one or more factors, such as low temperature storage, reduced a_w, and/or reduced pH, specifically designed to control growth of pathogens. Establishment of appropriate critical limits at specific processing steps ensures safety of the products, taking into account possible fluctuations during operations.

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Experience indicates, however, that optimal application of a HACCP plan or a GHP program may not guarantee that contamination from the processing environment will not occur. Even the best surgical operations in hospitals carry the possibility of contamination that may be life threatening to the patient. Thus, it is possible to reduce, but not prevent, or eliminate, the likelihood of contamination when food is exposed to a processing environment (whether in an open or closed system). When this potential is recognized, certain manufacturers may decide to establish procedures to monitor the implementation of GHP and, where contamination can result in unsafe foods, to establish a routine sampling program to assess control of the environment.

11.2 PRINCIPLES OF GHP

The basic elements of GHP are described in Codex Alimentarius Commission's document *General Principles of Food Hygiene* (CAC, 1997). Specific recommendations for individual products or product groups (e.g., canned foods, aseptically processed and packaged low-acid foods, spices and dried aromatic foods, infant formulae, frozen foods, fruits, and vegetables) have been addressed in several documents (CAC, 1994, 1995).

Product-specific documents also have been issued by other international organizations to provide guidance on GHP implementation (IDF, 1992; IOCCC, 1993; ECFF, 1996; EC, 1997) and hygienic design of premises and processing equipment (EHEDG, 1997).

Some of the more important prerequisite programs that can minimize contamination from the processing environment are:

- layout of processing lines and control of the movement of personnel and mobile equipment to minimize cross-contamination from raw materials to finished product.
- equipment design and location for cleanability.
- cleaning and disinfecting procedures that are targeted toward the pathogens of concern to the process.
- scheduled preventive maintenance to minimize breakdowns during operation.
- appropriate waste removal.
- training and behavior of personnel relative to the target pathogens.

Visual and other sensory inspections are valuable tools to assess adherence to, and the effectiveness of, GHP. While such inspections can be performed routinely by trained specialists, all workers should be trained to be constantly vigilant and to report deviations from normality. Microbiological surveillance for pathogens or appropriate indicators provides supporting information to assess whether the environment is under control.

The potential for contamination should be considered in the design and implementation of the GHP program and, if contamination is likely to occur, some means for assessing its effectiveness should be adopted. Each manufacturing facility must consider control measures that are appropriate for the conditions of operation, the food, the process, and the pathogen(s) of concern to minimize in-process contamination (Cordier, 1997; Tompkin *et al.*, 1999).

11.3 POST-PROCESS CONTAMINATION

Despite strict control of all CCPs to ensure destruction of pathogens in raw materials, foods may subsequently become contaminated within the food operation. This typically results from two general circumstances:

1. adding a contaminated ingredient following a kill step; or
2. an environmental contaminant.

11.3.1 Contaminated Ingredients

Many cases could be cited where pathogens were introduced into ready-to-eat foods through contaminated ingredients, such as cocoa powder used to manufacture chocolate (*Salmonella*; Gästrin *et al.*, 1972), paprika used to coat potato chips (*Salmonella*; Lehmacker *et al.*, 1995), onions added to cheese curd (*Clostridium botulinum*; De Lagarde, 1974), sprouts added to sandwiches (*Escherichia coli* O157:H7; CDC, 1997), and hazelnut purée added to yogurt (*C. botulinum*; O'Mahoney *et al.*, 1990).

Identifying high-risk ingredients during the hazard analysis when establishing a HACCP plan may involve epidemiologic or other published data, expert knowledge, and company databases on the incidence of pathogen(s) in the processing plant or in product. This should lead to the adoption of control measures, such as selection of suppliers, sampling plans for sensitive ingredients, or modification of recipes or processes. The addition of contaminated ingredients to ready-to-eat foods will not be further discussed. The principles described in the previous chapters are applicable to controlling this route of contamination.

11.3.2 Environmental Contamination

Foods cooked in the container in which they are sold and foods packaged using aseptic packaging systems are protected from contamination. Foods that are pasteurized, cooked, or undergo other processes that reduce pathogens and are subsequently exposed to the environment may acquire microorganisms before filling or packaging. Manufacturers of such foods take every reasonable precaution to prevent contamination from occurring after the food is cooked and before it is packaged, but it is impossible to prevent completely.

Some ready-to-eat foods are not cooked, but receive mild treatments that may alter their microflora, or they acquire microflora from the processing environment. For example, a range of fish products (e.g., pickled, gravad, or cold-smoked) receive very mild treatments, yet they will much more likely carry a microflora that reflects the microorganisms associated with the post-processing equipment and environment.

Ready-to-eat foods typically have been subjected to a wide range of processing conditions, some of which are intended to eliminate pathogens. Subsequent handling, storing, conveying, sorting and packaging, etc., create opportunities for contamination. Even the environment to which the foods are exposed can be a potential source of pathogens. Pathogens such as *Salmonella* or *Listeria monocytogenes*, and also spore-formers, can be introduced into a food operation by a number of vectors. Conditions permitting, they may become established and multiply, particularly in sites in the processing environment that are difficult to clean and disinfect. If this occurs, during production the pathogens may be transferred to product contact surfaces in the vicinity of the site and pass downstream with the flow of the product. In recent years, evidence has accumulated indicating that some pathogens can become established in the processing environment and persist for long periods, even years.

The probability of contamination of the food product increases with prevalence; the concentration of the pathogen is also influenced by the type of process, the hygiene pro-

cedures, and whether those procedures will spread the pathogen if they are not properly performed (Holah *et al.*, 1993). Severe failure of one or more steps in GHP may lead to high levels of pathogens in the processing environment and to product contamination (Langfeldt *et al.*, 1988; Steuer, 1992).

11.3.3 Examples of Outbreaks Due to Recontamination

Numerous outbreaks of illness due to the consumption of processed foods that have been recontaminated have been reported and described (Table 11–1). The number of implicated foods from industrial establishments, however, is relatively small in comparison to the total, with most outbreaks being related to foods prepared in food service operations, catering operations, homes, etc. (Beckers *et al.*, 1985; ICMSF, 1988; Motarjemi & Käferstein, 1997). The size of the outbreaks associated with industrially manufactured foods, however, can be very large due to the quantity of product and distribution throughout a country or region. In addition, some outbreaks have been known to last several months before they are recognized and the source of the manufactured food is identified. The causes and origin of the contamination are seldom published, but sufficient data are available to demonstrate the importance of post-process contamination.

The following examples illustrate the importance of adequate GHP procedures and the establishment of sites or niches supporting microbial growth and survival.

Salmonellosis due to consumption of chocolate presents a typical example. In an outbreak due to *S. eastbourne* (Craven *et al.*, 1975), inadequate separation between areas for storage and handling of raw and roasted cocoa beans led to contamination of roasted beans. Analysis of environmental samples identified the outbreak strain at different points, in particular on the wheels of a forklift used in both the raw and roasted bean areas.

In a large listeriosis outbreak in Switzerland due to the consumption of a soft cheese, Vacherin Mont d'Or, the *L. monocytogenes* serotype causing the outbreak was traced back to several ripening centers collecting otherwise sound cheeses from different small cheese makers. In particular, the serotype causing the outbreak was found on the trays used for cheese storage during maturation (Bille, 1990).

11.4 ESTABLISHMENT AND GROWTH OF PATHOGENS IN THE FOOD PROCESSING ENVIRONMENT

Effective GHP procedures targeted toward the pathogens of concern require knowledge of the incidence, distribution, fate, and behavior of the pathogens in food processing environments. A fundamental question that must be considered for each manufacturing facility is whether contamination can occur due to microorganisms that are merely transient in the premises or pathogens that become established in the premises.

11.4.1 Transient Versus Established Microorganisms

Transient microorganisms are introduced into a food plant through raw materials, humans, packaging supplies, etc., and do not become established in the environment and multiply. Transients, however, can contribute significantly to the types and numbers of microorganisms on the resulting food. Each raw agricultural commodity carries a wide variety of microorganisms and, possibly, certain pathogens (ICMSF, 1998). In general, industrial operations convert raw commodities into further processed products under con-

Table 11–1 Examples of Outbreaks Attributed to Environmental Contamination

Product	*Pathogen*	*Comments*	*Reference*
Ice cream	*S. enteritidis*	Pasteurized ice cream mix in tanker truck previously used for transporting raw liquid eggs	Hennessy *et al.* (1996)
Infant formulae	*S. ealing*	Contamination from the processing environment, insulation material of the drying tower	Rowe *et al.* (1987)
Soft cheese	*S. berta*	Cheese ripening in buckets previously used for chicken carcasses	Ellis *et al.* (1998)
Cooked sliced ham	*S. typhimurium*	Cooked ham placed into containers previously used for curing raw pork	Llewellyn *et al.* (1998)
Chocolate	*S. napoli*	Possibly contaminated water used in double-walled pipes, tanks, and other equipment	Gill *et al.* (1983)
Chocolate	*S. eastbourne*	Contamination from the processing environment	Craven *et al.* (1975)
Butter	*L. monocytogenes*	Contamination from the processing environment	Lyytikainen *et al.* (2000)
Hot dogs	*L. monocytogenes*	Contamination from the processing environment	Anonymous (1999)
Canned salmon	*C. botulinum*	Contamination from the processing environment, cooling water	Anonymous (1984); Stersky *et al.* (1980)
Lasagne	*S. aureus*	Growth of *S. aureus* in the processing equipment, improper cleaning	Woolaway *et al.* (1986); Aureli *et al.* (1987)
Different foods	*E. coli* O157:H7	Contaminated meat grinder and equipment at retail level	Banatvala *et al.* (1996)
Chocolate milk	*Y. enterocolitica*	Probably during manual mixing of pasteurized milk and chocolate syrup	Black *et al.* (1978)

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