Microorganisms in Foods 7

Microbiological Testing in Food Safety Management

ICMSF



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Chapter 6

Concepts of Probability and Principles of Sampling

6.1 Introduction
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6.3 Population and Sample of the Population
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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.

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6.4 CHOOSING THE SAMPLE UNITS

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

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

6.5 THE SAMPLING PLAN

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

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

6.6 THE OPERATING CHARACTERISTIC FUNCTION

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

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

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

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

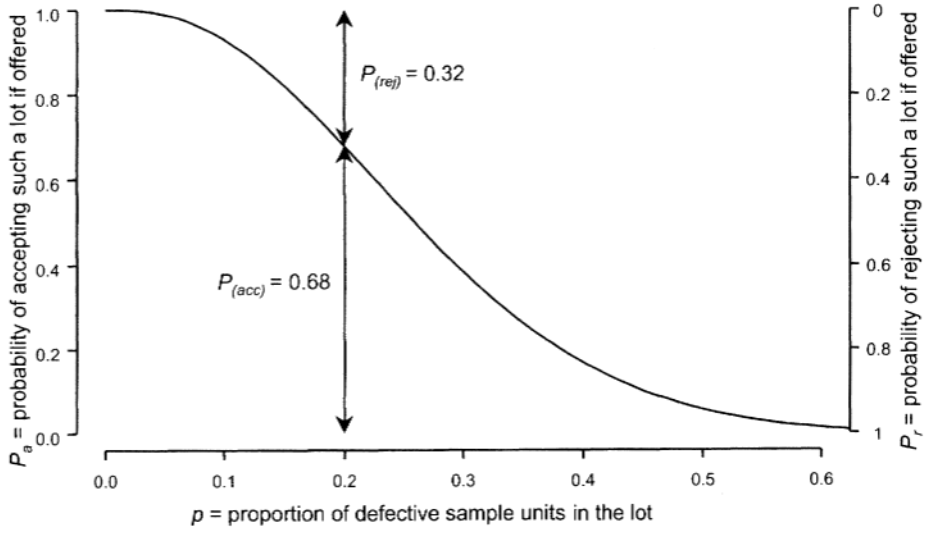


Figure 6–1 The operating characteristic curve for a sampling plan with $n = 10$, $c = 2$, i.e., the probability of accepting lots, in relation to the proportion defective among the sample units in the lot being examined. If, for example, the lot comprises 20% ($p = 0.2$) defective sample units, the lot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

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

6.7 CONSUMER RISK AND PRODUCER RISK

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

6.8 STRINGENCY AND DISCRIMINATION

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

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

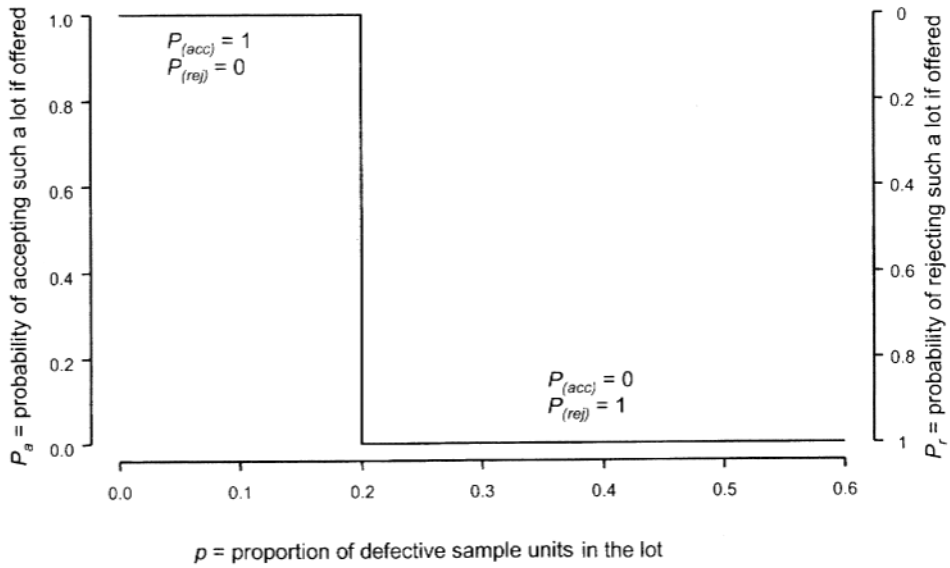


Figure 6.2 The operating characteristic curve (OC) for the idealized situation of complete discrimination between lots with a proportion of defective sample units below 20% and lots with such a proportion above 20%.

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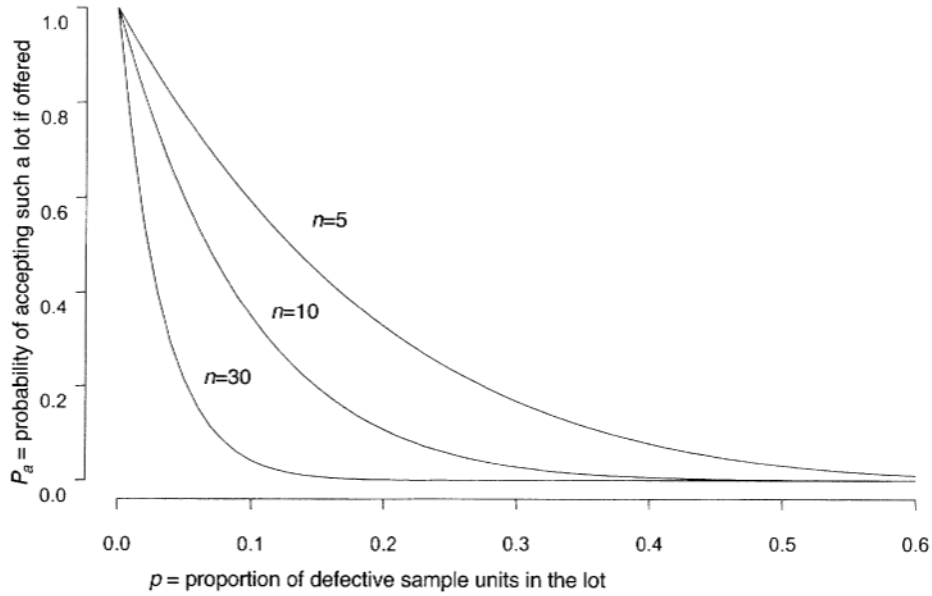


Figure 6–3 Operating characteristic curves for sampling plans with $n = 5$, $n = 10$, $n = 30$ and $c = 0$. With increasing numbers of sample units examined (n), the slope of the OC curve becomes steeper.

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

6.9 ACCEPTANCE AND REJECTION

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

6.10 WHAT IS A LOT?

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

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

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

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

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

6.11 WHAT IS A REPRESENTATIVE SAMPLE?

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

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

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

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

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

6.12 CONFIDENCE IN INTERPRETATION OF RESULTS

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

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

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

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

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

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

6.13 PRACTICAL CONSIDERATIONS

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

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

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

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

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

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

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

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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
10.7 Discrepancies between Original and Retest Results
10.8 Summary
10.9 References

10.1 INTRODUCTION

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

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

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

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

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

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

10.2 THE CONCEPT OF ZERO TOLERANCE

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

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

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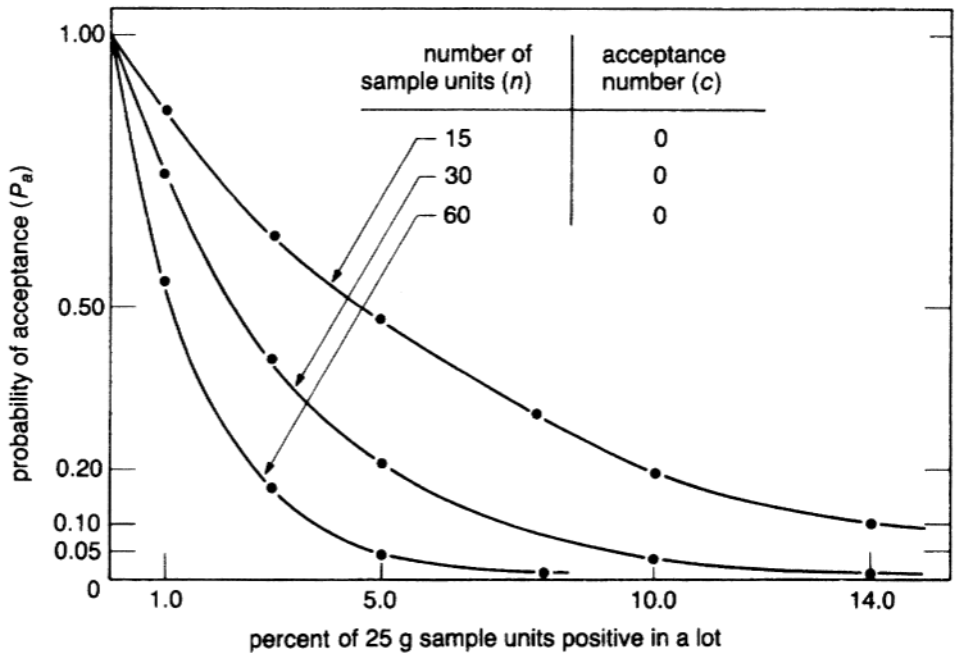


Figure 10–1 Relations between the sampling plan and the degree of security conferred—operating characteristic curves for three sampling plans proposed for *Salmonella*.

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

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

10.3 THE NEED FOR COMPROMISE

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

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

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

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

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

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

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

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

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

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

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

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

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

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

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

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

10.5 PROBLEMS IN THE IMPLEMENTATION OF STRINGENT SAMPLING PLANS

10.5.1 Conceivable Alternative Plans

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

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

10.5.2 Fallacious Procedures

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

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

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

10.6 RELATION TO COMMERCIAL PRACTICE

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

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

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

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

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

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

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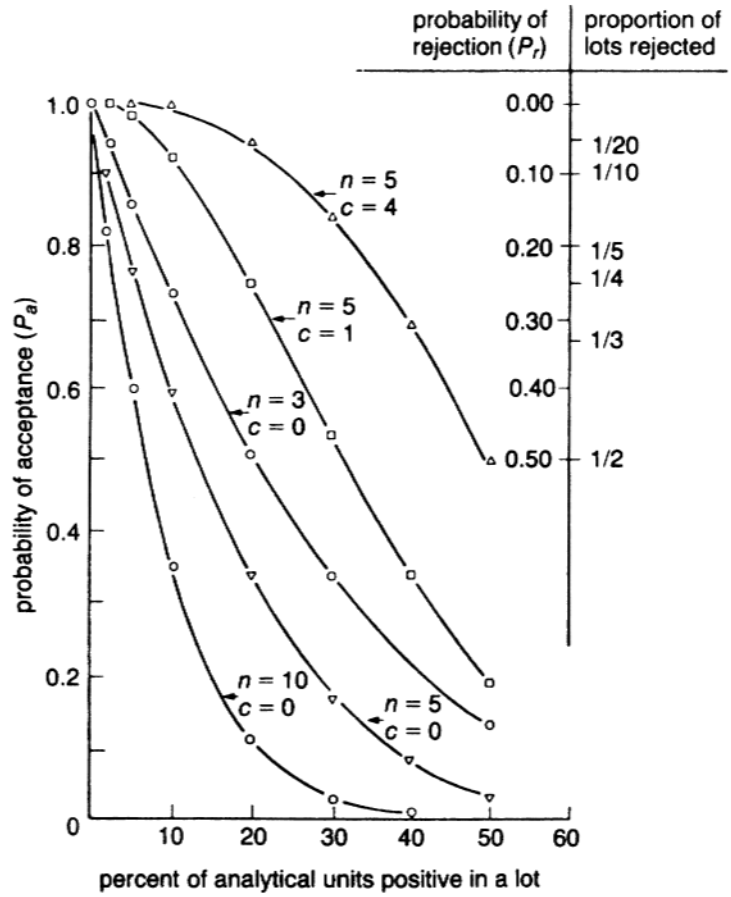


Figure 10–2 Operating characteristic curves for sampling plans with small numbers of analytical units.

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

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

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

10.7 DISCREPANCIES BETWEEN ORIGINAL AND RETEST RESULTS

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

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

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

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

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

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

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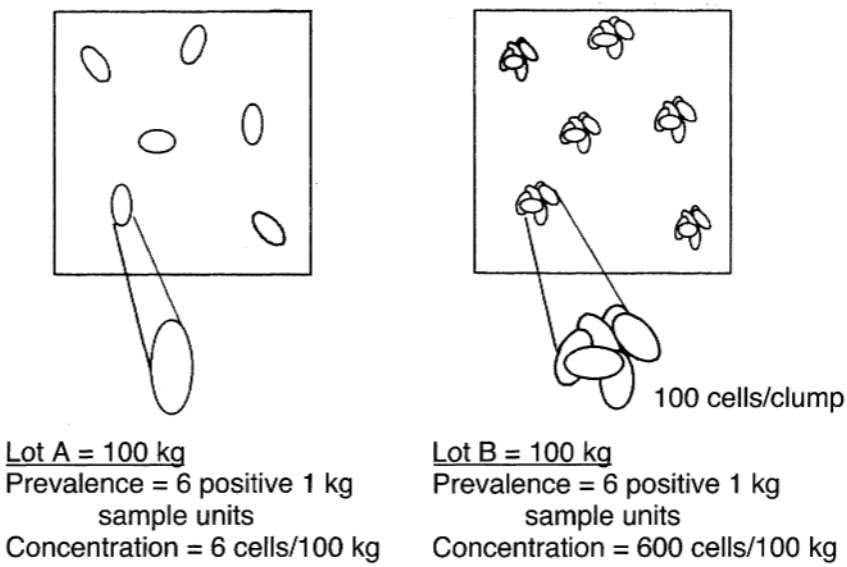


Figure 10–3 Illustration of two lots of product that upon analysis, may yield the same prevalence, but have 100-fold different concentrations.

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

10.7.1 Nonrandom Distribution

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

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

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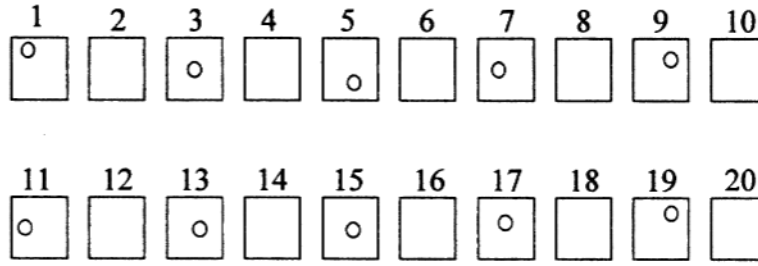


Figure 10–4a Illustration of regular (nonrandom) distribution throughout a production lot of 20 boxes (Lot A).

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

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

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

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

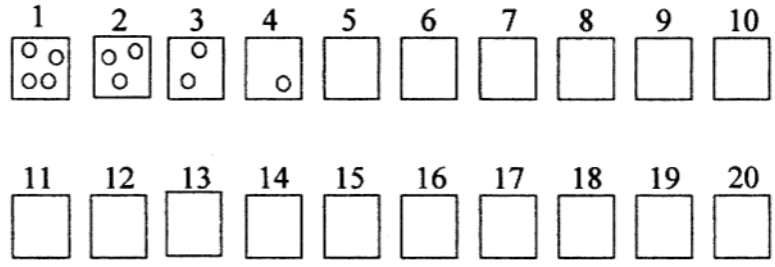


Figure 10–4b Illustration of heterogeneous (nonrandom) distribution throughout a production lot of 20 boxes (Lot B).

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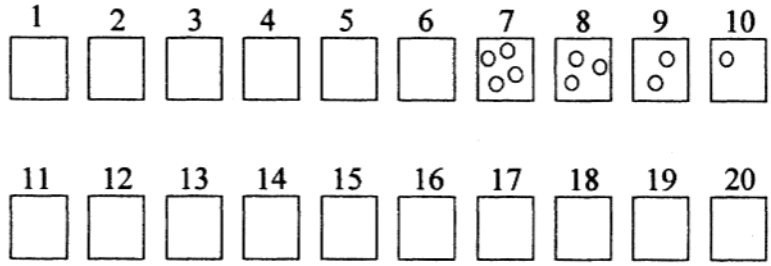


Figure 10–4c Illustration of heterogeneous (nonrandom) distribution throughout a production lot of 20 boxes (Lot C).

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

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

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

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

10.7.2 Low Prevalence of Contamination in a Lot or Batch

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

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

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

10.7.3 Low Prevalence of Contamination across Many Batches

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

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

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

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

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10.7.4 Change in the Concentration of Contamination

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

10.8 SUMMARY

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

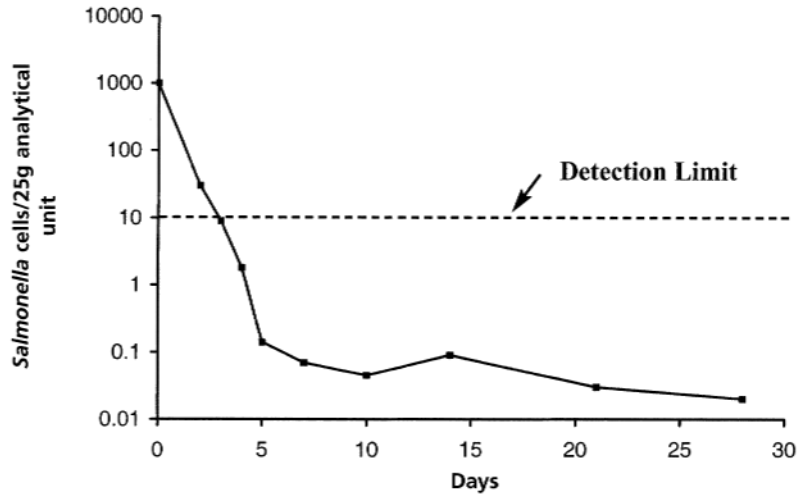


Figure 10–5 An example of a survival curve for *Salmonella* under dry conditions.

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ena described above can be involved. Contamination may be heterogeneously and non-randomly 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.

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Chapter 12

Sampling, Sample Handling, and Sample Analysis

12.1 Introduction
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12.1 INTRODUCTION

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

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

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

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

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

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

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

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

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

12.2 COLLECTION OF SAMPLE UNITS

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

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

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points in the food chain to judge whether imported or commercially available foods comply with current legislation. Intensive sampling by the same authorities would be carried out during an outbreak to identify its source. In commerce, sampling is performed to confirm compliance with specifications between suppliers and customers. Samples taken at intermediate steps of the processing line, from surfaces of equipment in contact with the product or from the processing environment, allow the food processor to verify adherence to Good Hygienic Practices (GHP) and the efficacy of preventive measures.

12.2.1 Steps in the Sampling Procedure

Initial sampling represents the step where physical samples are taken, according to a preestablished sampling plan, at specified sampling points. *Sample handling* encompasses all subsequent steps including the description, recording, and labeling of the samples, their intermediate storage before dispatch, their transport to the analytical laboratory, and their reception, registration, and storage up to the preparation of the analytical unit(s) before analysis. All steps up to the analysis must be controlled to ensure the quality of the sample units and to allow reliable traceability. The guidelines provided in this chapter are considered good practice and should help to optimize results.

12.2.2 Containers

Unless sample units comprise products in their original packaging, such as sachets, tins, boxes, bottles, or cups, the samples drawn must be placed in clean and sterile containers. The containers used should be of appropriate size and have a sufficiently wide opening to allow clean and easy transfer of the samples. Depending on the sample unit, different containers may be used, such as plastic bottles, jars, or bags, metallic cans, or boxes. Glass containers, however, should not be used in manufacturing facilities where breakage could lead to the presence of glass in the products.

Using sterile disposable containers is the most convenient, but for reasons of cost this may not be possible. If reusable containers are used, they should be thoroughly washed, rinsed, dried, and sterilized before further use. Depending on the material, containers are sterilized either at 121 °C for 15 min in an autoclave, or for at least 1 h at 160 °C in a hot air oven. Details of the different options for the sterilization of containers can be found in APHA (1992).

It is important to prevent samples from leaking during handling and, in particular, during transport. Screw caps are the most appropriate closing devices for jars and bottles, and plastic bags must be sealed securely. If rubber or cork is used for caps or closures, it must be confirmed that no adverse interactions with the samples can occur.

12.2.3 Sampling Utensils and Devices

Utensils used to collect the sample units should be sterile and adapted to permit the most appropriate and representative sampling according to the type of food. Sterile scissors, knives, saws, can openers, and other tools can be used to open boxes, packs, sacks, or cans. Samples of the actual food product can then be collected using sterile scoops, spoons, triers, probes, drill bits, forks, cork-borers, pipettes, or swabs, depending on the type of food sampled. Examples and further details can be found in FAO (1990).

For environmental sampling, utensils need to be adapted to the sampling site, with no limitations to their type. Sponges or swabs are most suitable to sample food contact sur-

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faces, floors, or walls. Scrapers, scoops, spatulas, or brushes are suited to collecting residues in cracks, crevices, or underneath equipment. Pipettes (plastic) can be used to collect water in drains, and air sampling devices can be used to monitor the air.

Containers and utensils should be packaged individually and be presterilized, thereby avoiding the need for disinfection between samples. Disposable plastic gloves are recommended when collecting certain samples (e.g., sponge samples from equipment), a new glove being used for each sample. Sterilization of utensils by means of a torch should be avoided and may even be dangerous (e.g., risking explosions in dusty environments).

Sampling ports or taps directly installed on equipment, such as tanks, are usually sterilized in situ by means of steam. They require particularly careful attention (design, cleaning, and sterilization procedures) to avoid contamination of the product.

12.2.4 Sampling Procedures

The method of sampling should be adjusted to fit the purpose. For lot acceptance determinations, the intent is to collect sample units that are representative of the lot. To assess control of the processing environment, a routine procedure should have been developed including sample sites and method of sampling. When investigating the source of a problem, the sampling sites, foods, ingredients, times, frequencies, etc. are not predetermined and are left to the discretion of the sampler. While it is desirable to make every effort to collect samples that fulfill the intended purpose, the safety of the individual collecting the sample, and others who may be affected, takes precedence. Under dangerous circumstances, sample collection should not be attempted. Likewise, if the method of sampling can jeopardize the food and render it injurious to consumers, the sampling procedure should be changed or the sample should not be collected.

Samples should be collected by persons previously trained and instructed, using appropriate methods and, above all, aseptic techniques. The timing and sampling point in the food chain or a food process may be crucial for data interpretation. When regulatory authorities collect samples at a port-of-entry, timing is determined by the arrival of trucks or ships. Samples collected by authorities to verify compliance with legal requirements may be collected during storage, distribution, retail, or food service. Samples collected at different steps in the processing line, such as after production and packaging and before and after cleaning, allow food manufacturers to verify adherence to GHP and the efficacy of Hazard Analysis Critical Control Point (HACCP) plans.

For packaged products, sampling is straightforward and need only follow preestablished sampling plans. For ultra high temperature products, for example, routine samples are taken at defined intervals during processing, while *event samples* are taken after startup, after stops in production, or after changes of paper rolls and strips to monitor and assess the impact of such events (Cordier, 1990). In other circumstances, packed units may be taken from pallets according to a predetermined scheme.

If the packaging material needs to be opened to collect the sample unit, appropriate precautions must be taken to avoid contamination. External surfaces must be cleaned to remove dust and soil and, if multiple layers of packaging are used (e.g., bags of flour, sugar, or other dry products), the outer layer(s) can be removed. This allows access to cleaner surfaces, which may then be disinfected, if necessary, before opening or cutting.

Sufficient quantities should be collected to allow additional analyses to be performed as necessary, or for unforeseen inquiries.

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If packages show signs of "blowing" (swelling), extra care should be exercised to avoid dissemination of contaminated material. In such circumstances, the whole package should be sent to the laboratory.

Ideally, the food should be mixed before collecting the sample units, but this may not be practical or possible. For liquid samples, such as milk, ice cream mixes, or beverages stored in vats or tanks equipped with stirrers, it is usually relatively easy to obtain representative samples. If there is no evidence of recent stirring and mixing, thorough mixing with a sterile ladle is recommended before collecting the sample.

In some foods, however, sampling specific components, sections, or layers of multicomponent products is desirable. Particular care should be exercised to achieve a good separation. Examples of special considerations are provided in Table 12–1.

Water (potable water, processing water) should be sampled from taps or built-in sampling devices after flushing sufficient water through those taps or built-in devices to obtain a representative sample. A sterile ladle or other means can be used for water or brine solutions in open systems. Addition of a neutralizing solution is necessary to neutralize disinfectant residues, if present.

Air sampling in processing environments may be performed to assess the microbiological status of air. Many techniques have been described, from simple passive collection using sedimentation plates to active air samplers based on impaction or impingement. Those methods and available equipment are reviewed by Henningson & Ahlberg (1994).

Special consideration should be given to sampling during investigation of foodborne outbreaks since the sampling can differ considerably from routine inspections. Using professional judgment, samples of food or equipment surfaces should be collected at the most appropriate locations where contamination, survival, or growth might have occurred (Bryan, 1999).

12.2.5 Sample Labeling

Sample units must be clearly labeled and identified to permit good traceability. This can be achieved by writing descriptive terms or numbering each sample unit directly on the container or a firmly attached label, making sure that the ink cannot be washed off.

In addition, a sampling report should be prepared with relevant details stating, for example, the time of sampling, sample site, particular observations on packaging, etc. The comments may vary widely depending on the purpose of sampling and what is to be learned from the analysis. Thus, the report should indicate the reason for sampling and, if known by the person collecting the samples, the types of analyses to be performed. If necessary, for example, for legal reasons or in case of dispute, the report should be signed by the person responsible for the sample collection as well as by representatives of the parties concerned. In such instances, sample containers may be sealed with an official seal that makes tampering impossible.

12.3 INTERMEDIATE STORAGE AND TRANSPORTATION

Collected sample units should be transported to the laboratory as promptly as possible. However, in certain situations, intermediate storage cannot be avoided before samples are dispatched, e.g., when daily dispatch is impossible. For environmental sampling, samples may be collected at regular intervals from specific sites over a period of time and, when assembled, dispatched. When samples are stored, the storage conditions must

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Table 12–1 Special Considerations When Sampling Food Commodities

Phase	*State*	*Form*	*Food Ingredient*	*Recommendations for sampling*
Solid	Dry	Fine powder	Dry milk, cocoa powder	From center of container unless problems suspected at the surface
		Large part	RTE* cereals, dry pasta	Select representative components and blend before drawing sample; may be rehydrated directly
		Blocks	Dates, sugar, cheese	If hard, use sterile hammer and chisel (also mortar and pestle) to draw sample; try to draw samples from several locations
		Multicomponent	Soup mix, spices	Entire unit or selected components drawn for sample
Solid	Moist	Particles	Ground meat	Multiple portions drawn from one or more packages or units
		Chunk	Tote of beef primal cuts	Selected pieces or liquid weep (drip) from large tote
		Blocks	Whole poultry, cheese	Whole bird rinsed in diluent or media; try to draw samples
		Carcass	Beef	Prescribed suspected area swabbed or sponged for testing
		Sausage	Sliced, cooked	Rinse or blend
			Fermented sausage	Selected areas for selected analyses, e.g., test for *S. aureus* near the surface, and enterohemorrhagic *E. coli* in the center
		Multicomponent	Cream dessert, stew	Selected areas for selected analyses, e.g., test for *S. aureus* near or in cream filling; test for *C. perfringens* in liquid portion of the stew
Solid	Frozen	Particles	IQF† peas/beans	Frozen sample either "stomached" or rinsed with diluent
		Blocks	Frozen egg	Collected with (sterile) funnel and drill
		Multicomponent	Pizza	Sector sampled while frozen unless specific components tested
Liquid		Single phase	Water	Membrane filter kept in conditions that avoid death or multiplication
		Multiple nonmiscible phases	Salad dressing	Well-mixed representative sample, or specific layer
		Homogeneous multiple phases	High calorie supplement	Representative weighed sample

*RTE—ready-to-eat
† IQF—instant quick frozen

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prevent changes, i.e., microbial growth or death. Storing dry or shelf-stable products or samples poses relatively few problems, but wet and perishable samples are more delicate and need to be refrigerated or even frozen. The storage conditions considered most appropriate must be established to ensure that the target flora remains unchanged. Loss of viability during frozen storage and subsequent thawing can be a particular problem (e.g., *Campylobacter jejuni* vegetative cells of *Clostridium perfringens*).

For transportation, all samples must be packed to avoid breakage and spillage. Where necessary, containers should be protected by additional packaging material. Finished products, raw materials, and environmental samples should be packaged separately or shipped separately to avoid possible problems of cross-contamination.

Samples of perishable chilled or frozen products should be shipped in insulated containers to maintain the appropriate temperature with a refrigerant, such as ice, dry ice, or freezer packs. For particularly sensitive or important samples, temperature recorders or indicators should be included to record in-transit temperatures that could affect the analytical results.

12.4 RECEPTION OF SAMPLES

When received at the analytical laboratory, sample units should be inspected visually for damage or spillage, temperature checked as necessary, and the samples cross-checked against the sample report. Information that could influence the analytical results should be noted on the sample report. Today laboratory information management systems are widely used, allowing for easy and complete registration, thus ensuring good traceability.

12.5 SAMPLE ANALYSIS

12.5.1 Withdrawing Analytical Units

Samples should be analyzed as promptly as possible and, if not, stored under conditions that do not permit either death or multiplication of the target microflora.

Paramount in the process of withdrawing sample units is to use aseptic technique to prevent contamination of the analytical unit. The first step of the preparation for microbiological analysis is to withdraw a representative analytical unit from each sample unit. Samples must be mixed thoroughly to obtain a representative analytical unit. Liquid, semi-liquid, and, to some extent, powders in containers with a headspace, can be mixed by inverting or shaking the container. Analytical units should be removed as soon as possible after mixing. The external parts of packages or containers are first disinfected using chlorinated or iodinated solutions. Opening of packages is performed taking all necessary measures to prevent contamination. Sterile utensils are used to open packages, mix, and withdraw the necessary aliquots.

12.5.2 Dilution and Homogenization of the Analytical Unit

Weighing the analytical unit into the appropriate diluent (first dilution) must be performed aseptically. With dusty products, it is recommended to work under a hood or in a laminar flow cabinet to avoid dispersion of dust that might be contaminated with pathogens.

Most analytical methods recommend weighing the analytical unit with a precision of ± 0.1 g of the desired weight. The size of the analytical unit has a significant effect on the coefficient of variation of the sample weight. The larger the analytical unit, the lower

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the coefficient of variation and the better the accuracy. For practical reasons, however, analytical units of 10 g up to 50 g are typical for quantitative methods and are diluted 1:10. For qualitative analyses, 25 g analytical units are frequently analyzed individually or as composites of 100 g, 200 g, or more.

For residue collected from the environment, only small quantities, commonly 1 g or less, may be available. Nevertheless, such samples can provide very valuable information.

Some products, such as liquids, semi-liquids, and paste-like samples, are normally mixed and dispersed rather easily. For products such as margarine, butter, chocolate, etc., gentle warming (to about 40 °C) improves dispersion and dissolution of the food matrix and facilitates the release of microorganisms. Other types of samples, in particular solids, require special treatments using homogenizers or blenders. Diluted homogenized sample units can then be further processed according to the protocols of specific analytical methods. Detailed information on sample dilution and homogenization is provided in various documents, such as ISO documents (1999).

Preparation of the first dilution, either for qualitative (enrichment) or quantitative (direct) analyses, can have a marked impact on the microbial population in the sample. This is often due to changes in the physico-chemical characteristics of the suspension as compared to the food matrix. Where the food does not confer protection, the composition of the diluent is important to prevent or minimize shock/injury to the microorganisms that can result from rapid changes in the ionic concentrations. This has long been recognized and is discussed, for example, by Keller *et al.* (1974).

Use of stomachers or blenders usually has no impact on the viability of microorganisms. An exception is high-speed blenders that may damage certain cells, for example anaerobes, due to exposure to oxygen (Ray, 1979).

Dry foods must be rehydrated slowly to avoid die-off due to osmotic shock (e.g., freeze-dried cells) (Ray *et al.*, 1971). This was confirmed by van Schothorst *et al.* (1979), who showed that the conditions of rehydration of foods can have a major impact on the recovery of salmonellae. Slow rehydration, e.g., using a soaking procedure, resulted in higher recovery rates than shaking.

In foods, the ratio between dead, injured, and healthy cells varies, as does the extent of injury to individual cells. Numerous studies have been devoted to this subject and reviews are available (Ray *et al.*, 1971; Mackey, 2000).

12.6 RECOVERY OF INJURED CELLS

Injury of microbial cells can have a significant impact on the final results and must be taken into account during analysis. Injured or damaged microbial cells should be allowed time to repair that damage before growth is initiated. This "time to repair" is seen as an increased lag phase and affects the incubation time of both solid and liquid media. Time spans recommended in standard methods are generally those optimal to recover the maximum number of microorganisms. Reducing this incubation period can lead to a significant reduction in counts, or in the case of qualitative methods, to false negatives. This was shown for *Salmonella*, for example, when preenrichment for 6 h gave lower recoveries than preenrichment for 24 h. Extending preenrichment to 48 h did not increase the number of *Salmonella*, and numbers were sometimes lower than that at 24 h (D'Aoust *et al.*, 1992; Hammack *et al.*, 1993).

Food components can markedly affect recovery of both injured and healthy cells, as is shown by detection of *Salmonella* in different food matrices. Rapid rehydration of dehydrated products, such as milk powder or feeds, reduces the recovery rate (Van Schothorst

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et al., 1979; D'Aoust & Sewell, 1986). Dehydrated culinary products, such as soups or concentrated bouillons, and raw materials, such as spices, onions, etc., require higher dilution rates (1:20 up to 1:100) or the addition of substances to neutralize the inhibitory effects of salt and certain food components (Andrews *et al.*, 1995). Bacteriostatic and/or bactericidal substances present in cocoa may inhibit growth of *Salmonella* during the preenrichment (Zapatka *et al.*, 1977). Inhibition is neutralized by adding casein or nonfat dry milk to the preenrichment broth (Poelma *et al.*, 1981; IOCCC, 1990). In fatty foods, surfactants such as Tween 80 improve recovery (D'Aoust *et al.*, 1982). Hydrocolloids may affect and inhibit recovery of salmonellae by thickening and changing the pH of the preenrichment broth. Using appropriate dilutions, adding enzymes to reduce viscosity, and adjusting pH have been shown to improve handling and recovery (Amaguaña *et al.*, 1996, 1998). Gel formation of gelatin during incubation will affect recovery, and dilution (1:20) or using papain to reduce the viscosity are recommended (Jay et al, 1997; Amaguaña *et al.*, 1998).

Environmental samples can be heavily contaminated and addition of malachite green to the preenrichment broth to inhibit competitive microorganisms has been shown to enhance recovery of salmonellae (Van Schothorst & Renaud, 1985).

Optimization of preenrichment conditions is therefore essential for the detection of salmonellae and should be considered for other pathogens. Increasing the number and type of selective enrichment broths and selective plating media will not improve detection of injured and healthy cells if they are not permitted to recover and grow during preenrichment.

Selective agents in direct plating media can have an adverse effect on particular microorganisms (Ray, 1979). This is of particular concern if low levels need to be detected. Selective agents, such as sodium lauryl sulphate, Oxgall, brilliant green, bile salts, sodium desoxycholate, crystal violet, and others, are used in different media to detect Enterobacteriaceae, coliforms, or *Escherichia coli*. Comparative studies with basal agar supplemented with one of these selective components have shown inhibition rates between 0 and 99% for *E. coli* (Ray, 1979). This has been confirmed by others using different experimental approaches, underlining the need for careful choice of media in the microbiological analysis of foods.

Numerous attempts have been made to overcome this problem either through choice of selective agent, introducing a resuscitation step in the procedure, use of a nonselective medium, or addition of betaine or pyruvate to enhance recovery of injured or stressed cells (Ray, 1979; Johnson & Busta, 1984; Mackey *et al.*, 1994; Marthi & Lightfoot, 1990).

12.7 ERRORS ASSOCIATED WITH METHODS AND PERFORMANCE OF LABORATORIES

The errors associated with quantitative methods, such as colony count techniques, differ from those for qualitative methods, such as presence/absence tests. Errors affecting the quality of data obtained by analytical laboratories have been discussed in detail (Jarvis, 1989). Quality of results is characterized by the accuracy of the method, i.e., the ability to provide results equal to, or close to, the real value. The repeatability (r) of a method reflects the difference between two single results obtained when the same sample is analyzed by the same analyst under identical analytical conditions. The reproducibility (R), on the other hand, represents the difference obtained between two laboratories. Examples of r and R values for different analytes are provided in Table 12–2.

Over recent years, considerable efforts have been made in the accreditation of laboratories. This will have a positive outcome, provided accreditation is not just considered as a system or a commercial asset. If properly implemented, it should be used for continual

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Table 12–2 Examples of Repeatability (*r*) and Reproducibility (*R*) for Different Analytes in Mashed Potatoes, Rehydrated with Cultures. (Data provided by the Silliker Laboratories).

Estimation of laboratory variance (*Repeatability*) using the average variation of homogeneity samples for proficiency test materials. Each set examined by the same technician (20 tests).

Method	$s^{2\dagger}$	*s*	*CV*‡ *(%)*	*Repeatability (r)*
Aerobic mesophilic counts (*n*=80)	0.0126	0.1122	2.46	0.317
C. perfringens spores (*n*=40)	0.103	0.321	7.29	0.908
Coliforms (*n*=80)	0.057	0.239	6.42	0.677
Coliforms (MPN)* (*n*=80)	0.109	0.331	9.29	0.935
E. coli (MPN) (*n*=40)	0.150	0.387	9.73	1.096
Yeasts (*n*=40)	0.023	0.152	3.31	0.425
Molds (*n*=40)	0.022	0.150	4.16	0.424

Estimation of the variance among laboratories (*Reproducibility*) using the average variation from 13 proficiency test sets (1995–1998).

Method	s^2	*s*	*CV (%)*	*Reproducibility (R)*
Aerobic mesophilic counts (*n*=1669)	0.068	0.261	5.4	0.37
C. perfringens spores (*n*=660)	0.353	0.594	15.2	1.68
Coliforms (*n*=1439)	0.138	0.371	10.0	1.05
Coliforms (MPN) (*n*=1066)	0.191	0.437	11.4	1.24
E. coli (MPN) (*n*=706)	0.435	0.659	17.8	1.87
Yeasts (*n*=701)	0.129	0.359	8.7	1.02
Molds (*n*=735)	0.084	0.290	8.9	0.82

†*s*–standard deviation
‡CV–coefficient of variation
*MPN–most probable number

improvement of analytical performance and laboratory procedures, such as the quality of the media used and their preparation and control of incubator and water bath temperatures. Skills and training of personnel, standardization of laboratory practices, and so on, should also be addressed.

Participation of laboratories in proficiency tests organized and offered by national, professional, or commercial organizations and requested by accreditation bodies also represents an opportunity for improvements (Black & Craven, 1990; Peterz, 1992; Berg *et al.*, 1994). Such proficiency tests facilitate benchmarking performance of the laboratory and identification of weaknesses that need improvement. It must, however, be realized that the type of samples provided for proficiency testing has limitations related to the preparation and the viability of microbial population. Consequently, check samples for proficiency testing are not available for all food matrices. The concentration of pathogens is frequently relatively high and a competitive flora is not always included in check samples. Such samples do not, therefore, accurately assess the laboratory's ability to detect very low numbers of injured cells that may occur in actual food samples. The use of reference materials containing very low levels of injured cells, such as the *Salmonella* product developed in the Netherlands (Foundation, 2001), may be more useful in assessing the laboratory perfor-

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mance or reliability of a method. Reference materials have been developed for various microorganisms (Peterz & Steneryd, 1993; In't Veld *et al.*, 1995).

Simplified or alternative methods are often used to cope with the large number of analyses and to obtain results more quickly. This is legitimate and can accommodate a sudden influx of samples, e.g., environmental, to detect a source of contamination. This is more effective than applying more cumbersome standard methods that limit the number of samples that can be analyzed.

It is extremely important to underline the need to use alternative methods that have been validated. This not only allows more results to be obtained sooner, but also guarantees reliability of results. A number of validation procedures exist, ranging from a simple peer review of alternative methods by an expert panel to extremely thorough procedures based on extensive comparative and collaborative studies (Andrews, 1996; Lombard *et al.*, 1996; Rentenaar, 1996; Scotter & Wood, 1996). The development of a technical protocol within Microval (Rentenaar, 1996) represents an example of how to standardize validation procedures and to develop an International Organization for Standardization/Comit Europen de Normalisation standard that will be issued during 2001.

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Chapter 15

Salmonella in Dried Milk

15.1 Introduction
15.2 Risk Evaluation
15.3 Risk Management
15.4 Product and Process Criteria
15.5 GHP and HACCP
15.6 Acceptance Criteria for Final Product
15.7 References

15.1 INTRODUCTION

Milk is widely used worldwide as an important part of the diet, especially for younger children. Raw milk is a very perishable product and efforts to preserve it have led to the development of a range of dairy products (Anon., 1995). One of the earliest means of preserving milk was natural fermentation (ICMSF, 1998), but modern technologies have improved the old preservation methods and added new ones (Robinson, 1986a, b; Varnam & Sutherland, 1994; ICMSF, 1998 at Chap. 16). In particular, drying has developed into one of the most used technologies. In addition to preserving the most nutritious elements, drying offers the advantage of weight reduction, making transportation more convenient. Dried milk has been shipped in large quantities around the world for decades. In many tropical countries, producing and/or importing dried milk provides children with this nutritious food.

Milk is pasteurized, or even sterilized, before drying to render the product safe because drying cannot be relied upon to kill pathogens in sufficient numbers (Daemen & van der Stege, 1982). Unfortunately, very occasionally recontamination has occurred and, especially when dried milk was used in infant formula, this led to cases or outbreaks of salmonellosis (Anon., 1997; Becker & Terplan, 1986; Gelosa, 1994; Louie, 1993; Usera *et al.*, 1996; Rowe *et al.*, 1987). Vast quantities of dried milk are consumed daily worldwide without evidence of causing salmonellosis. This may indicate that recontamination seldom occurs, or that low numbers of salmonellae do not cause an infection and that multiplication of *Salmonella* in the reconstituted milk must occur to cause illness. Data on the infective dose of salmonellae in children are not well documented (FAO/WHO, 2000) because infections due to dried milk are rare, and when they occur (Collins *et al.*, 1968; Furlong *et al.*, 1979), the number of salmonellae in the reconstituted product is rarely determined. Several aspects of the risk evaluation of *Salmonella* in dried milk will be discussed and serve as an example of setting a food safety objective (FSO).

15.2 RISK EVALUATION

15.2.1 Hazard Identification

All *Salmonella* serovars, with the exception of *Salmonella typhi* and *S. paratyphi*, which behave very differently, are considered in this risk evaluation. Salmonellae are

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Gram-negative rods that can grow in milk with $a_w > 0.95$ and pH > 4.9 at temperatures between 8–45 °C (ICMSF, 1998). Salmonellae are not particularly heat resistant and when present in milk are readily killed by heat treatments such as the various time-temperature combinations used in commercial pasteurization (Thomas *et al.*, 1966; Read *et al.*, 1968). Salmonellae are widespread in most regions of the world and animals can be infected by a wide range of serovars (ICMSF, 1996; Ziprin, 1994). During infection, animals may excrete salmonellae in high numbers. Consequently, water and dust may become contaminated and, particularly when cows are shedding salmonellae, milk may be contaminated during milking. When contaminated water or dust enters a dairy plant, salmonellae may gain entry to production lines and the product may become contaminated (Van Schothorst & Kleiss, 1994).

15.2.2 Exposure Assessment

Pasteurization of milk was one of the first examples of a technology applied to render safe a potentially hazardous food. During pasteurization, at least a 10-log reduction of salmonellae is normally obtained by minimal processing conditions (72 °C for 15 s) (ICMSF, 1996). Often an even greater killing effect is achieved in the production of whole milk powder, because temperatures of 80–85 °C are applied for at least 5 s to inactivate most lipolytic enzymes.

Milk pasteurization is a process that can be closely controlled. Handling the dried milk is, however, more critical because many potential routes for recontamination exist (IDF, 1991, 1994). The frequency of recontamination is unpredictable, but when good hygienic practices are maintained, it is rare and the numbers of salmonellae involved are very low. Normal end-product analysis of dried milk will not reveal such low levels of recontamination; consequently, accurate estimates of the frequency of contamination and the true concentration are difficult to establish. In addition, milk powder must be reconstituted before consumption. Although the procedures recommended for preparing, storing, and using reconstituted milk are well documented, the potential opportunities for multiplication are not known. However, based on commercial experience and epidemiologic evidence, it is reasonable to assume that the presence of *Salmonella* in the product is infrequent and the concentration at the moment of consumption is low.

15.2.3 Hazard Characterization

This part of the risk evaluation describes the effect of the identified hazard on an individual or particular groups within a population. Salmonellosis is normally caused by the ingestion of salmonellae in food or water (Silliker & Gabis, 1986). The incubation period may vary from 5 h to 5 days (ICMSF, 1996). Symptoms include nausea, stomach cramps, vomiting, diarrhea, and fever (D'Aoust, 1991). Especially the very young, very old, and immunocompromised persons may become infected with low doses (< 100 cfu g^{-1}) of salmonellae, and their symptoms may be more severe (Blaser & Newman, 1982; Glynn & Palmer, 1992). Salmonellae may cause death among the aged and other vulnerable persons. Moreover, salmonellosis may leave sequelae, such as rheumatoid arthritis (Archer, 1985; Keat, 1983; Smith, 1994). When estimating risk, it is thus very important to specify which type of illness is considered and the target population.

The type of food also may influence the dose-response relationship. Experience has shown that in foods with a high fat content (e.g., chocolate, cheese, or fermented meats),

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low numbers of salmonellae (less than 10 cfu g^{-1}) may provoke illness (D'Aoust *et al.*, 1975; D'Aoust, 1985; Graven *et al.*, 1975; Greenwood & Hooper, 1983; Hedberg *et al.*, 1992; Lehmacher *et al.*, 1995). On the other hand, classical volunteer studies conducted with salmonellae in fluids indicated that up to 10^6 salmonellae were necessary (McCullough & Eisele, 1951a, b, c,). While data are not available for the number of cases of salmonellosis caused by reconstituted milk powder, it is reasonable to state that the number is very low in terms of number of servings per annum. Since reconstituted dry milk is often consumed by children, a vulnerable population group, a worst case scenario approach is used in this risk evaluation, i.e., it is assumed that one *Salmonella* per serving of milk powder may lead to gastroenteritis in children.

15.2.4 Risk Characterization

The world production of whole milk powder is estimated to be more than one million tons (10^{12} g) per annum. Assuming a serving size of 10 g, 10^{11} servings are consumed annually. Outbreaks and cases of salmonellosis due to the direct consumption of dried milk are rare (Collins *et al.*, 1968; Furlong *et al.*, 1979), but in this example it will be assumed that fewer than 1000 cases of salmonellosis result from contaminated milk powder annually. This gives a probability of illness of < 1 in 10^8 servings. In the absence of accurate data, it is not possible to validate this estimate, but this is most likely an overestimation of the actual risk.

15.3 RISK MANAGEMENT

15.3.1 Acceptable Level of Consumer Protection

In most industrialized countries, the prevalence of salmonellosis from all sources averages about 50 cases per 100,000 population per annum (Gomez *et al.*, 1997). For this example, a public health goal could be stated as less than 1 case per 100,000 population per annum associated with consumption of dried milk. This means the product will not significantly add to the current burden of disease.

15.3.2 Establishing a Food Safety Objective

If the number of servings consumed annually is assumed to be one glass of milk per day (i.e., 10 g powder) by one-third of the world's population, this would mean that about 10 million servings per 100,000 persons would be consumed per annum or 10^8 g. Considering the estimated probability of infection to be < 1 in 10^8 servings, this would mean that the consumption of dried milk would be associated with < 1 case per 100,000 population per annum.

Using a worst-case scenario, i.e., that 1 *Salmonella* cell per serving could provoke illness, the following FSO could be proposed:

The concentration of *Salmonella* in dried milk powder must be < 1 cfu 100 kg^{-1} at the time it is reconstituted for serving and consumed.

This FSO reflects a public health goal of < 1 case per 100,000 population per annum. The same FSO also could have been established by reviewing annual production records and the number of illnesses reported or estimated, as was calculated in the risk characterization section.

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For this particular food-pathogen combination, no change in risk is expected until the milk powder is reconstituted, after which, the time and temperature of holding will determine whether salmonellae can grow and the rate of growth. The potential for growth and increase in risk, such as during subsequent storage, must be addressed through education, such as labeling and other means. In this example, the FSO is intended for milk powder that is consumed immediately following reconstitution.

The safety of reconstituted milk also can be affected by contamination introduced from other sources, such as the water used to reconstitute the milk, utensils, container, and the person involved in preparing the milk. Other pathogens may be of significance (e.g., viruses, shigellae) in addition to salmonellae. These concerns are not considered in this example.

15.3.3 Control Measures

In principle, several control measures are available to help prevent *Salmonella* contamination in powdered milk and thus to meet the FSO. First, elimination of salmonellae at the dairy farm would prevent contamination during milking and further handling. However, this is very difficult to achieve, because salmonellae are widely distributed in nature, including farm environments (Van Schothorst, 1986). A second option is to eliminate salmonellae from the whole factory environment, but this also is difficult to achieve. Consequently, efforts to eliminate salmonellae should be concentrated on the equipment and environment following pasteurization (Stadhouders, 1975; Jarl & Arnold, 1982; Terplan & Becker, 1989). In current industrial practice, a series of control measures are applied, from collection of the milk until the powdered milk is packaged, to avoid contamination to the extent possible. Collectively, the control measures ensure the FSO has been met. In this example, the control measures are described only briefly. More detailed information can be found in the literature (IDF, 1991, 1994). Control measures include:

- *Controlling initial levels in raw materials*—Effective hygienic measures are applied at the farm level and the collected milk is refrigerated to below 7 °C to prevent multiplication.
- *Reducing levels during pasteurization of raw milk*—Raw milk is pasteurized to eliminate any salmonellae present (initial concentration).
- *Preventing an increase in levels by avoiding recontamination*—Recontamination during drying, handling, storing, and packaging is prevented by adopting a number of good hygienic practices (GHPs) that minimize the chance of contamination, e.g., use of filtered air; good separation of the wet and dry processing steps.
- *Providing consumer information*—A risk management option is to provide consumers with information on the product's label. This information provides instructions on reconstituting the milk powder, including precautions for what should not be done during and after reconstitution. Governments may want to include this information in general food hygiene information programs.

In this example, testing the final product cannot be used as a control measure to achieve the required FSO. Even with the most stringent sampling plan recommended (i.e., two-class sampling plan, $n = 60$, analytical unit = 25 g), the mean concentration of *Salmonella* would have to be at 1 cfu in 526 g or greater before a positive lot could be

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detected with a 95% probability, assuming a log-normal distribution and a standard deviation of 0.8 (Legan *et al.*, 2001).

15.3.4 Performance Criterion

A performance criterion is the required outcome of a step, or combination of steps, that contributes to ensuring that an FSO is met. Performance criteria are usually applied at steps where hazards can either be reduced or increased if appropriate control measures are not applied. Performance criteria may be more stringent than the FSO to ensure that the FSO is met. For milk powder that must be reconstituted before consumption, a more stringent performance criterion may be adopted to account for the slight abuse during preparation and use.

To arrive at a performance criterion for the control measures during manufacturing, the equation from Chapter 3 can be used:

$$H_0 - \Sigma R + \Sigma I \leq \text{Performance criterion}$$

$$H_0 - \Sigma R + \Sigma I \leq -8$$

where:
ΣR = total (cumulative) decrease of the hazard
ΣI = total (cumulative) increase of the hazard
H_0 = initial level of the hazard
Performance criterion, H_0, R, and I are expressed in $\log_{10}$ units.

The basic control measures to achieve the FSO and its related performance criterion consist of killing the salmonellae that may occasionally be found in fresh milk and preventing recontamination of the heat-treated raw material.

15.3.4.1 Controlling Initial Levels in Raw Materials

Since the effectiveness of the heat treatment depends on the number of salmonellae, hygienic measures must be taken at the farm level to keep contamination of salmonellae as low as reasonably possible. The collected milk must then be cooled rapidly and maintained below 7 °C to prevent multiplication. Tankers must be refrigerated and/or insulated. Upon receipt at the dairy plant, the temperature should be checked as one of the several physico-chemical tests. Total viable counts and coliforms may be determined as a check on the effectiveness of the chilling and the general hygienic measures taken. In many countries, maximum total counts have been established as microbiological standards.

The first stages of milk powder production have little or no influence on the number of salmonellae in the milk. During clarification, filth, clumps of milk particles, etc., as well as an excess of animal cells (for instance leukocytes), are removed. A separator is used to remove a portion of the cream. When this equipment is adequately and frequently cleaned, separation has no influence on the number of salmonellae. Depending on the type of milk powder produced, some of the cream may be added back to the fluid milk to obtain the required fat content. Storing and handling the fat, holding and standardizing the milk, etc. should be performed in a manner that prevents contamination with salmonellae and does not permit their number to increase.

Given the above hygienic precautions, the initial number of salmonellae in raw milk is usually very low, and no more than 1 cfu ml^{-1} (i.e., $H_0 = 0$).

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15.3.4.2 Reducing Levels during Pasteurization of Raw Milk

Pasteurization is of great importance and is normally identified as a critical control point (CCP) in a Hazard Analysis Critical Control Point (HACCP) system. The temperature is monitored with a thermograph. Most equipment has a flow diversion valve and when the temperature drops below the target value, the milk is diverted to a holding vat for repasteurization; thereby, the flow rate is also monitored and maintained at the required rate and survival of salmonellae is prevented. During cooling, an overpressure should be kept on the pasteurized milk side of the heat exchanger to prevent recontamination. If the pasteurizer does not function correctly or if salmonellae are introduced during cooling, the required low level of salmonellae may not be achieved. Fouling of equipment and buildup of biofilms must be prevented. Inspections and/or tests to detect micro-leaks are used in good hygiene and maintenance practices.

If the initial concentration of salmonellae is assumed to be no more than 1 cfu g^{-1}, then to achieve a performance criterion of 1 cfu per 10^8 g, an 8 $\log_{10}$ reduction needs to be achieved through pasteurization, i.e., $\Sigma R = 8$ (see Figure 15–1).

$$H_0 - \Sigma R + \Sigma I \leq \text{Performance criterion}$$

$$0 - \Sigma R + \Sigma I \leq -8$$

$$\Sigma R = -8$$

15.3.4.3 Preventing an Increase in Levels by Avoiding Recontamination in the Factory

Following pasteurization, the milk moves through an evaporator to concentrate it before spray drying. The temperatures used for condensing may further reduce the number of salmonellae, but evaporation is normally not important for the safety of the milk powder. Balance tanks are often used between condensing and spray drying to compensate for differences in flow rates. The balance tanks should be located in clean, *Salmonella*-free environments to prevent recontamination. The air entering the balance tank could be filtered as an additional preventive measure.

At this stage, it is critical to maintain a barrier between the wet operations of the factory and the dry operations where the spray dryer and the rest of the production line are

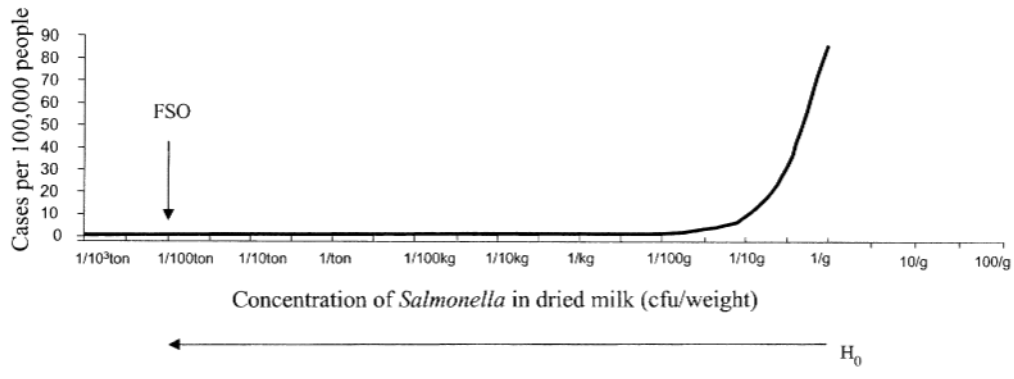


Figure 15–1 Pasteurization of raw milk and preventing recontamination in the factory.

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located. People, forklift trucks, etc. should not enter the dry zone without taking precautions to prevent introducing contamination. Air entering this dry sensitive area should be of controlled hygienic quality, entry of pests should be prevented, humidity should be low, and dry cleaning should be the routine procedure used.

The condensed milk is transported via a high-pressure pump to a spray nozzle or another spraying device installed at the top of the spray dryer. Hot air is blown into the aerosol of fluid milk that has been generated by the spray nozzle. The water is evaporated and milk powder falls to the bottom of the dryer. The hot air, which may still contain very fine particles of milk powder, is passed through cyclones to remove the milk particles from the air. These particles are returned to the milk powder at the bottom of the spray dryer.

The powder needs further cooling, and additional air is blown over the film of milk powder leaving the spray dryer. This air must be of high hygienic quality, because if it were to contain salmonellae, the cells would contaminate the final product. After cooling, the powder is passed over a sieve to remove clumps. It is then transported, either mechanically or pneumatically, to a hopper, which feeds the packaging machine or intermediate storage bins. Maintaining a clean, dry environment that consistently tests negative for salmonellae is critical for all the steps that occur within the dry areas of the plant, including the packaging operation.

All the lines where the milk is still in a fluid phase can be cleaned with Cleaning In Place installations or manually using water, cleaning agents, brushes, etc. The environment in this area of the plant is also wet cleaned. In contrast, the spray tower and its environment, as well as the sections of the line and surrounding environment after the spray dryer, are all dry cleaned whenever possible. Occasionally wet cleaning is necessary, but in these instances the equipment or surrounding environment are dried as soon as possible afterward. Drains and pipes containing water should be properly sealed. Condensation on cold pipes should be prevented; when insulation is used, care should be taken to prevent buildup of moisture and microbial growth, a potentially significant source of contamination. The principle applied here is that salmonellae cannot multiply when water is not available.

If the initial concentration of salmonellae is assumed to be no more than 1 cfu g^{-1} and an 8 $\log_{10}$ reduction is achieved through pasteurization (i.e., $\Sigma R = 8$), then, to achieve a performance criterion of 1 cfu per 10^8 g, there must be no contamination (i.e., $\Sigma I = 0$) (see Figure 15–1).

$$H_0 - \Sigma R + \Sigma I \leq \text{Performance criterion}$$

$$0 - 8 + \Sigma I \leq -8$$

$$\Sigma I = 0$$

15.4 PRODUCT AND PROCESS CRITERIA

The process criterion for the pasteurization would consist of heating the milk for at least 15 s at 72 °C, or for 5 s at 80 °C.

The most serious problem in controlling the hazard from salmonellae in dried milk is preventing recontamination. To achieve that, many GHP-related measures are necessary. In practice, this means eliminating salmonellae from the equipment and its immediate

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environment and preventing salmonellae from entering this environment with air, dust, pests, packaging materials, personnel, etc. Although it is difficult to quantify such low contamination rates, a recontamination rate of < 1 *Salmonella* per 10^8 g would be required. To put this into perspective, 10^8 gram is 100 tons of product, and that implies preventing recontamination during 30 h of production using a medium-sized spray-drying tower.

15.5 GHP AND HACCP

The basis for the production of safe milk powder is the application of GHPs that have been developed through many decades of industrial production of this product. All milk powders receive a heat treatment during one or more stages of production, and consequently preventing salmonellae in the end product depends mainly on preventing recontamination after the final heat treatment.

During development of the HACCP plan, the sources of *Salmonella* contamination and possible multiplication have to be identified, control measures put into place, and monitoring procedures described and implemented. A well-known source of salmonellae is milk residues, which may be found on the roof of spray tower buildings. Birds may contaminate this powder, and when the secondary air intake is close to the roof, this air may transfer salmonellae to the powder if the air filtration is not controlled. Salmonellae may also occur in the dust or mud outside the factory and may even gain access in noncritical areas, such as warehouses for raw materials or finished goods. Operators, maintenance personnel, visitors, etc. should, therefore, change their shoes before entering the dry areas of the milk powder production line. Movement of air throughout the various rooms should be well controlled. A slight overpressure should be maintained in the spray tower building, as well as in the packaging room. Again, the quality of this air should be carefully controlled. The packaging material could be a source of salmonellae, so control measures to ensure its high hygienic quality should be in place. Many other good hygienic practices are put in place to prevent recontamination, as described elsewhere, for instance in the IDF bulletin, *Recommendations for the Hygienic Manufacture of Milk and Milk Based Products* (IDF, 1994).

15.5.1 Monitoring and Verification from the Raw Milk to the Consumer

The quality of the incoming milk, and thus the potential level of contamination with *Salmonella*, is not under direct control of the dairy plant. However, the plant can influence the hygienic conditions at the farm and during transportation of the milk. The hygienic condition of incoming milk can be monitored by a number of tests, such as freezing point determination, methylene blue tests, filth tests, tests for aerobic mesophilic microorganisms, cell counts, acidity, etc. If necessary, the incoming milk could be tested microbiologically to check, for example, that a level of 1 *Salmonella* ml^{-1} is not normally exceeded, as a verification procedure.

The time and temperature specified as process criteria are monitored in most dairy plants with continuous temperature and flow rate recorders of different designs and levels of sophistication.

Elimination of *Salmonella* from the equipment and its environment and preventing their introduction requires a number of hygienic measures that need careful surveillance (see Chapter 10). Testing for indicator microorganisms, as well as for salmonellae, can

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check the effectiveness of these hygienic measures. If no salmonellae are found in the most likely locations and if the numbers of indicators, such as Enterobacteriaceae, are in the normal order of magnitude, it can be assumed that the performance criteria are achieved.

Testing end products for salmonellae is, in principle, not necessary as a routine monitoring procedure. Greater reliance must be placed on monitoring the HACCP system and assessing control of the environment through visual inspections and microbiological testing. When the process and environment records indicate that the operation is under control, microbiological testing would not be sufficiently sensitive to determine whether the performance criterion has been met. Nevertheless, many in industry often test a few samples for the presence of salmonellae as part of their verification program. While a positive result would indicate that the process was not under control, a negative result cannot be relied upon to ensure that the performance criterion and FSO have been met (see sections 15.3.3 and 15.6.3).

15.6 ACCEPTANCE CRITERIA FOR FINAL PRODUCT

15.6.1 Organoleptic

Milk powder has certain organoleptic characteristics that can easily be checked at any stage in the food chain. Milk powder should be dry, free flowing, and easily dissolve in cold or lukewarm water. It has a distinct white or creamy white color, without brown (Maillard reaction) or black (burned) discoloration or particles. It should not have a rancid or other abnormal aroma. Unopened packages should not be damaged or show any sign of insect infestation. Although these organoleptic acceptance criteria are more related to quality than to safety, they indicate that GHP were adhered to during production, storage, and distribution. As mentioned above, deviations from GHP could lead to safety problems. Atypical product should, therefore, not be accepted without further inquiries or investigational sampling.

15.6.2 Chemical and Physical

A number of quality-related tests, such as moisture level, fat and protein content, solubility, etc., are used in commerce (CAC, 1999). Most of them are unrelated to the safety of the product. Sometimes tests for aflatoxin M, antibiotics, heavy metals, pesticides, etc. are performed to check compliance with legislation. Moisture or water activity tests are normally not necessary to determine whether growth of pathogens might have occurred. When moisture is raised to a_w levels allowing microbial growth, the powder becomes totally unacceptable and would be rejected through normal organoleptic examination.

15.6.3 Microbiological

Microbiological examination often includes a test for aerobic plate counts (APC), coliforms, or Enterobacteriaceae, and sometimes *Salmonella* (see also ICMSF Book 2, (1986)). For APC, case 2 is selected with a three-class plan consisting of $n = 5$, $c = 2$, $m = 3 \times 10^4$, and $M = 3 \times 10^5$. Case 2 is appropriate to this situation in which these counts indicate the normal level of microorganisms found in these products, without direct link to GHP or HACCP.

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Table 15–1 Performance of Sampling Plans for *Salmonella* Expressed as the Mean Concentration Detected with 95% Probability Using a 25 g Sample Size and Assuming a Log-Normal Distribution and a Standard Deviation of 0.8

Case	10	11	12
No. of Samples	$n = 5$	$n = 10$	$n = 20$
Mean Concentration	32/1000g (1 cfu/32g)	12/1000g (1 cfu/83g)	5.4/1000g (1 cfu/185g)
Case	13	14	15
No. of Samples	$n = 15$	$n = 30$	$n = 60$
Mean Concentration	7.4/1000g (1 cfu/135g)	3.6/1000g (1 cfu/278g)	1.9/1000g (1 cfu/526g)

Case 5 can be used for coliforms with the following values: $n = 5$, $c = 1$, $m = 10$, and $M = 10^2$. Case 5 relates to indicators of adherence to GHP and HACCP. Lots of milk powder meeting this criterion should not be rejected, unless other findings raise concern.

The following guidance can be used for *Salmonella* testing. Cases 10, 11, and 12 normally apply to foods being tested for *Salmonella* and result in 5, 10, and 20 samples, respectively. The analytic unit from each sample is typically 25 g. For milk powder, case 11 is most appropriate because there may be no change in the level of *Salmonella* between when the powder is tested at the factory or port-of-entry and when the powder is reconstituted for serving. When the powder is intended for a high-risk population, the number of samples can be increased to 15, 30, and 60, with 30 samples being the most appropriate. The performance of these sampling plans can be related to a mean concentration of bacteria that could be detected with a probability of 95% using the approach of Foster (1971) and Legan *et al.* (2001) and assuming a log-normal distribution with a standard deviation of 0.8. The mean concentrations controlled appear in Table 15–1. Thus, for case 11, there would be 95% confidence of detecting *Salmonella* if present at a level of 1 cfu 83 g^{-1} or greater and if the level of contamination is log normally distributed throughout the lot with a standard deviation of 0.8.

These criteria are applicable when no information is available on the history of the lot or the supplier's control system. For a full discussion on the need for testing and choice of sampling plans, the reader is referred to the previous chapters.

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Listeria monocytogenes in Cooked Sausage (Frankfurters)

16.1 Introduction
16.2 Risk Evaluation
16.3 Risk Management
16.4 Process and Product Criteria
16.5 GHP and HACCP
16.6 Acceptance Criteria for Final Product
16.7 References

16.1 INTRODUCTION

This chapter considers *Listeria monocytogenes* in cooked sausage (i.e., frankfurters) as an example of a microbial hazard that is capable of growth in a wide variety of perishable ready-to-eat (RTE) foods. The use of performance criteria, process criteria, and validation in relation to the Hazard Analysis Critical Control Point (HACCP) plan is described. In addition, the importance of Good Hygienic Practices (GHP) and an example of a sampling program to assess control of a pathogen in the environment is discussed. In this example, the pathogen of concern is psychrotrophic and can establish itself as a resident and multiply in the refrigerated areas of the food operation, as well as in the refrigerated food. This chapter describes the application of principles introduced in previous chapters. Hypothetical values are used throughout the chapter wherever assumptions were necessary to illustrate a concept or procedure. No attempt has been made to validate their accuracy.

Sausage is one of the oldest forms of processed food and may actually have been the first processed meat product. Thousands of years ago, primitive civilizations began processing meat when they discovered that procedures such as salting, sun drying, and cooking could prolong the edible qualities of meat products, in addition to improving flavor. Almost all races and societies have used this type of food processing method at some point during their development. The term "sausage" is derived from the Latin word *salus*, meaning chopped or minced meat preserved by salting. In 1987, Frankfurt-am-Main, over the protests of Vienna (Wien), which considers itself the originator of the "frankfurter," celebrated the 500th birthday of its claim to being the birthplace of the frankfurter (hot dog). Hundreds of different sausage varieties are available, including cooked, uncooked, smoked, fermented, cured, etc., as well as varieties using combinations of these processes.

Cooked sausages, which include the ever-popular frankfurter as well as bologna and various luncheon meats, are prepared from mixtures of comminuted beef, pork, chicken,

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and/or turkey to which salt, sugar, sodium nitrite, and spices are normally added. When making frankfurters, the meat/ingredient mixture, commonly referred to as the sausage emulsion, is stuffed into natural or artificial casings, which are then twisted to form sausage links. Frankfurters are typically cooked to an internal temperature of $\geq$ 70 °C to (a) coagulate protein, (b) fix the cured color, and (c) destroy certain pathogens and bacteria that would cause spoilage. Although not an absolute requirement, frankfurters and other similar sausages are commonly subjected to natural smoke. Alternatively, a commercially available liquid smoke solution can be added to the sausage emulsion or atomized onto the surface of frankfurters before or during heating. After cooking, frankfurters are cooled, packaged, and refrigerated for shipment to wholesale and retail markets (Figure 16–1). Skinless frankfurters, which are very popular, are produced in a similar manner, except that the artificial casing is mechanically peeled from the sausage after cooking.

Cooking renders the sausages free of *L. monocytogenes*. However, post-processing contamination can occur and in rare instances has led to illness, such as the 1998–1999 multistate outbreak with 101 confirmed cases, including 15 adult deaths and six miscarriages/stillbirths (CDC, 1999). It should be noted that multiplication of *L. monocytogenes* on frankfurters is probably necessary for disease to occur, but data on the dose-response relationship in humans are not available. This and other aspects of the risk analysis of *L. monocytogenes* in frankfurters are discussed below. Several reports dealing with risk assessment and *L. monocytogenes* have been published and contain additional background information (Farber *et al.*, 1996; Hitchins, 1996; Buchanan *et al.*, 1997; Bemrah *et al.*, 1998; FAO/WHO, 2000; Buchanan & Lindqvist, 2000; Ross *et al.*, 2000; FDA, 2001).

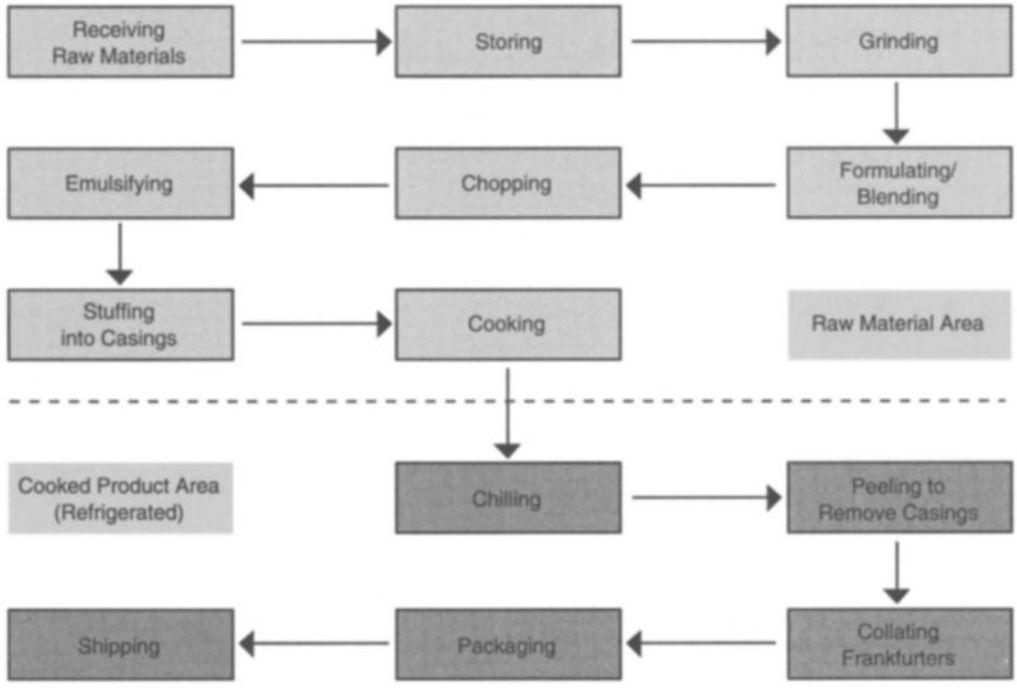


Figure 16–1 Flow diagram for typical frankfurter operation.

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16.2 RISK EVALUATION

16.2.1 Hazard Identification

Epidemiologic data suggest that most exposures to *L. monocytogenes* are foodborne (Ciesielski *et al.*, 1988; Broome *et al.*, 1990; Farber & Peterkin, 1991; McLauchlin, 1993; Mead *et al.*, 1999). Although listeriosis occurs infrequently, at somewhere between 2–6 cases annually per million of population, between 20–30% of the cases are fatal (McLauchlin, 1993; Rocourt, 1996; Mead *et al.*, 1999). Thirteen serotypes of *L. monocytogenes* have been identified, but most illnesses have been associated with only three, namely serovars 4b, 1/2a, and 1/2b. Serovar 4b accounts for close to half of all the cases in Europe, while serovars 4b, 1/2a, and 1/2b are more equally represented in North America (Farber & Peterkin, 1991; McLauchlin, 1993; Rocourt, 1996; Rocourt & Bille, 1997). However, the potential for all serovars to cause human illness cannot be discounted.

All *L. monocytogenes* serotypes are considered in this risk evaluation. *L. monocytogenes* is a Gram-positive, non-spore-forming rod. It is facultatively anaerobic, growing at temperatures between −0.4 and 45 °C, pH values 4.39–9.4, a water activity of 0.92 or higher, and a salt concentration of 10% or less (ICMSF, 1996). It is catalase positive, oxidase negative and produces β-hemolytic colonies on blood agar. The organism is widely distributed in nature and can be found on fruits and vegetables, in soil, water, sewage, silage, slaughterhouse waste, milk from healthy and mastitic cows, as well as human and animal feces (Farber & Peterkin, 1991, 1999). *L. monocytogenes* can be found as part of the normal flora of many animal species and humans, and it has been estimated that between 2 and 6% of humans and animals are transient carriers of the organism. Fecal excretion of the organism is not necessarily an indication of disease, and the role of healthy carriers in the epidemiology of listeriosis is still unclear (Rocourt, 1996).

L. monocytogenes is inactivated by heat treatments of 70 °C or above, used in cooking or pasteurization. However, products, such as frankfurters, that are purchased as a precooked sausage are sometimes eaten without further cooking or may be reheated by a variety of methods (frying pan, grilling, simmering water, microwave). The practice of reheating in a microwave oven was associated with one sporadic case of listeriosis in an elderly woman who had cancer (CDC, 1989). Meat products, such as cooked sausages, can be recontaminated between cooking and packaging in the processing facility. During extended refrigerated storage (e.g., ≥ 35 days), the organism could multiply to potentially hazardous levels on the surface of the frankfurters and in the exudate, especially if the product is temperature-abused. The free exudate in the package has a higher concentration of cells than the product itself and may be a source of cross-contamination in the kitchen.

16.2.2 Hazard Characterization

Human listeriosis is now recognized as a disease primarily caused by ingestion of *L. monocytogenes* in food. This also includes secondary transmission between a mother and her fetus or neonate. Despite its widespread presence in the environment, illness due to this organism occurs infrequently. The outcome of listeriosis can be severe, with an estimated case fatality rate of between 20–30% in the segments of the population most at risk, i.e., immunocompromised individuals, pregnant women, the very young (i.e., fetuses and neonates), and the elderly. This spectrum of individuals is estimated to com-

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prise 15–20% of the population (Buchanan *et al.*, 1997) and is expected to increase, reflecting the trend in longer life expectancy and an overall aging population.

Unlike the symptoms caused by many foodborne pathogens, diarrhea and other gastrointestinal symptoms are not common symptoms of listeriosis, although the patient may experience malaise and a mild fever. In several foodborne listeriosis outbreaks, patients have exhibited only these mild symptoms (Salamina *et al.*, 1996; Dalton *et al.*, 1997). However, the majority of reported cases have been the invasive type of listeriosis, symptoms of which include meningitis, encephalitis, and septicemia. In pregnant mothers, unrecognized and untreated listeriosis can lead to abortion of the fetus, stillbirth, or premature delivery of a sick child. Serious sequelae, such as mental retardation and hydrocephalus, also have been reported following cases of foodborne listeriosis (Büla *et al.*, 1995).

There are no experimental dose-response data available for *L. monocytogenes* in humans (i.e., the minimum infectious dose (MID) is uncertain). As with many other foodborne pathogens, the MID will depend on factors such as the virulence of the strain, the amount of food consumed, the levels of the organism in the food, and the immune state of the host. Some animal data are available, mainly using the mouse model (Audurier *et al.*, 1980; Golnazarian *et al.*, 1989; FAO/WHO, 2000), but extrapolation of mouse data to the human situation is tenuous at best. While dose-response studies using human volunteers for certain pathogens have sometimes been conducted, this is not feasible with *L. monocytogenes*, since the risk of severe morbidity or mortality is too great. An alternate approach used with RTE foods is to combine epidemiologic data with estimates of the number of organisms present in the incriminated food. However, because outbreaks are an uncommon occurrence and it is difficult to obtain sufficient material in most cases, the amount of reliable data is scant and the information insufficient to draw reliable conclusions. The type of food consumed, perhaps, may influence the dose-response relationship.

When a risk estimate for *L. monocytogenes* is being made, it is important to determine which type of illness is being considered (i.e., mild versus invasive) and for which part of the population (i.e., "normal" versus "high-risk"). Since frankfurters are consumed by all segments of the population, different risk estimates ideally should be used for normal versus high-risk individuals.

Foodborne listeriosis appears generally to follow one of three scenarios. Scenario 1 consists of isolated cases for which information about the food is seldom available. Scenario 2 consists of an outbreak or cluster of cases involving a single lot of contaminated food. Both of these events typically involve errors in food handling that lead to a single lot of food becoming contaminated and an opportunity for multiplication before the food is consumed. Once the implicated food is eliminated, further cases cease to occur.

Scenario 3 consists of an outbreak involving anywhere from a few cases to several hundred cases scattered by time and location. This type of outbreak typically involves an unusually virulent strain that has become established in the food processing environment and contaminates multiple lots of food over days or months of production. Experience in cooked meat and poultry operations indicates that a niche or site within the cooked product environment wherein *L. monocytogenes* becomes established and multiplies is commonly involved. The sites may be impossible to reach and clean with normal cleaning and sanitizing procedures. In fact, the processing environment typically appears visually clean and acceptable. The sites serve as a reservoir from which the pathogen is dispersed during a food processing operation, contaminating food contact surfaces and, thereby, the

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food. The niche usually affects only the product being handled (e.g., filled, sliced, conveyed, assembled, packaged) along one packaging line and not the product on an adjacent line. Microbiological testing is necessary to detect the niche. Examples of niches include hollow rollers on conveyors, cracked tubular support rods on equipment, the space between close fitting layers of metal-to-metal or metal-to-plastic, worn or cracked rubber seals around doors, on-off valves and buttons for equipment, and saturated insulation.

In all three scenarios, there is an opportunity for *L. monocytogenes* to multiply in the food before it is consumed. Food processors should establish systems to prevent scenario 3 events and minimize the risk of scenarios 1 and 2.

16.2.3 Exposure Assessment

L. monocytogenes is found as a frequent contaminant of RTE foods (Farber & Peterkin, 1991, 1999). Many different foods have been involved in both sporadic cases and foodborne outbreaks, with the approximate levels detected ranging from 100 to 1×10^9 cfu g^{-1} (Table 16–1). The meat industry has a long history of providing safe food, including frankfurters, notwithstanding the recent large listeriosis outbreak (CDC, 1999). Qvist & Liberski (1991) found *L. monocytogenes* to be present in four of 67 (6%) frankfurter samples, while Wang & Muriana (1994) detected the organism in seven of 93 (7.5%) packages of retail frankfurters examined. The US Department of Agriculture (USDA) monitoring program for RTE meats after packaging at the manufacturing facility found the prevalence of *L. monocytogenes* in small-diameter cooked sausages to be 5.3, 4.8, 4.1, 3.7, 3.3, 3.5, and 1.8% annually from 1993 to 1999, respectively.

Frankfurter processing involves a step-heating schedule to allow for the formation of the "skin," for setting of the emulsion, and to allow the curing reaction to occur. This step also reduces *L. monocytogenes* numbers. Zaika *et al.* (1990) prepared frankfurters from a sausage emulsion inoculated to contain around 10^8 cfu g^{-1} of *L. monocytogenes*. After stuffing, all frankfurters were thermally processed (without smoke) according to a standard commercial heating schedule. Zaika and colleagues found that *L. monocytogenes* decreased approximately 1000-fold in frankfurters heated to an internal temperature of 71.1 °C. Hence, based on those data, cooking frankfurters to an internal temperature of 71.1 °C should eliminate higher levels of *L. monocytogenes* ($\sim 10^3$ cfu g^{-1}) than are likely to occur in raw frankfurter emulsions. In addition, post-process pasteurization can eliminate *L. monocytogenes* from the surface of frankfurters that have been contaminated between cooking and packaging. This procedure is currently practiced commercially by certain manufacturers throughout the world.

The cooking step for frankfurters can be controlled in facilities operating with effectively designed and implemented HACCP plans, but preventing contamination of the cooked product during cooling and packaging is much more difficult. Data gathered by the American Meat Institute in 1988 pointed to frankfurters as a likely carrier of *L. monocytogenes* and suggested that uncontrolled environmental conditions before packaging may play a major role in contaminating the finished product (Anon., 1989). Environmental sampling of a turkey frankfurter plant, whose product was linked to a case of listeriosis, found that contamination of the majority of frankfurters occurred at a single point during the peeling process, just prior to packaging (Wenger *et al.*, 1990). In retrospect, a properly applied environmental sampling program (see Chapter 10) might have detected the site and, if corrected, prevented contamination of the final product.

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Table 16–1 Foodborne Outbreaks due to *L. monocytogenes*

Location (year)	*No. of Cases (deaths)*	*Perinatal/ nonperinatal*	*No. cfu g^{-1}*	*Foods associated*	*Serotype*	*Reference*
Boston, USA (1979)	20 (5)*	0/20	NK†	Raw celery, tomatoes, lettuce‡	4b	Ho *et al.*, 1986
New Zealand (1980)	29 (9)	22/7	NK	Shellfish, raw fish	most cases 1/2b	Lennon *et al.*, 1984
Maritime Prov., Canada (1981)	41 (17)	34/7	NK	Coleslaw‡	4b	Schlech *et al.*, 1983
Massachusetts, USA (1983)	49 (14)	7/42	NK	Pasteurized milk‡	4b	Fleming *et al.*, 1985
California, USA (1985)	142 (48)	93/49	$10^3 - 10^4$	Queso blanco fresco cheese	4b	Linnan *et al.*, 1988
Switzerland (1983–1987)	122 (31)	65/57	$10^4 - 10^6$	Raw milk cheese	4b	Bille, 1990
Connecticut, USA (1989)	9 (1)	2/7	NK	Shrimp‡	4b	Riedo *et al.*, 1994
United Kingdom (1987–1989)	355 (94)§	185/129‖	$< 10^2 - 10^6$	Pâté‡	4b; 4b(x)	McLauchlin *et al.*, 1991
W. Australia (1990)	11** (6)	11/0	8.8×10^3	Pâté‡	1/2a	Kittson, 1992
Tasmania, Australia (1991)	4(0)	0/4	1.6×10^7	Smoked mussels	1/2b	Mitchell *et al.*, 1991
New Zealand (1992)	3 (2)	2/1	NK	Smoked mussels	1/2a	Baker & Wilson, 1993; Brett *et al.*, 1998
France (1993)	39 (12)	31/8	$< 100 - 1.0 \times 10^4$	Pork rillettes (pâté)	4b	Goulet, 1995
France (1992)	279 (85)	92/187	NK	Pork tongue in jelly	4b	Goulet *et al.*, 1993

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Italy (1993)	23 (0)	0/23	NK	Rice salad‡	1/2b	Salamina *et al.*, 1996
USA (1994)	45 (0)	1/44	2.9×10^{11}	Chocolate milk	1/2b	Dalton *et al.*, 1997; Proctor *et al.*, 1995
France (1995)	20 (4)	9/11	NK	Raw milk soft cheese	4b	Goulet *et al.*, 1995
Sweden (1994–1995)	6 (1)††	2/4	$< 100 - 2.5 \times 10^{6}$	Cold-smoked rainbow trout	4b	Ecklow *et al.*, 1996
Canada (1996)	2 (0)	0/2	2×10^{9}	Imitation crab meat	1/2b	Farber *et al.*, 2000
Sweden (NK)	5 (0)	0/5	1.9×10^{5}	Cold-smoked rainbow trout	1/2a	Miettinen *et al.*, 1999
Finland (1999)	18 (4)	0/18	$<100 - 1.1 \times 10^{4}$	Butter	3a	Lyytikäinen *et al.*, 1999
USA (1998–1999)	101 (20)	NK	NK	Frankfurters, sliced deli meats	4b	CDC, 1999

* For 2 of these 5 deaths, an underlying disease, not listeriosis was apparently the cause of death.
† Not known.
‡ Foods only epidemiologically linked.
§ Outcome only known for 252 cases.
‖ Only 314 patients categorized.
** Includes two sets of twins.
†† Not known if death due to listeriosis or leukemia.

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Glass & Doyle (1989) reported that this pathogen can proliferate on vacuum-packaged, artificially contaminated (~0.01 *L. monocytogenes* cfu g^{-1}) retail frankfurters during storage at 4.4 °C; populations increased by 2–5 $\log_{10}$ on samples that were judged organoleptically acceptable after 4 weeks. In another study, *L. monocytogenes* increased in number from 5×10^2 to 2.1×10^5 MPN g^{-1} on vacuum-packed frankfurters stored at 4 °C for 20 days. Interestingly, non-inoculated control products contained 1.2×10^2 MPN g^{-1} after the 20-day storage period, after initially testing negative for the organism (Bunčić, *et al.*, 1991). In a more detailed study of vacuum-packaged all-beef, poultry, or beef/pork frankfurters stored at 5 °C for up to 28 days, McKellar *et al.* (1994) found 40 of 61 (65.6%) frankfurters supported growth of *L. monocytogenes*, with an average increase of 1.26 $\log_{10}$ within a 14-day period. Initial lactic acid bacteria concentrations, as well as phenol and nitrite levels, varied considerably during storage, unlike sodium chloride levels. In addition, average pH levels decreased significantly during storage by 0.19 pH units. Several statistical models were derived in an attempt to describe adequately the growth and death of the organism in all frankfurter samples. Although no one model was found to be sufficient, the best model implicated initial and final lactic acid bacteria counts and initial pH as factors that influence the growth of *L. monocytogenes*.

16.2.4 Risk Characterization

Cooked sausages, of which the frankfurter is but one example, are consumed in large quantities throughout the world. It is estimated that 20 billion frankfurters are consumed each year in the US, for an average of 60 frankfurters per person per year. A retrospective case-control study conducted in 1986–1987 involving 154 listeriosis patients found that sporadic cases were significantly more likely to have eaten either frankfurters that had not been fully reheated or chicken meat that was still pink (Schwartz *et al.*, 1988). It was estimated that 20% of sporadic cases were linked to the two food products. A subsequent case-control study identified as potential causes Mexican-style and feta cheeses, foods purchased from the deli section of grocery stores and, again, undercooked chicken, but not frankfurters (Schuchat *et al.*, 1992).

The population of the US in 1998 was 270 million. From 1996–1999, the estimated incidence of listeriosis from the FoodNet system was 5, 5, 6, and 5 cases per million per year, respectively (CDC, 2000) or about 1,350 cases per year. If it is assumed that 1% (13.5 cases) of the total listeriosis cases might be due to frankfurters and there are 20×10^9 servings of frankfurters per year, then that equals 13.5 cases for every 20×10^9 servings among the total population.

Approximately 20% of the US population (54 million) is immunocompromised. If it is assumed that this high-risk population consumes frankfurters at the same rate as the general population (i.e., about 60 frankfurters per year), then the total number of frankfurters consumed by this subpopulation would be $(54 \times 10^6) \times 60 = 3240 \times 10^6$ or 3.24×10^9. If it is assumed that listeriosis is restricted to this immunocompromised population and 1% (13.5) of the total listeriosis cases might be due to frankfurters, then there would be 13.5 cases for every 3.24×10^9 servings among the high risk population.

The risk associated with frankfurters, however, is likely to be related to growth of *L. monocytogenes* and handling/preparation practices. Between 1993–1999, the prevalence of *L. monocytogenes* among the lots sampled by USDA-Food Safety and Inspection Service declined from 5.3 to 1.8%. Using a single dose approach (Buchanan *et al.*, 1997), one could assume that all cases of listeriosis are associated with the proportion of frank-

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furters having a high level of *L. monocytogenes* (e.g., $\geq 1 \times 10^3$ cfu g^{-1}). Although there are no data for the proportion of frankfurters that may have this level, it will be assumed that 5% contain *L. monocytogenes* but only 10% of those have a high level (i.e., $\geq 1 \times 10^3$ cfu g^{-1}) when purchased. The total number of contaminated frankfurters having a high level of *L. monocytogenes* purchased by the high-risk population then would be $3.24 \times 10^9 \times 0.05 \times 0.10 = 16.2 \times 10^6$.

If it is further assumed that the method of handling and reheating is inadequate to eliminate the high level of *L. monocytogenes* in 10% of the servings, then the frequency of exposure to a high level of *L. monocytogenes* would be $16.2 \times 10^6 \times 0.10 = 1.62 \times 10^6$. If 1% of all listeriosis is associated with eating franks, then the 13.5 cases would more likely result from the estimated 1.62×10^6 franks having a high level of *L. monocytogenes* that are handled and prepared in such a manner that illness can result among susceptible individuals.

Thus, the probability (P) that the high-risk population would acquire listeriosis from frankfurters containing high levels of *L. monocytogenes* would be P = 13.5 cases per 1.62×10^6 servings (i.e., approximately one case per 120,000 servings) or $P = 8.3 \times 10^{-6}$.

Since numerous assumptions have been made and reliable data are lacking, it is impossible to validate this estimate or to calculate attendant uncertainties. For example, the estimate assumes that all the strains of *L. monocytogenes* detected on frankfurters are equally virulent, which is unlikely. The estimate does suggest, however, that the risk of acquiring listeriosis from frankfurters on a per serving basis is exceptionally low, even among the high-risk population. Experience indicates that when frankfurters are contaminated with a highly virulent strain, cases may occur among the high-risk population (CDC, 1999).

The draft risk assessment conducted jointly by the US Food and Drug Administration and the USDA estimated the median number of cases of listeriosis per serving of frankfurters to be 3.0×10^{-6}, 5.0×10^{-8} and 5.9×10^{-9} for the perinatal, elderly, and intermediate age-based populations, respectively (FDA, 2001). The perinatal population consisted of fetuses and neonates (16 weeks after fertilization to 30 days after birth) with exposure occurring in utero from contaminated food eaten by the pregnant mother. The elderly group included individuals who were 60 or more years of age. The intermediate age group included the remaining population, including susceptible individuals not included as perinatal or elderly, such as cancer, AIDS, and transplant patients, for whom there was insufficient data for them to be considered as a separate population.

On a per serving basis, *reheated* frankfurters were of low risk relative to 19 other categories of foods studied, ranking 15th out of 20 for the three population groups. Frankfurters consumed *without reheating* were of high risk, ranking first or second among the 20 categories for the three population groups, assuming that from 1–14% of frankfurters are consumed without reheating. Overall, frankfurters ranked number 4 or 5 among the 20 food categories for the three population groups when the median estimates were used to predict the relative risk for listeriosis on a per annual consumption basis, again assuming that 1–14% of frankfurters are consumed without reheating (FDA, 2001).

16.3 RISK MANAGEMENT

16.3.1 Acceptable Level of Consumer Protection

In most industrialized countries, the annual incidence of listeriosis is between 2–6 cases per million of the population (Rocourt & Bille, 1997). For the risk characterization,

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an estimate of 1% of the total cases of listeriosis was used for the cases that might be associated with frankfurter consumption, i.e., 13.5 cases. An example of a public health goal could be to reduce the number of cases attributable to the consumption of frankfurters by some value (e.g., 50% reduction to no greater than 6.75 cases per million of the population).

16.3.2 Establishing a Food Safety Objective

Due to its widespread presence in the environment, eradication of *L. monocytogenes* from the food supply is impossible. There is general agreement that when the organism is ingested in low numbers, even susceptible individuals have little chance of developing listeriosis. A realistic food safety objective (FSO) must therefore be established that would, as far as possible, recognize that it is not possible with current technology to eliminate contamination of foods with *L. monocytogenes*. When total prevention is not possible, measures must be put into place to control recontamination to an acceptable level. To achieve these goals, it is essential that GHP and HACCP programs that are specific to the control of *L. monocytogenes* be applied at all stages of manufacture, storage, transport, and retail. In addition, research should continue to develop additional barriers to control the growth of *L. monocytogenes* in meat and poultry products. This might include the addition of food additives and/or the use of a competitive flora (McMullen & Stiles, 1996).

It is currently recognized that on a daily basis, humans are consuming *L. monocytogenes* in foods at levels of at least 100 cfu g^{-1} and not becoming ill. For example, data from Denmark show an overall contamination rate for cold-smoked salmon products of around 25–32%, with 4–5% of those samples containing greater than 100 cfu g^{-1} (Huss, 1997). Even with the large amounts of Danish cold-smoked salmon being consumed worldwide, there have been no recorded cases of listeriosis due to this product. In addition, countries such as Canada have established an action level for *L. monocytogenes* of 100 cfu g^{-1} for low risk products and have not seen an increase in the baseline levels of human listeriosis. This is also consistent with the estimated dose-response curve for *L. monocytogenes* from Buchanan *et al.* (1997), which includes an estimate for immunocompromised individuals, is reasonably conservative, and allows for the possibility of a single cell causing serious illness (Figure 16–2). Finally, epidemiologic data indicate that foods involved in listeriosis outbreaks are those in which the organism has multiplied and in general have contained levels well in excess of 100 cfu g^{-1} (see Table 16–1). Thus, on the basis of epidemiologic and prevalence data, the following FSO is proposed:

The concentration of *L. monocytogenes* in frankfurters should not exceed 100 cfu g^{-1} at the time of consumption.

This proposal is consistent with an earlier recommendation from the International Commission on Microbiological Specifications for Foods (ICMSF) (ICMSF, 1994). It also is compatible with a conclusion of the World Health Organization (WHO)/Food and Agriculture Organization (FAO) risk assessment for *L. monocytogenes* in RTE foods that a more strict tolerance of "not detected in 25 g" does not provide a higher level of protection (Ross *et al.*, 2000). Indeed, the incidence rate for listeriosis in the US, which has maintained the "negative in 25 g" tolerance, is not lower than other industrialized countries that have applied a tolerance of 100 cfu g^{-1}.

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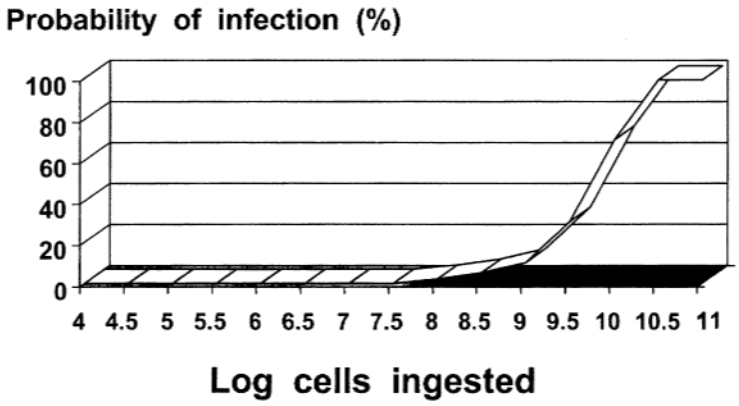


Figure 16–2 Estimated dose response curve for *Listeria monocytogenes*.

16.3.3 Control Measures

It is anticipated that industry can easily meet the proposed FSO for the product at the time of manufacture. However, if frankfurters are recontaminated after the commercial cooking, by the time they are consumed, there is potential for concentrations to exceed 100 g^{-1}, especially with a long refrigerated shelf-life, no additional barriers to growth, and/or temperature abuse. To reduce the incidence of listeriosis (e.g., to fewer than 5 cases per million population) by specifically reducing the number of cases due to frankfurters (e.g., from 13.5 to no greater than 6.75 cases), then other risk management options need be considered.

A flow diagram for a typical frankfurter operation is shown in Figure 16–1. HACCP systems and GHP procedures must be used to achieve the expected level of control and meet the FSO. It is important to consider available control measures in the context of the steps used in manufacture. In this example, controlling *L. monocytogenes* so the concentration will not exceed 100 cfu g^{-1} when frankfurters are consumed will involve combinations of the following control measures:

- *Controlling initial levels in raw materials*—Handling raw materials during storage and preparation to minimize an increase in numbers due to contamination or growth.
- *Reducing levels during cooking of frankfurters in the manufacturing process*—Cooking to eliminate *L. monocytogenes* in the raw meat emulsion. Establishing a performance criterion for cooking that results in process criteria (i.e., critical limits) that can be incorporated into the HACCP plan.
- *Preventing recontamination between cooking and packaging*—Minimizing recontamination between cooking and packaging by adopting GHP measures, e.g., separating raw from cooked product, and an environmental management and monitoring program.
- *Reducing levels in cooked product after packaging (in-pack pasteurization)*—Applying commercially feasible pasteurization processes for pasteurizing the product after packaging.
- *Preventing increase in levels between packaging and preparation for serving*—Controlling the increase of *L. monocytogenes* that subsequently may occur with

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recontamination and growth in packaged frankfurters during storage and distribution. Examples of control measures might include: adding safe, acceptable additives to control growth of *L. monocytogenes* on frankfurters, using code-dating practices and improved chill chain management to preclude an unacceptable increase in *L. monocytogenes*, or freezing the product.

- *Reducing levels prior to consumption*—Improving storage and handling practices and inactivating *L. monocytogenes* through effective cooking and reheating prior to consumption. These outcomes could be achieved as control measures in food service operations or through education of consumers, particularly the more susceptible population and their health care providers.

16.3.4 Performance Criteria

A performance criterion is the required outcome of one or more control measures at a step or combination of steps applied to achieve an FSO (see Chapter 3). Performance criteria are usually applied at steps where hazards can either be reduced or where hazards may increase.

To arrive at a performance criterion for the control measures needed to meet an FSO for frankfurters, the equation from chapter 3 can be used:

$$H_0 - \Sigma R + \Sigma I \leq FSO$$

$$H_0 - \Sigma R + \Sigma I \leq 2.0$$

where:

FSO = Food safety objective
H_0 = Initial level of the hazard
ΣR = Total (cumulative) reduction of the hazard from processing, etc.
ΣI = Total (cumulative) increase of the hazard

FSO, H_0, R, and I are expressed in $\log_{10}$ units.

Manufacturers of frankfurters should assume that *L. monocytogenes* will be present in all species and types of raw meat and poultry. This is evident from reports in the literature and results from surveys such as the USDA baseline studies for ground beef, ground turkey, and ground chicken (Table 16–2) (USDA, 1995a; USDA, 1995b; USDA, 1996). Data of this nature can be used to establish the initial concentration (H_0). Another option would be to generate similar data from raw emulsions collected at the facility prior to cooking.

The USDA baseline studies reported the prevalence of *L. monocytogenes* in ground beef, chicken, and turkey to be in the range of 18–35% when analyzing 25 g samples. Further analysis of the positive samples yielded low numbers of *L. monocytogenes*. It can be concluded from the data that concentrations of 100 g^{-1} or fewer would be expected under normal conditions. Further reducing the likelihood of higher concentrations is the effect of blending and emulsification that occurs in the manufacturing process. These steps disperse the cells more uniformly throughout the meat emulsion and result in an overall lower number g^{-1} with little likelihood of localized pockets of higher concentrations.

16.3.4.1 Controlling Initial Levels in Raw Materials

Consideration should be given to multiplication of *L. monocytogenes* that might occur as raw meat and poultry is stored and prepared for cooking. It is important to note that

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Table 16–2 USDA Baseline Survey Results for Ground Beef, Ground Turkey, and Ground Chicken

	Ground beef (563 samples)	*Ground turkey (165 samples)*	*Ground chicken (162 samples)*
Prevalence based on 25 g samples	18%	23%	35%
Geometric mean for positive samples only	3.9/g	3.8/g	2.24/g
95% confidence level	2.2–7.2/g	0.4–2.9/g	1.06–4.7/g

Samples found positive by the qualitative method were further analyzed to determine the number of *L. monocytogenes* g^{-1} of ground meat. The results for the positive samples were as follows:

Number of L. monocytogenes/g	*Ground beef (99 samples)*	*Ground turkey (52 samples)*	*Ground chicken (59 samples)*
< 0.03	45.2	82.5	56.1
0.03–0.29	0.0	0.0	0.0
0.3–2.9	30.2	6.7	29.3
3.0–29.9	15.0	0.0	10.5
30–299.9	9.6	10.9	3.2
300 or higher	3 samples had > 110/g	0.0	0.9

Note: These data indicate, for example, that in the case of ground beef, 563 samples were analyzed using 25 g each. Only 99 samples were found to contain *L. monocytogenes*. Upon further analysis of the 99 samples, 90.4% had fewer than 30 cfu $gram^{-1}$. From an overall perspective, only 3 of the total 563 samples had greater than 110 *L. monocytogenes* $gram^{-1}$, the upper limit of detection used for testing.

In the case of ground turkey, 165 samples were analyzed using 25 g each. Only 52 were found to contain *L. monocytogenes*. Upon further analysis of the 52 samples, 89.2% had fewer than 30 cfu $gram^{-1}$. From an overall perspective, the highest number detected among the 165 samples was a sample containing 93 cells $gram^{-1}$.

the samples for the baseline studies were collected, packaged, and shipped in refrigerated containers to the laboratory. Samples were considered acceptable for analysis if they were received at the laboratory by the day after collection and with a temperature of 10 °C or less. Hence, the samples were at least one day older than when collected and some temperature abuse may have occurred.

To further place the possibility of multiplication during preparation into perspective, the data in Table 16–3, derived from the USDA Pathogen Modeling Program, indicate that from 4–9 days at 4 °C would be required for *L. monocytogenes* to increase 10-fold in meat or poultry with no added salt (0.5%) (Buchanan & Whiting, 1996). Food MicroModel (Ver 3.02) similarly predicts that it would take around 6 days for a 10-fold increase of *L. monocytogenes* (4 °C, 0.5% NaCl, pH 6.5). Longer times would be required in raw materials containing added salt. For example, the mechanically deboned poultry meat used for manufacturing frankfurters typically contains added salt and sodium nitrite and is chilled to well below 4 °C before shipping to the frankfurter manufacturing plant. The values in Table 16–3 include the lag phase estimated for each set of parameters. The length of the lag phase that actually occurs in raw meat under commer-

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Table 16–3 Days for a 1 Log_{10} (10-Fold) Increase in *L. monocytogenes* at 4 °C and pH 6.0

	Days for 10-fold increase			
	Aerobic conditions (e.g., meat at top of container)		*Anaerobic conditions (e.g., meat below surface of container)*	
Sodium chloride (%)	*No $NaNO_2$ Added*	*150 μg g^{-1} $NaNO_2$ added*	*No $NaNO_2$ Added*	*150 μg g^{-1} $NaNO_2$ added*
0.5	5.3	8.9	4.1	8.8
1.0	5.6	9.4	4.5	9.6
1.5	6.0	10.0	4.9	10.5
2.0	6.4	10.6	5.3	11.5
2.5	6.8	11.4	5.8	12.4
3.0	7.3	12.3	6.2	13.4

cial conditions has not been determined, but the raw meat and/or poultry used for the manufacture of frankfurters is normally less than 7 days from the date of slaughter.

From the data in Table 16–2 it is evident that the numbers of *L. monocytogenes* are very low in raw meat and poultry. Furthermore, the data in Table 16–3 indicate that significant multiplication during preparation is unlikely. For this example, it will be assumed that the initial concentration may under certain circumstances increase 10-fold prior to commercial cooking. Thus, in this example, the initial number in the raw materials prior to cooking is assumed to be no more than 1000 g^{-1} (i.e., $H_0 = 3$).

16.3.4.2 Reducing Levels during Cooking of Frankfurters in the Manufacturing Process

If it is assumed that initial numbers of *L. monocytogenes* in the raw materials could be as high as 1000 g^{-1} (i.e., $H_0 = 3$), then a performance criterion for the reduction step (i.e., cooking) to meet the FSO would, theoretically, only need to be a 1 log_{10} reduction as given below and illustrated in Figure 16–3a.

$$H_0 - \Sigma R + \Sigma I \leq FSO$$

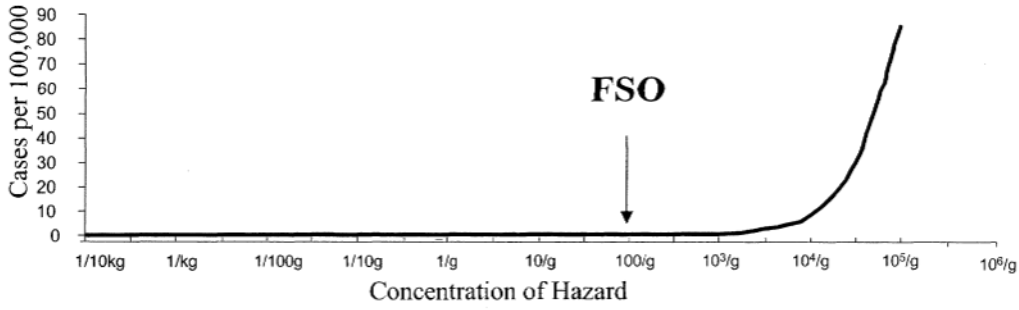
$$3 - \Sigma R + 0 \leq 2.0$$

$$\Sigma R = 1$$

However, if any viable cells remain after cooking, by the time the frankfurters are consumed, there is the potential for concentrations to exceed 100 g^{-1}, especially with a long refrigerated shelf-life, no additional barriers to growth, and/or if temperature abuse occurs. Therefore, to ensure that survivors do not remain and multiply during distribution and storage, it is necessary to apply a more stringent cooking process. For this example, it will be assumed that a 6 log_{10} reduction (R = 6) during cooking will achieve the desired result, given an initial concentration of no greater than 1000 g^{-1} ($H_0 = 3$). Thus, a 6 log_{10} reduction would result in a final concentration of ≤ 1 cfu kg^{-1} after cooking. This could be expressed as a performance criterion:

The concentration of *L. monocytogenes* after cooking shall be ≤ 1 cfu kg^{-1}.

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1) FSO could be met with only 1 $\log_{10}$ reduction:

$\leftarrow H_0$

$\Sigma R = 1$

2) Cooking during manufacture (6 $\log_{10}$ reduction, $\Sigma R = 6$) is applied to reduce levels to meet a performance criterion of $\leq 1\ kg^{-1}$ ($\leq 10^{-3}$ per g)

$\leftarrow H_0$

$\Sigma R = 6$

Figure 16–3a Control measure: cooking.

This is demonstrated in the following equation and illustrated in Figure 16–3a.

$$H_0 - \Sigma R + \Sigma I \leq 1/kg\ (\leq 10^{-3}/g)$$

$$3 - \Sigma R + 0 \leq -3$$

$$\Sigma R = 6$$

If the initial number is less than 1000 g^{-1} through raw material selection and/or preventing any increase during handling and storage (e.g., $\leq 100\ g^{-1}$), then a 5 $\log_{10}$ reduction would be adequate to meet the performance criterion of $\leq 1\ kg^{-1}$.

16.3.4.3 Prevent Recontamination Following Cooking

L. monocytogenes is a very hardy organism and can be recovered from different locations in the plant environment, depending on the level of control. In the absence of an effective control program, the organism can persist for prolonged periods in frankfurter production environments. During a 1989 investigation, the same strain of *L. monocytogenes* was recovered from a patient, from leftover frankfurters in the patient's refrigerator, and 4 months later from the frankfurter peeler in the manufacturing facility (Wenger *et al.*, 1990). In another outbreak in the US associated with frankfurters, it was reported that removing a refrigeration unit resulted in new or increased contamination of production equipment (CDC, 1999). This outbreak involved cases from August 2, 1998 through January 16, 1999, suggesting that multiple lots of product were implicated. Attempts to verify that the strain had become established in the environment, however, were unsuccessful. Thus, even if process criteria are met for cooking, recontamination of processed

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product is a possibility unless proper attention has been paid to the GHPs that are targeted toward control of *L. monocytogenes*.

Certain measures that fall within the realm of GHP have been found to be important for control of *L. monocytogenes* in cooked meat and poultry operations. The measures include plant layout, equipment design, maintaining equipment, cleaning and sanitizing procedures that are specific to listeriae control, maintaining a clean and dry environment, use of enclosed steam to sanitize equipment as a scheduled routine procedure, and low temperature storage (Tompkin *et al.*, 1999).

Two factors determine the effectiveness of a listeriae control program: routine environmental testing and the response to a positive finding. Without an environmental testing program, it is not possible to assess control. Furthermore, in the event a positive product contact sample is detected, corrective actions should be initiated to eliminate the source of contamination, thereby minimizing the risk of product contamination. To verify control, plants should implement an environmental monitoring program for *L. monocytogenes* or an indicator, such as *L. innocua* or *Listeria spp.* The program must be specific to each plant and should detail the areas to be sampled, the analytic method to be used, the frequency of sampling, and the action to be taken when *Listeria spp.* are detected (Tompkin *et al.*, 1992, 1999).

An effective monitoring program to assess control of the cooked product environment should consider the following strategies:

- preventing the establishment of *L. monocytogenes* in niches or other sites that can lead to contamination of RTE foods,
- implementing a sampling program that can assess in a timely manner whether the environment in which RTE foods are produced is under control,
- responding to each positive product contact surface sample as rapidly and as effectively as possible, and
- verifying that the problem has been corrected and providing data (e.g., tabulated, trends in graphical form) to facilitate short- and long-term assessment of control.

Experience in frankfurter operations indicates that sites within the cooked product environment wherein *L. monocytogenes* becomes established and multiplies (i.e., niches) are important contributing factors to product contamination. The sites serve as a reservoir from which the pathogen is dispersed and contaminates product contact surfaces. Furthermore, an environmental sampling program, such as described in Chapter 11, is necessary to assess whether the environment is under control and to detect the presence of a niche.

Response to a positive product contact sample (e.g., a sponge sample collected from a conveyor belt used in a frankfurter operation) is best when the following items are considered. Assuming an effective control program is in place, the primary source of *L. monocytogenes* in frankfurter operations is contamination from one or more niches. In general, contamination flows downstream with the process flow, much like a river. To regain control, it is necessary to locate the source where growth and contamination are occurring. This can best be accomplished by creating a map of the cooked product rooms and the equipment layout (see Chapter 11). The results of samples collected from each piece of equipment should be recorded on the map, including both positive and negative results. The layout map should then be reviewed for patterns. Which sites are more frequently positive? Where in the process flow do the first positives occur? It is important when seeking the source to analyze all the sponge samples separately and not as com-

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posites. In addition, sampling should be aggressive both in terms of location and frequency throughout the period of operation. Unfortunately, niches can rarely be detected unless the equipment is operating and product is being produced. While pursuing the source of contamination, consideration must be given to the possibility that a niche may not be involved (contamination could result, e.g., from someone touching the floor or other unclean surface and returning to handle exposed product).

When equipment has been identified as the source, the following steps can be effective. First, dismantle the equipment, collecting samples of suspicious material as this is being done. Second, replace obvious defective parts (e.g., hollow rollers) and clean and sanitize the equipment as it is being reassembled. If this procedure fails to eliminate the cause (or source) of contamination, it may be necessary to remove sensitive electronics, oil, and grease, and heat the equipment. This can be accomplished by placing the equipment in an oven and heating to an internal temperature of 71 °C with high humidity. Alternatively, the equipment can be shrouded with a tarp and steam can be injected into the space until the equipment reaches an internal temperature of 71 °C. Internal temperatures can be monitored with strategically placed thermocouples.

In frankfurter operations the following are examples of identified sources of contamination:

- rubber gaskets around the doors and other openings to the brine chill system
- saturated insulation on pipes carrying refrigerant to the brine chill system
- peelers that remove artificial casings before packaging
- casing removal systems
- hollow rollers on conveyors
- on/off valves and buttons for various equipment
- within complex collating equipment
- hollow support rods on the frames of equipment.

It is difficult to assign a performance criterion to recontamination, as any recontamination has the potential to reach high numbers through growth during the subsequent distribution and storage.

To ensure that the performance criterion of $\leq 1\ kg^{-1}$ (i.e., $\leq 10^{-3}$ per g) is not exceeded due to recontamination, there must be no recontamination and "I" in the following equation must be zero. This is illustrated in Figure 16–3b.

$$H_0 - \Sigma R + \Sigma I \leq 1\ kg^{-1}\ (\leq 10^{-3}\ \text{per g})$$

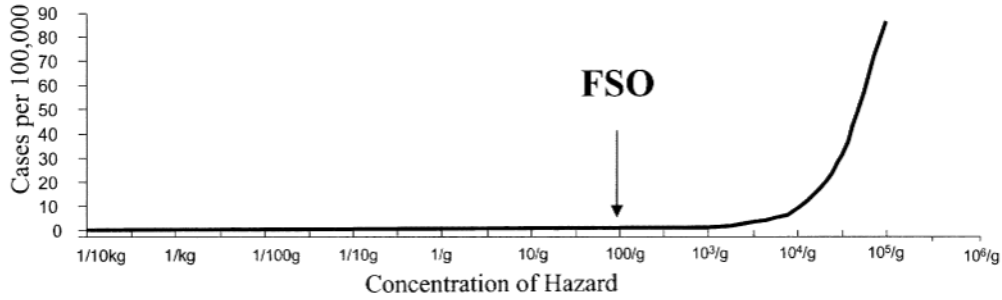
$$3 - 6 + \Sigma I \leq -3$$

$$\Sigma I = 0$$

16.3.4.4 Reducing Levels in Cooked Product after Packaging (In-Package Pasteurization)

Experience indicates that recontamination after cooking is the most common reason for the presence of *L. monocytogenes* in packaged, cooked sausages, such as frankfurters. If the increase in concentration due to recontamination is assumed to be as high as $10\ g^{-1}$, then an in-package pasteurization treatment could be applied as a means to achieve a 4 log reduction and still meet the performance criterion of $\leq 1\ kg^{-1}$ (i.e., 10^{-3} per g). As demonstrated in the following equation, if cooking provides an initial 6 log reduction and recontamination results in 10 cfu g^{-1}, then an in-package pasteurization treatment could be used to provide a final 4 log reduction and meet the performance criterion of ≤ 1/kg.

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Preventing recontamination between cooking and packaging
through effective GHPs and an environmental sampling program

$$H_0 - \Sigma R + \Sigma I \leq \text{Performance criterion (1 kg}^{-1} \text{ or} \leq 10^{-3} \text{ per g)}$$

$$3 - 6 + \Sigma I \leq -3$$

$$\Sigma I \leq 0$$

Figure 16–3b Control measure: preventing recontamination.

Hence, the combined reduction steps for cooking and in-package pasteurization result in a 10 log reduction (i.e., $\Sigma R = 10$). This is illustrated in Figure 16–3c.

$$H_0 - \Sigma R + \Sigma I \leq 1 \text{ kg}^{-1} (\leq 10^{-3} \text{ per g})$$

$$3 - \Sigma R + 4 \leq -3$$

$$\Sigma R = 10 \text{ (Cooking 6D, In-pack pasteurization 4D)}$$

16.3.4.5 Preventing Increase in Levels between Packaging and Preparation for Serving

In this and the next example, an in-package pasteurization treatment is not applied. If the increase in concentration due to recontamination is again assumed to be as high as 10 g^{-1} (i.e., a 4 $\log_{10}$ increase from 1 kg^{-1}) and if the FSO of 100 g^{-1} at time of consumption is to be met, there can be no greater than a 10-fold increase during storage and distribution before the food is consumed (see also Figure 16–3d).

$$H_0 - \Sigma R + \Sigma I \leq \text{FSO}$$

$$3 - 6 + 4 \leq 2$$

$$\Sigma I \leq 1$$

If recontamination occurs, then an increase of 10-fold or more must be controlled by adding inhibitory ingredients, freezing the product for distribution, and/or establishing use by date coding that are within the time for a 10-fold increase.

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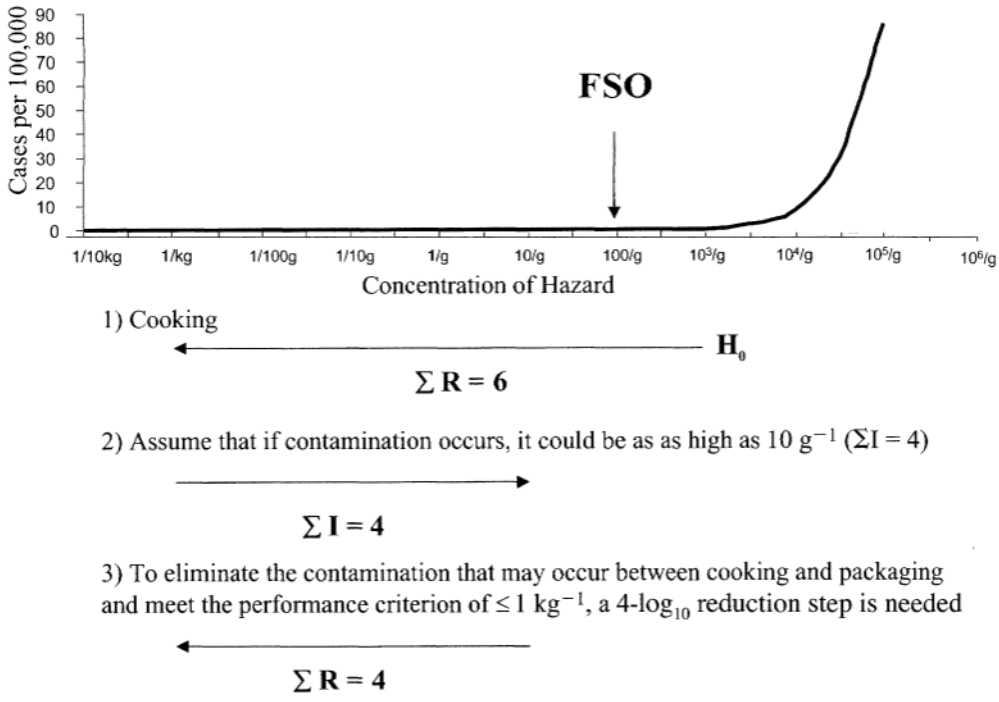


Figure 16–3c Control measure: reducing levels using in-package pasteurization.

16.3.4.6 Reducing Levels Prior to Consumption

If the product is recontaminated with *L. monocytogenes* to a level of 10 g^{-1} (I=4) and growth does occur during subsequent distribution and storage, then the handling and preparation procedures become very important for susceptible consumers. If it is assumed that there could be a 5 $\log_{10}$ increase due to growth (I=5) between when the frankfurters are cooked in the factory and when they are reheated for serving, then the combined increase after cooking would be $\Sigma I = 4 + 5$ for a total increase of 9 $\log_{10}$. At the time of preparation, the frankfurters would have a level of 10^6 g^{-1}. This could be represented as follows (see also Figure 16–3e).

$$H_0 - \Sigma R + \Sigma I \leq FSO$$

$$3 - 6 + 9 \leq FSO$$

$$H_0 - \Sigma R + \Sigma I \leq 6$$

Therefore, to meet the required FSO of ≤ 100 g^{-1}, an additional reduction step during preparation for serving would be required prior to consumption, as follows.

$$H_0 - \Sigma R + \Sigma I \leq FSO$$

$$3 - \Sigma R + 9 \leq 2$$

$$\Sigma R \leq 10$$

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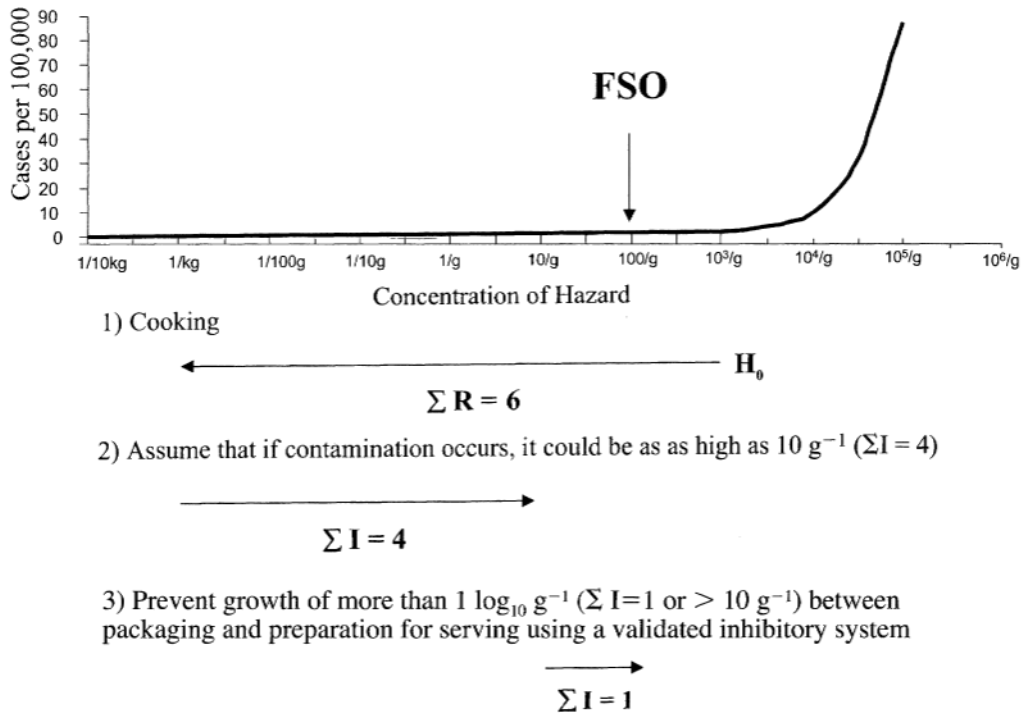


Figure 16–3d Control measure: use of inhibitor(s) to prevent growth after packaging.

After taking account of the 6 $\log_{10}$ reduction (i.e., $\Sigma R = 6$) that occurs when the frankfurters were cooked during manufacture, the reduction during preparation for serving must be at least as follows (Figure 16–3e).

$\Sigma R \leq 4$

The final reheating before serving then must reduce the level of *L. monocytogenes* by 4 $\log_{10}$ to ensure that the frankfurters will meet the FSO of 100 g^{-1} at the time they are consumed.

16.4 PROCESS AND PRODUCT CRITERIA

Each manufacturer must determine the parameters for cooking that will provide the desired product quality and cost, and, in this example, ensure that the performance criterion (i.e., $< 1\ kg^{-1}$) is met for cooking. The parameters adopted by one manufacturer may differ from those adopted by others. This is due to differences in equipment, desired product quality, type of raw materials (e.g., beef, pork, chicken and/or turkey), type of casing (e.g., natural, artificial), method of applying smoke (e.g., natural, liquid), fat content, and other factors. To meet the performance criterion requires knowledge of the heat sensitivity of *L. monocytogenes*, particularly in frankfurter emulsions.

Historically, the minimum internal requirement set by USDA for cooked sausage was 64.4 °C. For frankfurters, industry typically applied processes to achieve at least 68.3 °C. Since about 1980–1985, many processors have increased the temperature to 71 °C, or

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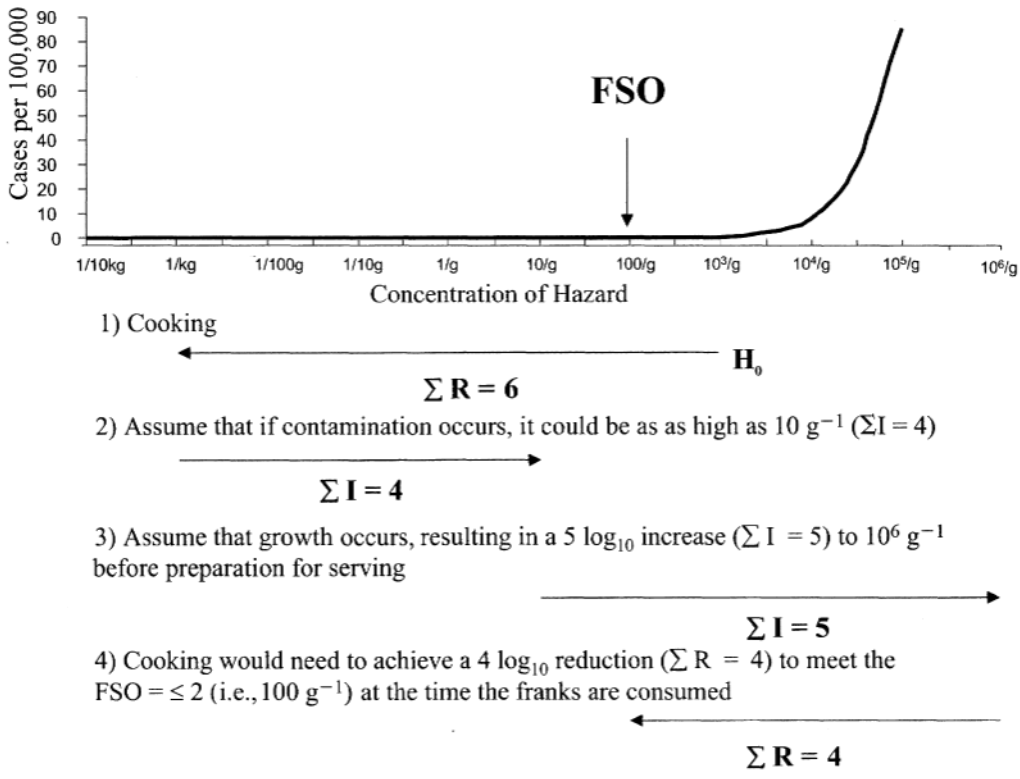


Figure 16–3e Control measure: reducing levels prior to consumption.

higher, to achieve greater consistency in product quality and refrigerated shelf-life. Even with the minimum requirement of 64.4 °C, however, there was no evidence that cooked sausages were implicated as a source of foodborne illness due to survival of enteric pathogens, probably due to the low concentration of enteric pathogens present initially and their sensitivity to heat. In the case of *L. monocytogenes*, the data in Table 16–4 indicate that survival also is unlikely to occur. Even with extensive testing for the pathogen since 1988, there has been no documented incident in which *L. monocytogenes* surviving the heat process was the underlying reason for a contaminated cooked sausage.

The thermal process can be validated by various means. One could be to conduct thermal destruction tests in the laboratory using frankfurter emulsion inoculated with a mixture of *L. monocytogenes* isolates from meat operations. Another may be to perform in-house challenge experiments with nonpathogenic surrogate organisms, such as *L. innocua*. Various items, such as provided in Chapter 3, should be considered when conducting such studies to validate a thermal process. A third approach to validation is to review the published literature for data on thermal resistance. For example, numerous studies have been published that report thermal destruction values for *L. monocytogenes* in various meat and poultry products (ICMSF, 1996; Farber & Peterkin, 1999; Zaika *et al.*, 1990; Fain *et al.*, 1991).

The data summarized in Table 16–4 provide lethality values for *L. monocytogenes* in meat, poultry, and seafood cooked to temperatures within the range of 60–65 °C. A number of factors influence the rate of death, but from the data, it can be concluded that even

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Table 16–4 Reported Thermal Destruction Values for *L. monocytogenes* in Meat, Poultry, and Seafood Products

Product	*Temp (°C)*	*D value* (min.)*	*Reference*
Ground meat with cure mix	64	2.28	Farber (1989)
Ground meat	64	<1.01	
Beef	65	0.93	Mackey *et al.* (1990)
Chicken leg	65	0.53	
Chicken breast	65	0.52	
Buffer	65	0.29	Boyle *et al.* (1990)
Meat slurry	65	0.75	
Lean beef	62.8	0.6	Fain *et al.* (1991)
Fatty beef	62.8	1.2	
Beef steak	63.9	2.2	Gaze *et al.* (1989)
Chicken breast	63.9	1.6–1.8	
Ham—control culture	60	1.8	Carlier *et al.* (1996)
Ham—heat-shocked culture	60	3.5	
Ham—"resistant" culture	60	1.0	
Mussels	62.2	1.85	Bremer & Osborne (1995)
Crabmeat	60	1.3–2.6	Harrison & Huang (1990)
Lobster meat	62.8	1.1	Budu-Amoako *et al.* (1992)
Salmon	65	0.9–1.2	Embarek & Huss (1993)
Cod	65	0.27–0.28	
Cured sausage blend of 66% pork and 33% beef	63.9	3.3	Farber & Brown (1990)
Cured sausage blend of 66% pork and 33% beef	30 min. at 47.8 °C, then heat to 63.9 °C	4.2	
Cured sausage blend of 66% pork and 33% beef	60 min. at 47.8 °C, then heat to 63.9 °C	4.7	
Cured sausage blend of 66% pork and 33% beef	120 min. at 47.8 °C, then heat to 63.9 °C	8.0	

*D value = time in minutes at a specified temperature for a one $\log_{10}$ (i.e., ten-fold) reduction in the number of *L. monocytogenes.*

these lower temperatures are effective for killing *L. monocytogenes* in a wide variety of foods. Such data can be used to arrive at alternative thermal processes for sausage products, such as frankfurters, and to consider the disposition of products in the event of a deviation. The adequacy of a thermal process based on an internal temperature of 70 °C can be determined from the data in Table 16–5.

An example of a default process criterion to achieve a 6 $\log_{10}$ reduction for in-pack pasteurization can be found in the draft Code of Practice developed by a Belgium-Dutch Chilled Meals Working Group, which recommends an internal heat treatment of 70 °C for 2 minutes for elimination of *L. monocytogenes* in chilled, long-life pasteurized foods (Lund & Notermans, 1993). The advantage in using a performance criterion rather than a default process criterion is that it allows flexibility in how the performance criterion is achieved. This would facilitate the development of new technology that may offer novel ways to achieve the same end result.

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Table 16–5 Reported D values for *L. monocytogenes* at 70 °C

Product	*D value (min)**	*Reference*
Beef	0.14	Mackey *et al.* (1990)
Chicken leg	0.11	
Chicken breast	0.13	
Meats (predicted value)	0.13	
Meat slurry	0.23	Boyle *et al.* (1990)
Beef slurry (pH 5.9)	0.23	
Phosphate buffer (pH 7.2)	0.15	
Chicken	0.16, 0.20	Gaze *et al.* (1989)
Beef steak	0.20, 0.14	
Turkey meat emulsion	0.35	Fain *et al.* (1991)
Salmon, cod	0.13–0.17	Embarek *et al.* (1993)
Sucrose solution, a_w 0.98	0.15 @ 68.3C	Sumner *et al.* (1991)
Sucrose solution, a_w 0.96	0.30 @ 68.3C	
Raw milk	0.05 @ 68.9C	Bradshaw *et al.* (1985)
Raw milk	0.015@ 71. 7C	

*D value = time in minutes at a specified temperature for a one $\log_{10}$ (i.e., ten-fold) reduction in the number of *L. monocytogenes.*

Product criteria that are intended to prevent growth after packaging could be validated by performing challenge studies using surface-inoculated frankfurters to assess the likely increase (ΣI) of *L. monocytogenes* to $\geq$ 100 g^{-1} before the recommended use-by date. Challenge studies could involve, for example, using commercial product inoculated and packaged in a laboratory with a mixture of five isolates of *L. monocytogenes* and then stored at temperatures to which the product would be exposed during storage and distribution. Many factors should be considered when conducting such studies (see Chapter 3). Predictive modeling also can provide an estimate of the pathogen's behavior and possible growth or inactivation (McKellar *et al.*, 1994; Armitage, 1997; McClure *et al.*, 1997; McDonald & Sun, 1999; Buchanan & Whiting, 1996).

Examples of additives that have been found effective for inhibiting *L. monocytogenes* include sodium lactate, sodium acetate, and sodium diacetate (Schmidt, 1993; Schmidt & Leistner, 1993; Qvist *et al.*, 1994; Wederquist *et al.*, 1994, 1995; Blom *et al.*, 1997; Tompkin, 1999; Juncher *et al.*, 2000; Hoogenkamp & Wales, 2000).

A uniform procedure has not been developed to validate code-dating practices to ensure the safety of perishable foods with extended shelf-life (e.g., frankfurters and other cooked meat or poultry products). As discussed in Chapter 3, a number of factors must be considered when validating the effectiveness of one or more steps along the food chain. The following factors should be considered when performing a validation study for code-dating frankfurters:

- physiological state of the inoculum (e.g., 24 h broth culture)
- method of inoculation (surface)
- inoculum level (per package or per g)
- source and number of strains

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- virulence of the strains (outbreak strains should be avoided in most laboratories)
- temperature(s) of storage
- natural competitive flora (commercial v. pilot plant product)
- product formulation (fermentable carbohydrate and rate of acid production).

16.5 GHP AND HACCP

Certain aspects of GHP must be controlled to minimize recontamination of cooked products, as has been discussed in this chapter and Chapter 11. In this example, a performance criterion of ≤ 1 kg^{-1} was established for the cooked product and for in-package pasteurization to ensure that the FSO of ≤ 100 g^{-1} is met. This led to the establishment of process criteria that could be adopted as critical limits in the HACCP plan. The critical limits typically consist of a holding time and internal temperature, their selection being influenced by the cooking system (e.g., continuous or batch oven). If, for example, 71 °C is adopted, the critical limit for cooking frankfurters during their manufacture could be stated as "an internal temperature of 71 °C", a hold time not being necessary.

With a properly designed and implemented HACCP plan, there would be no benefit to testing the cooked product for further validation of the process or to verify that each lot has been cooked correctly. If an event occurs requiring such testing, then a sampling plan of $n = 5$, $c = 0$, $m = 0/25$ could be used. This would provide 95% confidence of detecting a positive lot if the mean count was 0.03 cfu g^{-1} or greater.

16.6 ACCEPTANCE CRITERIA FOR FINAL PRODUCT

16.6.1 Organoleptic

No organoleptic criteria are applicable to assess the likely presence of *L. monocytogenes* in frankfurters.

16.6.2 Chemical and Physical

No chemical or physical criteria are applicable to assess the likely presence of *L. monocytogenes* in frankfurters.

16.6.3 Microbiological

When it is known that a product has been pasteurized in the pack, sampling final product would provide no added value and is not recommended. Likewise, for those plants that are using a validated kill step for cooking and where an effective environmental sampling program documents that the risk of recontamination is being controlled, there is little value in testing end product. The reason is that a comprehensive management system can maintain frequencies of contamination to less than 0.5%. Under these circumstances, the frequency of defective units is too low for detection with any practical sampling plan (see Chapters 6, 7, and 17).

Where product is crossing international borders and nothing is known about the product or the manufacturing process, end-product testing may be appropriate. The ICMSF has provided guidance on sampling plans for *L. monocytogenes* in a variety of foods and conditions (van Schothorst, 1996). For frankfurters that have not been in-pack pasteur-

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ized and, if contaminated, growth could occur (e.g., no added inhibitors), ICMSF sampling case 12 ($n = 20$, $c = 0$) would apply. When case 12 is applied and 25 g analytical units are used in a two-class sampling plan, which would be typical for *L. monocytogenes*, there is 95% probability that a positive lot would be detected when the mean concentration is 1 cfu in 186 g or greater. This assumes a log-normal distribution and standard deviation of 0.8. Thus, the sampling plan performance would be adequate to detect lots that exceed an FSO of $\geq 100\ g^{-1}$. If the frankfurters are intended specifically for highly susceptible individuals (e.g., hospitals), then case 15 would apply ($n = 60$, $c = 0$). When case 15 is applied and 25 g analytical units are used, there is a 95% probability that a positive lot would be detected when the mean concentration is 1 cfu in 526 g or greater.

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E. coli O157:H7 in Frozen Raw Ground Beef Patties

17.1 Introduction
17.2 Risk Evaluation
17.3 Risk Management
17.4 Control Measures
17.5 Acceptance Criteria
17.6 Statistical Implications of the Proposed Sampling Plan
17.7 References

17.1 INTRODUCTION

This chapter is concerned with *Escherichia coli* O157:H7 in frozen raw ground beef patties. The information follows the principles outlined in earlier chapters. The unique severity of the pathogen and its impact among children under five years of age warrant special consideration. In this example, the merits of microbiological testing will be explored as a potential control measure to enhance the safety of ground beef. A significant portion of this material was published as a discussion paper (Tompkin & Bodnaruk, 1999). This example draws primarily on data from the US. The information may be applicable in other countries with similar prevalence rates in ground beef and where undercooking is a risk factor.

Ground beef is an important raw agricultural commodity. In 1992 in the US, the types of cattle from which ground beef was derived were steers, 39.1%; heifers, 19.2%; cows, 34%; and bulls, 7.7%. Imported boneless manufacturing beef composed 15% of the total US ground beef supply. Imports into the US account for almost half of the world trade in fresh, chilled, and frozen beef. Ninety percent of the beef imported into the US is from Australia, New Zealand, and Canada. Other sources include Costa Rica, Honduras, and the Dominican Republic. The annual average quantity of beef imported into the US has been over 600,000 tons (USDA, 1994). Annual per capita consumption was estimated to be about 11.8–12.7 kg from 1982–1998 (AMI, 2000). Ground beef is consumed in the form of hamburger and as a major ingredient in a variety of foods.

E. coli O157:H7 is among the more newly recognized foodborne pathogens, being first identified during an outbreak in 1982 in which ground beef was implicated. It has since been a steadily increasing cause of foodborne illness worldwide. It is recognized that there are numerous serotypes of enterohemorrhagic *E. coli* (EHEC), some of which may be more important in certain regions of the world than *E. coli* O157:H7 (WHO, 1997; Acheson, 2000).

Since 1982, ground beef has become recognized as a major vehicle for transmission of *E. coli* O157:H7 in the US. In general, the pathogen is deposited on the surface of beef carcasses during the slaughtering process. After chilling, the carcasses are cut into large portions for sale as bone-in or boneless beef cuts (e.g., round, loin, rib, chuck). During this

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process, the larger portions are trimmed of excess fat and other tissue. The trimmings commonly are used in the manufacture of raw ground beef and a wide variety of ready-to-eat products (e.g., sausages). The same process of trimming meat and fat occurs at other steps along the food chain, with much of it being used for ground beef. Wherever it may occur along the food chain, the process of trimming and subsequent grinding distributes the pathogen throughout the ground meat. The most common scenario leading to illness has involved undercooking, survival of the pathogen, and subsequent infection, particularly among the more susceptible consumers. Cross-contamination in kitchens and food service establishments from ground beef to other ready-to-eat foods also has occurred.

It is extremely difficult to compare data from different countries on incidence and prevalence due to the use of different methods of sampling/reporting. However, data for different countries indicate that *E. coli* O157:H7 is an international concern, with the prevalence in meat ranging from around 0.1%–5% and prevalence in cattle ranging from around 1.5%–28% (Table 17–1).

17.2 RISK EVALUATION

17.2.1 Hazard Identification

E. coli is a species of Gram-negative, facultatively anaerobic, rod-shaped bacteria commonly found in the lower part of the intestine of warm-blooded animals. *E. coli* O157:H7 is a particular serotype of the group referred to as enterohemorrhagic *E. coli* (EHEC). This is a subgroup of the verocytotoxigenic *E. coli* (VTEC) that has been shown to cause human illness. VTECs produce verotoxins, or shiga-like toxins, that are closely related to the toxin produced by *Shigella dysenteriae* (Cassin *et al.*, 1998). Most EHEC isolates are acid-tolerant, capable of surviving in acid foods and during passage through the stomach (Benjamin & Datta, 1995; Arnold & Kaspar, 1995; Leyer *et al.*, 1995; Cheville *et al.*, 1996).

Table 17–1 Incidence of *E. coli* O157:H7 Infection and Prevalence in Meat and Cattle in Various Countries

Country	*Incidence HUS/VTEC*†*	*Prevalence (%) in Meat*	*Prevalence (%) in Cattle*
Argentina	HUS 7.8 per 100,000	0.4 bovine meat	
Australia	HUS 2.79 per 100,000		
Canada	VTEC 3.0 per 100,000		
Denmark	VTEC 0.1 per 100,000	0.13 minced beef	1.5
Germany		0.7 beef	
		5.0 minced beef	
Japan		0.3 carcass	1.4
Netherlands	30 per year	0.08 beef/pork	11.1
England and Wales	VTEC 1.29 per 100,000	1.5 beef	15.6
US	0.74 per 100,000	1.0	5
			28‡

*HUS = hemolytic uremic syndrome
†VTEC = verocytotoxigenic *E. coli*
‡using improved isolation methods

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Cattle appear to be the main reservoir of *E. coli* O157:H7. Contamination of carcasses during slaughter is the primary route that ultimately leads to contamination of ground beef. Other foods (e.g., lettuce, sprouts, fruit juices, vegetables, raw milk) and water also have been implicated as vehicles of transmission. Person-to-person is an important mode of transmission, particularly in day care centers. Direct contact with animals carrying the organism is also a recognized source of infection (WHO, 1997).

17.2.2 Hazard Characterization

E. coli O157:H7 infection can result in moderate to severe disease or death, with most deaths occurring in children under five years of age and in the elderly (AGA, 1994; Tarr, 1994). Studies conducted in FoodNet sites in the US suggest that from 13–27 cases of infection occur in the community for each confirmed case that is reported (Mead *et al.*, 1999). Within the FoodNet sites, the annual laboratory-confirmed case rate per 100,000 population has been from 2.1–2.8 in the four years of 1996–1999 (CDC, 2000).

The time to onset of symptoms following exposure ranges from three to nine days, with an average of four. The duration of illness ranges from two to nine days; however, with complications, the illness may last many months and lead to permanent damage or even death. Symptoms may include hemorrhagic colitis: grossly bloody diarrhea, severe abdominal pain, vomiting, but no fever; hemolytic uremic syndrome (HUS): prodrome of bloody diarrhea, acute nephropathy, seizures, coma, and death; and thrombotic thrombocytopenic purpura: similar to HUS but also fever and central nervous system disorder (ICMSF, 1996).

The potent toxins of *E. coli* O157:H7 can initially cause a particularly severe form of human disease, hemorrhagic colitis. About 10% of these patients can go on to develop HUS, characterized by acute renal failure, hemolytic anemia, and thrombocytopenia, which is particularly serious in young children and elderly people. On average, 2–7% of patients with HUS die, but in some outbreaks among the elderly, the mortality rate has been as high as 50% (WHO, 1997). Long-term renal dysfunction occurs in about 10–30% of survivors of HUS. The estimate for the prevalence of HUS in North America is about 3/100,000 children under five years of age per year (Mahon *et al.*, 1997).

From outbreak data, it appears that fewer than 100 cells can cause disease among consumers (AGA, 1994), particularly those at greater risk (e.g., less than five years of age) (Mahon *et al.*, 1997). In the 1993 northwestern US outbreak involving undercooked hamburgers, it was estimated that as few as a dozen cells may have caused illness among children (Tarr, 1994).

There are estimated to be about 73,480 cases of illness attributed to *E. coli* O157:H7 per year in the US, of which 85% are estimated to be foodborne. The number of hospitalizations and deaths due to foodborne transmission of this pathogen was estimated to be 1,843 and 52 per year, respectively (Mead *et al.*, 1999). These values differ significantly from the earlier estimates of Mahon *et al.* (1997). The more recent estimate revealed more cases, but fewer deaths. The estimated costs of medical treatment and lost productivity range from $216–580 million/year (AGA, 1994).

17.2.3 Exposure Assessment

The source of *E. coli* O157:H7 on beef carcasses is the hide and intestines of the cattle being slaughtered. Prevalence among cattle being held for slaughter is similar to or

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slightly higher than the prevalence on the external surface of hides of recently slaughtered animals (Hancock, 1998).

The percent of cattle with *E. coli* O157:H7 in their feces was initially reported to be typically less than 5% (Hancock, 1998). A later study involving a more sensitive analytical method found 28% of the cattle entering slaughtering plants to be actively shedding *E. coli* O157:H7 or nonmotile *E. coli* O157 in their feces during July and August, the months of highest prevalence. Eleven percent of the hide surfaces also were positive (Elder *et al.*, 2000).

Studies have shown that colonization of cattle is of short duration (1–2 months); long-term carriers have not been found. The typical pattern of shedding in a herd followed over time is one of epidemics of shedding interspersed with longer periods with rare or non-shedding animals. These epidemics occur mainly during warm weather, suggesting that environmental proliferation may play an important role in the epidemiology of *E. coli* O157:H7 (Hancock, 1998). It is important to note that *E. coli* O157:H7 does not cause any adverse effects in cattle. Its presence in a herd or individual animal can be detected only by microbiological testing.

While some cross-contamination may occur from carcass-to-carcass through contact with common equipment and workers' hands, there is no published evidence that *E. coli* O157:H7 has ever become established and multiplied in a slaughtering/chilling/fabrication operation and contaminated subsequent lots of beef.

Technology is currently available that can:

- minimize carcass contamination during the slaughtering process,
- reduce the likelihood of microbial attachment to exposed tissues, and
- decontaminate carcasses, such as with steam, hot water, or organic acid sprays prior to chilling.

Collectively, the above control measures can significantly reduce, but not eliminate, the likely presence of enteric pathogens on raw beef. Systems for decontamination will likely involve multiple control measures during slaughtering, evisceration, and chilling (Dorsa *et al.*, 1996; Dorsa, 1997; Castillo *et al.*, 1998; Nutsch *et al.*, 1998; Sofos & Smith, 1998; Sofos *et al.*, 1999; Bacon *et al.*, 2000).

It is reasonable to assume that when present, *E. coli* O157:H7 is on the surface of carcasses, not in internal tissues. Rapid chilling of adequately spaced carcasses should retard *E. coli* O157:H7 growth on carcass surfaces. Surface dehydration during chilling is an additional factor that can restrict growth.

After chilling, it is not likely that *E. coli* O157:H7 will multiply during subsequent fabrication because the lower temperature limit for multiplication of *E. coli* O157:H7 is 7–8 °C (ICMSF, 1996). Furthermore, estimates for growth under optimum laboratory conditions in broth indicate that:

- at 10 °C, the time to increase 10-fold is estimated to be 73 h, and
- at 15 °C, the time to increase 10-fold is estimated to be 25 h (Buchanan & Whiting, 1996).

Thus, in controlled chilling/fabrication operations, the concentration of *E. coli* O157:H7 should not increase. Conversely, in operations that do not address chilling/fabrication as important factors requiring control, some degree of multiplication may occur.

Epidemiologic data for outbreaks reported each year by the Centers for Disease Control and Prevention (CDC) indicate the risk of *E. coli* O157:H7 from beef continues

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to be associated with consumers who have not changed their handling/cooking habits to control the risk of the pathogen. These consumers do not understand the risks and/or do not have the knowledge to deal with a pathogen such as *E. coli* O157:H7. Some may be aware of the risk and have chosen to ignore recommendations for proper handling and cooking of ground beef. A 1996 survey indicated that 19.7% of the population consumed pink (undercooked) hamburger at some time during the previous 12 months (CDC, 1998a).

In its report of FoodNet for 1997, CDC stated: "In contrast with previous investigations, hamburgers eaten at fast food restaurants were not associated with infection, suggesting that recent changes in that industry may have reduced *E. coli* O157:H7 infections from that source" (CDC, 1998b). This is a conservative assessment because a review of the outbreaks reported each year by CDC shows no reported outbreaks from the larger fast food operations between 1993–2000. This verifies that the control measures adopted in those operations have been effective in managing the risk of *E. coli* O157:H7 in ground beef.

The improved controls in fast food operations do not appear to have carried through to other areas. A case-control study conducted at FoodNet sites has determined that undercooked ground beef continues to be a principal food source of *E. coli* O157:H7 infections (CDC, 1998b). Outbreaks have continued to occur in other food service establishments (e.g., restaurants, schools) and among consumers who prepare ground beef at home, on camping trips, or in other settings. Ground beef is the primary vehicle, not roasts, steaks, etc.

This information suggests that a continuing effort should be directed toward educating food handlers and consumers in proper handling and cooking of ground beef before serving. A goal should be established to reduce the percentage of consumers who consume rare ground beef from approximately 20% to some lower value. School systems and other food service establishments should consider additional training/education and other options (e.g., precooked beef products).

17.2.3.1 Survey Results from USDA-FSIS

The US Department of Agriculture (USDA)-Food Safety and Inspection Service (FSIS) established a sampling and testing program for ground beef in 1994 to:

- stimulate industry actions to reduce the presence of *E. coli* O157:H7 in raw ground beef (i.e., to encourage industry to institute and maintain effective control measures),
- encourage industry to sample and test raw ground beef routinely,
- find and remove from commerce product containing *E. coli* O157:H7, and
- expand the agency's information base and understanding in the control of *E. coli* O157:H7 (FSIS, 1994).

Results of the sampling and testing program are summarized in Table 17–2. The methods of sampling and analysis have been modified since 1995 to increase sensitivity of detection. During 1995–1997, a single 25 g sample was analyzed. Since the beginning of fiscal year 1998, five 65 g samples have been analyzed.

During the 1990s, industry implemented new control measures to reduce the occurrence of salmonellae and *E. coli* O157:H7 in ground beef. Due to the increased sensitivity of the methods used by FSIS, it is not possible to assess the effectiveness of changes implemented by industry or to determine whether the prevalence of *E. coli* O157:H7 in ground beef has, in fact, increased since the inception of the sampling program.

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Table 17–2 Results from the FSIS Sampling Program Beginning with Fiscal Year 1995

Fiscal Year	*No. Samples*	*No. Positive*	*% Positive*
1995*	5291	3	0.057
1996*	5326	4	0.075
1997*	5919	2	0.034
1998†	7529	14	0.19
1999†	8710	29	0.33
2000‡	>4,000	38	0.95

*25 g analytical units; †325 g analytical units, ‡325 g and improved isolation method (i.e., immunomagnetic beads to concentrate cells). The results for FY2000 are through August 11, 2000.

17.2.3.2 Data from Ground Beef Implicated in Illness and Lots Found Positive by USDA-FSIS

As ground beef has been implicated in outbreaks, samples from some implicated lots have been collected and analyzed. The ability to detect additional positive samples from across implicated lot(s) indicates a relatively high prevalence and concentration. In a few instances, a quantitative analysis was performed to determine the concentration of *E. coli* O157:H7. For example, in the 1993 outbreak that occurred in the northwestern US, frozen ground beef patties produced at a particular facility on November 19 and 20, 1992 were found positive. Positive samples were found in lots 4 and 9–17 in the production of November 19 and in lot 7 in the production of November 20. Quantitative analysis of some of the positive lots conducted by FSIS and CDC resulted in most probable number values in the range of 1–4 cells g^{-1}, with a single high value of 15 g^{-1} (FSIS, 1993).

Other evidence suggesting a relatively high prevalence and concentration of *E. coli* O157:H7 in certain lots of ground beef includes:

- large numbers of cases among consumers,
- multiple outbreaks involving consumers in different locations, and
- ability to detect *E. coli* O157:H7 with just a single sample from an implicated lot.

Follow-up sampling of positive lots of ground beef detected at retail stores has led to a positive sample from coarse ground beef used.

Data from the USDA-FSIS and lots implicated in outbreaks suggest that the vast majority of ground beef has a very low prevalence and concentration of *E. coli* O157:H7. In rare instances, a very small proportion of the lots contains a relatively high prevalence and concentration. It is because of this kind of skewed distribution in the number of bacteria that a log-normal distribution function is often used in microbiology. A hypothetical log-normal distribution of counts with a standard deviation would give the curve shown in Figure 17–1. Baseline studies on the numbers of bacteria in raw meat and poultry in the US and Canada provide further support to the general applicability of a log-normal distribution for the microbial population in these foods (FSIS, 1996; CFIA, 2000). When a log-normal distribution is assumed for these data, it is estimated that the standard deviation would be between 1.3 and 0.55. Figure 17–2 shows an estimated log-normal distribution (mean = 1.2, sigma =0.8) from counts obtained in the baseline study of the number of Biotype I *E. coli* in raw ground beef in the US compared to the

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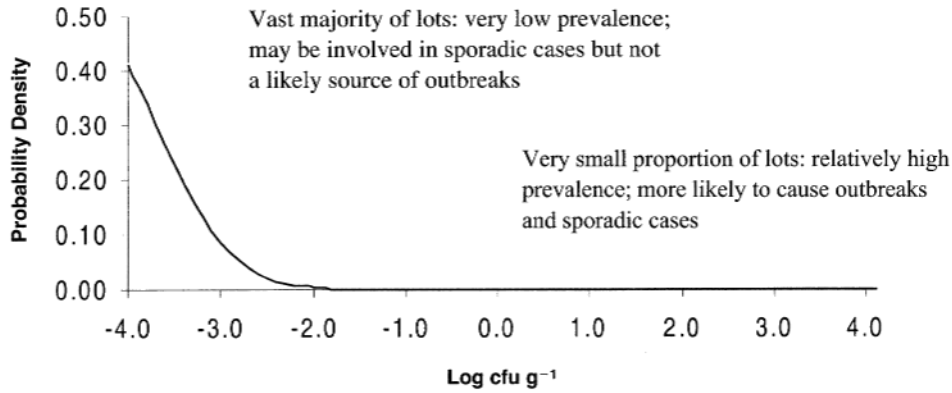


Figure 17–1 Illustration of the likely distribution in prevalence and concentration of *E. coli* O157:H7 in ground beef in North America.

hypothetical distribution for the counts of *E. coli* O157:H7 in raw ground beef. To consider the uses and limitations of sampling for *E. coli* O157:H7 in raw ground beef, we have assumed that the counts for this organism also have a log-normal distribution with a standard deviation value of 0.8, but a mean concentration < 1.2 g^{-1}. Although hypothetical, such a distribution of counts would be consistent with a prevalence of 1% positive in 325 g samples taken.

17.2.3.3 Lots with Low Prevalence and Concentration

The vast majority of ground beef has a very low prevalence and, presumably, low concentration of *E. coli* O157:H7. For example, the results of the FSIS sampling program suggest a background prevalence of about 1% or less when analyzing 325 g. This prevalence is likely influenced by the prevalence of *E. coli* O157:H7 that occurs in cattle at the time of slaughter and also reflects the level that may be normally achievable from slaugh-

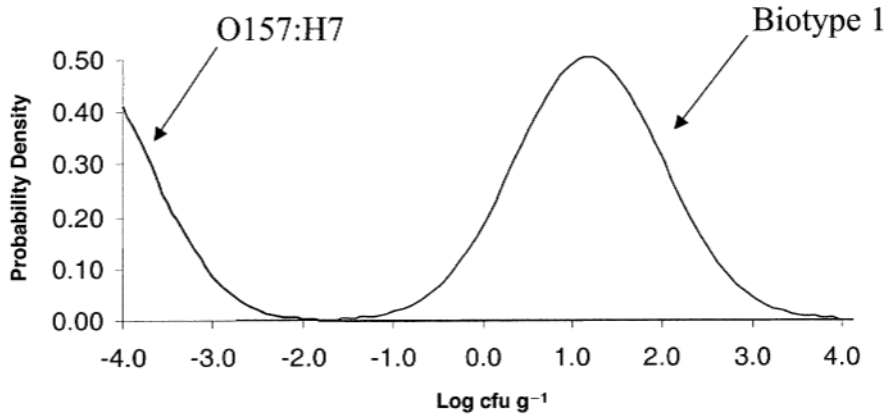


Figure 17–2 Comparison of hypothetical distribution in prevalence and concentration of *E. coli* O157:H to fitted distribution for *E. coli* Biotype I (mean = 1.2, sigma = 0.8) taken from the U.S. Nationwide Raw Beef Microbiological Survey Aug 1993–Mar 1994.

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ter to the manufacture of ground beef with existing technology. These lots have such a low prevalence of contamination that defective lots cannot be detected with any degree of confidence through routine microbiological testing. Possibly, these lots may be involved in sporadic cases, but seldom with outbreaks.

Considering the low prevalence, it is questionable that the FSIS sampling program alone, with 5,000–7,000 samples/year, would have a measurable impact on the number of cases/100,000/year attributable to *E. coli* O157:H7 in ground beef. While perhaps ineffective in ensuring consumer protection, the FSIS program does send a strong message to industry and consumers as to its expectations. This has been one factor that has resulted in the implementation of improved control measures in slaughtering operations.

It would be impractical to implement a routine sampling plan to detect and reject contaminated lots with a $\leq 1\%$ prevalence of contamination. For example, if the prevalence rate was 0.7%, the number (n) of samples required to detect the pathogen with a 95% probability would be 428 units. Even sampling to provide a 90% confidence level would require 329 sample units. Additional information describing the difficulty of detecting positive lots with low prevalence of contamination is evident in Table 17–3.

If the defect level is 0.5% and 30 sample units are tested, there is an 86% probability that all 30 samples will be found negative and the lot will be accepted. Even with 100 sample units, there is a 61% probability that all 100 samples will be found negative and the lot will be accepted. Clearly, microbiological sampling and testing are very ineffective for detecting lots with low prevalence of contamination.

17.2.3.4 Lots with Relatively High Prevalence

Experience indicates that a very small proportion of lots of ground beef have a relatively high prevalence (e.g., 5% or higher) and, presumably, higher concentration of cells (e.g., 1–10 g^{-1}). In these lots, it has been possible to find additional positive samples when resampled. Published information does not explain how these lots acquire a higher prevalence or concentration of *E. coli* O157:H7. It is strongly suspected that contamination results when trimmings from one or a few carcasses are introduced into the grinding process with other trimmings and cause a "comet-like" effect as described in Chapter 10 and Figure 10–4c. It is probable that these lots would more likely cause outbreaks as well as sporadic cases, if the product is undercooked or other foods are contaminated.

Due to the higher prevalence of contamination in these lots, it may be possible to apply a statistically valid sampling plan and, to the extent possible, exclude them from the mar-

Table 17–3 Probability of Accepting a Defective Lot with Indicated Proportion of Defective Sample Units

	No. of sample units			
% defective	*15*	*30*	*60*	*100*
0.1	0.99	0.97	0.94	0.90
0.5	0.93	0.86	0.74	0.61
1	0.86	0.74	0.55	0.37
2	0.74	0.55	0.30	0.13
5	0.46	0.21	0.05	0.01

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ket. Since it is not possible to anticipate which lots may be in this category, the intent of the sampling plan would be to detect them as they are produced.

17.2.4 Risk Characterization

The annual per capita consumption of ground beef in the US was estimated to be approximately 12 kg from 1982–1998 (AMI, 2000). If it is assumed that all the ground beef is consumed as hamburger patties weighing 125 g each, this would result in 96 servings/person/year. Since the total population in the US in 1998 was approximately 270 million, the total number of servings on a national basis would be about 26×10^9 servings per year. If the USDA survey data for FY 2000 are used, it can be assumed that the prevalence rate for *E. coli* O157:H7 in ground beef is about 1%. This prevalence rate was determined using five analytical units, each weighing 65 g. A positive result for any of the five analytic units was reported as a positive 325 g.

Data are not available for 125 g samples; however, for this example it will be assumed that the prevalence rate in 125 g samples is 0.7%. Given that assumption, it can be estimated that the number of raw hamburger patties containing *E. coli* O157:H7 each year is 26×10^7 (i.e., 1% of 26×10^9 servings). While a survey indicated that 19.7% of the population had consumed pink (undercooked) ground beef at some time during the previous 12 months, it is not likely that this occurred with each serving. For example, individuals purchasing hamburgers in fast food operations would very likely receive a hamburger that is well done and with no evidence of undercooking. This would significantly reduce the extent of exposure to undercooked hamburgers containing *E. coli* O157:H7. If it is assumed that undercooked hamburgers were consumed 5% of the time among the respondents of the survey, then, it can be estimated that the number of undercooked servings that contained *E. coli* O157:H7 before cooking would be about 26×10^5 [i.e., $5\% \times 19.7\% \times (26 \times 10^7)$]. If it is further assumed that the concentration of *E. coli* O157:H7 in the patties before cooking is 1 g^{-1} (H_0), then the number of cells would be 125/patty and their distribution within the patties would influence whether they are killed during cooking. It has been reported that an expected D value for *E. coli* O157:H7 in ground beef with 30.5% fat is 0.47 min at 62.8 °C (Line *et al.*, 1991). Cooking beef to this temperature typically yields a medium-rare (i.e., not raw) appearance. It is not likely that *E. coli* O157:H7 with an initial level of 1 g^{-1} would survive in a patty that is cooked to 62.8 °C unless, perhaps, all the cells are at the site of slowest heating within the patty. Thus, it may be possible for patties to appear undercooked (medium-rare), but have no viable cells remaining. As with many of the assumptions in this example, data are not available for the frequency of undercooking. Thus, it will be assumed for this example that 10% of the undercooked patties are insufficiently cooked to kill all the cells that are present. This leads to an estimate of approximately 26×10^4 patties/year potentially being consumed in the US that contain viable *E. coli* O157:H7.

The extent to which these patties would lead to illness would be influenced by many factors, in particular the susceptibility of the consumer. It is not likely that the most highly sensitive population, namely children less than five years of age and the elderly, would consume hamburger at a rate of 96 servings/year. In addition, these estimates assume that all the ground beef is consumed as patties. These estimates also ignore the fact that a significant portion of the patties are cooked in USDA inspected facilities and

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in fast food operations that apply control measures to ensure a minimum internal temperature of 70 °C.

17.3 RISK MANAGEMENT

17.3.1 Level of Consumer Protection

The exposure assessment revealed that about 25% of the *E. coli* O157:H7 outbreaks during 1982–1997 and 33% of illnesses were attributable to ground beef (FSIS, 1998). An estimate for sporadic cases is not available. Using those data, and assuming that there are 62,458 foodborne cases of *E. coli* O157:H7 per year, then the number of cases attributable to ground beef would be approximately 20,611 cases/year. An example of a public health goal for this food-pathogen combination would be to reduce or eliminate the number of cases attributable to ground beef. A 25% reduction in the number of cases attributed to ground beef, for example, would mean a reduction of 5,152 cases/year.

17.3.2 Establishing a Food Safety Objective

In this example, it is difficult to establish a clear relationship between the level of *E. coli* O157:H7 consumed and the probability of illness. However, outbreak data (AGA, 1994; Tarr, 1994) and risk estimates (Cassin *et al.*, 1998) suggest that fewer than 100, to as low as a dozen or so cells, can cause illness among consumers, particularly those at greater risk. Therefore, to establish a food safety objective (FSO) for this example, it is important for the value to be necessarily conservative to reflect the degree of uncertainty, the relatively low infective dose, and the severity of the illness. An example of an FSO would be:

The concentration of *E. coli* O157:H7 in raw ground beef shall not exceed 1 cfu 250 g^{-1}.

Since a concentration of 1 cfu 250 g^{-1} also can be expressed as -2.4 $\log_{10}$ cfu g^{-1}, the FSO also could be stated as the concentration of *E. coli* O157:H7 in raw ground beef shall not exceed -2.4 $\log_{10}$ cfu g^{-1}. The FSO is equivalent to no greater than 1 cell per two patties with each weighing 125 g, a common weight for commercially manufactured ground beef patties.

The proposed FSO should provide a margin of safety considering the thermal destruction that occurs even in undercooked patties. This FSO is only applicable to ground beef for sale in retail stores or for use in food service establishments. Ground beef for manufacturing products cooked in commercial establishments with Hazard Analysis Critical Control Point (HACCP) systems need not meet the proposed FSO because the required cooking processes have been validated to ensure destruction of at least 10^5 g^{-1} salmonellae or *E. coli* O157:H7.

17.4 CONTROL MEASURES

17.4.1 Good Hygienic Practices (GHP)/HACCP

A generic model HACCP plan has been developed for teaching the principles of HACCP to those involved in the manufacture of frozen ground beef patties (Bernard *et*

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al., 1997). The document discusses the strengths and limitations of GHP and HACCP for control of enteric pathogens in ground beef and provides guidance that may facilitate meeting the FSO by manufacturers of ground beef.

17.4.2 Controlling Initial Number (H_0)

The only means to control the concentration of enteric pathogens in raw ground beef is through raw material selection. The options would be limited to (1) supplier assurance programs and (2) pretesting trimmings before use. In addition, maintaining an effective time-temperature control program is needed to prevent growth of *E. coli* O157:H7 on carcasses during chilling/cutting and through grinding/freezing the ground beef patties.

17.4.2.1 Control through Supplier Assurance Programs

Control through supplier assurance programs entails selecting suppliers who have implemented interventions to minimize carcass contamination (e.g., enclosing bung in plastic bag and tying, an improved method of hoof and hide removal, use of steam vacuum on areas likely to be contaminated) during the slaughtering process and/or to decontaminate carcasses (e.g., hot water or steam pasteurization, organic acid spray) at strategic steps in the slaughtering process. As described earlier, it is likely that these control measures can significantly reduce, but will not likely eliminate, the presence of enteric pathogens (Dorsa *et al.*, 1996; Dorsa, 1997; Castillo *et al.*, 1998; Nutsch *et al.*, 1998; Sofos & Smith, 1998; Sofos *et al.*, 1999; Bacon *et al.*, 2000).

Suppliers should be audited following the guidelines in Chapter 4 and the results used as a basis for approval. Familiarity with the current use of interventions and their effectiveness should be incorporated into the audit. In addition, certain interventions (e.g., steam pasteurization, hot water sprays) may have been validated to achieve a minimum level of reduction under specified conditions of operation.

17.4.2.2 Control through an Incoming Raw Materials Screening Program

Control through an incoming raw materials screening program consists of using microbiological tests of incoming raw materials to identify and select the better, more reliable suppliers and to eliminate suppliers who exercise less control of microbial quality (Bernard *et al.*, 1997; Eisel *et al.*, 1997; Scanga *et al.*, 2000). These tests could involve indicator microorganisms as a general measure of GHP and good manufacturing practices.

Trimmings to be used for ground beef could be pretested for *E. coli* O157:H7 by various sampling plans (AMSA, 1999; Scanga *et al.*, 2000). One option would be to have the supplier sample the trimmings and have them analyzed while the raw materials are in transit. Slaughtering operations that use their own in-house trimmings to manufacture frozen ground beef patties could sample the trimmings before grinding.

Producers of ground beef should consider how much reliance to place on the interventions employed by suppliers and whether it is necessary, or effective, to test for *E. coli* O157:H7. If testing is used as a control measure, then consideration must be given to testing raw materials (i.e., trimmings) or finished product (i.e., frozen patties).

To meet the required FSO through raw material selection, the concentration of *E. coli* O157:H7 in the trimmings would have to be controlled so the ground beef will meet the FSO (i.e., FSO = $-2.4 \log_{10}$ or not exceed 1 per 250 g). This can be expressed in the following simple equation (described fully in Chapter 3).

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$$H_0 - \Sigma R + \Sigma I \leq FSO$$

$$H_0 - 0 + 0 \leq -2.4$$

$$H_0 \leq -2.4$$

Thus, the initial concentration of *E. coli* O157:H7 in the frozen ground beef patties must be -2.4 $\log_{10}$ cfu g^{-1} and, if present, there must be no growth ($\Sigma I = 0$).

There has been only one report of beef with a concentration of *E. coli* O157:H7 as high as 1,000/g. That occurred in a very small operation (i.e., a locker plant) that processed individual animals for local customers (Todd *et al.*, 1988). Such operations would be less able to exercise control over the slaughtering/chilling/fabrication process. In larger commercial operations, however, lower numbers of *E. coli* O157:H7 in trimmings and, ultimately, in ground beef would be expected. This would reflect the fact that few carcasses would carry the pathogen and some dilution of the pathogen would occur as trimmings are mixed with the large quantities of negative product. For these reasons, it may be assumed that the concentration of *E. coli* O157:H7 in trimmings from these operations would not likely exceed 100 g^{-1}. Even in the outbreak that occurred in the northwestern US, the highest concentration detected was 15 g^{-1}. Therefore, it is reasonable to assume that the initial level is unlikely to exceed 100 g^{-1} (i.e., $H_0 = 2$).

17.4.3 Reducing Levels (ΣR)

If an initial concentration of 100 g^{-1} is assumed as a worst case, then a performance criterion can be established for the reduction necessary to achieve the FSO of -2.4 $\log_{10}$ cfu g^{-1} (1 cell per 250 g) at the time the patties are consumed. This can be expressed as follows:

$$H_0 - \Sigma R + \Sigma I \leq FSO$$

$$2 - \Sigma R + 0 \leq -2.4$$

$$\Sigma R \leq 4.4$$

Thus, the performance standard for cooking the frozen patties could be stated as:

a 4.4D reduction of *E. coli* O157:H7 in the coldest area of the patty.

If the initial level (H_0) can be controlled to a lower level (e.g., $H_0 = 1$), then the performance criterion could be met with a milder cooking process (e.g., $\Sigma R \leq 3.4$).

The only means available to the ground beef producer to ensure that the patties will be cooked sufficiently to meet the FSO in restaurants, institutions, hotels, and other commercial operations would be to provide cooking instructions. This also would be necessary for consumer package labeling, but it is less certain whether consumers follow these instructions. In some regions of the world, regulations specify minimum cooking temperatures for ground beef. In the US, the Food Code (FDA, 1999) recommends that ground beef be cooked to an internal temperature of 66 °C for 1 min, 68 °C for 15 s or 70 °C for < 1 s. These time-temperature relationships provide a 6.5D or greater reduction for salmonellae, a pathogen of comparable heat resistance to *E. coli* O157:H7. Many states have adopted these recommendations. Food service operations that comply with this requirement would be applying a validated cooking process that would exceed the above performance standard. One reason for the greater thermal destruction required in the Food Code is that a value of 1000 g^{-1} was assumed for the estimated initial concen-

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tration of *Salmonella* and *E. coli* O157:H7 in ground beef ($H_0 = 3$), and a minimum 6D reduction was desired ($\Sigma R = 6$).

17.4.4 Options Involving Research and Education

Information from the above risk evaluation could lead to several additional risk management options. Examples could include:

- support research that may lead to on-farm control measures (e.g., competitive exclusion, vaccine, control of water supply for cattle). This option should be viewed as a long-term endeavor.
- support research that may lead to additional in-plant control measures to minimize contamination during slaughter and/or decontamination of carcasses.
- provide education to food handlers, particularly those in higher risk situations (e.g., non-fast food service establishments, homes), for proper handling and preparation of ground beef for serving.
- encourage the use of a thermometer and discourage reliance on appearance for estimating doneness when cooking ground beef.
- evaluate the barriers to acceptance of irradiation of ground beef and assess how they may be overcome.
- support research on new technologies to reduce the level of *E. coli* O157:H7 in ground beef while retaining acceptable quality.

17.5 ACCEPTANCE CRITERIA

17.5.1 Organoleptic Criteria

The presence of *E. coli* O157:H7 in raw ground beef cannot be assessed by organoleptic evaluation and, thus, meaningful organoleptic criteria cannot be established.

17.5.2 Chemical and Physical Criteria

The presence of *E. coli* O157:H7 in raw ground beef cannot be assessed by chemical or physical determinations and, thus, meaningful chemical or physical criteria cannot be established.

17.5.3 Microbiological Criteria

The following considers the merits of testing frozen ground beef patties to verify that the FSO has been met by the manufacturer.

While it may not be possible to detect with confidence lots with a low prevalence of contamination, it may be possible to detect some lots of higher prevalence that are more likely to be associated with outbreaks. Thus, one risk management option may be to implement a routine sampling program specifically intended to detect those lots of relatively higher prevalence and, perhaps, concentration. Rare as those lots may be, they are more likely to be associated with illness. It must be emphasized, however, that such a sampling and testing program cannot guarantee the safety of lots that test negative!

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The decision to test the frozen ground beef patties as a risk management option can be influenced by many factors, such as:

- the potential public health impact, particularly to young children, in the event *E. coli* O157:H7 is present.
- the intended customer (e.g., food service or home) and intended use (i.e., likelihood of undercooking).
- the effectiveness of product labeling and supporting education to reduce the likelihood of mishandling and undercooking.
- costs associated with illness and liability should product be implicated.

17.5.3.1 Choice of Sampling Plan

The sampling plans recommended in Chapter 8 consider risk to the consumer, including the effect certain microbial agents may have on certain sensitive populations. *E. coli* O157:H7 has been placed into a classification that requires the most stringent sampling plans (i.e., cases 13–15) and 15, 30, or 60 sample units from a lot.

The decision to select 15, 30, or 60 samples depends on whether the hazard would be expected to decrease, not change, or increase between when a food is sampled and when it is consumed (NAS-NRC, 1969; Foster, 1971; NRC, 1985; ICMSF, 1986).

E. coli O157:H7 cannot multiply in frozen ground beef patties; extended holding times above 7–8 °C are needed for even a 10-fold increase. Thus, case 15 does not apply.

Since cooking will kill *E. coli* O157:H7, this suggests that a sampling plan of $n = 15$, $c = 0$ (case 13) would be appropriate. However, approximately 20% of consumers continue to consume rare ground beef. While it would be expected that partial cooking may reduce the number of *E. coli* O157:H7, cooking to a rare state cannot be depended on to eliminate the hazard. This leads to a sampling plan of $n = 30$, $c = 0$ (case 14) as being appropriate for raw ground beef. Recovery of *E. coli* O157:H7 from frozen ground beef implicated in outbreaks is evidence that freezing cannot be relied upon to eliminate the pathogen, further supporting selection of case 14.

It is important to reemphasize the limitations of testing food for the purpose of detecting and eliminating positive lots. For example, even with the proposed sampling plan of $n = 30$, $c = 0$, there is a 21% probability of accepting a lot when the proportion of positive sample units throughout the lot is 5%. There is a 74% probability of accepting a lot when the proportion of positive sample units is 1% (see Table 17–3).

17.5.3.2 Lot

The National Academy of Sciences-National Research Council Salmonella Committee (NAS-NRC, 1969) defined a lot as:

- an identifiable and identified unit of production, distinguishable from other units, that has been packaged
- within a determined period of time
- without major interruptions of flow, shutdowns, or other changes (such as the use of different sources of raw materials) that could be expected to cause one portion of the lot to differ significantly in integrity from another and that is
- complete and
- accessible for inspection and testing.

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The ICMSF has defined a lot as follows:

> In the commercial sense, a lot is a quantity of food supposedly produced under identical conditions, all packages of which would normally bear a lot number that identifies the production during a particular time interval, and usually from a particular line, retort, or other critical processing unit. Statistically, a lot is considered as a collection of units of a product from which a sample is drawn to determine acceptability of the lot (ICMSF, 1986).

In this example, the maximum size of a lot consists of all the ground beef produced on common equipment from cleanup to cleanup. Smaller lots could be established (e.g., hourly).

17.5.3.3 Sample and Analytical Units

A sample unit is a representative sample collected at random from a lot that is a small-scale replica of the lot itself and from which a smaller analytical unit may be drawn for analysis.

- The sample unit is one frozen patty.
- The analytical unit is 25 g.

Ideally, the sample units would be submitted to a certified laboratory and analyzed by an official method, or its equivalent, for *E. coli* O157:H7. The method can be adjusted for analysis of 15 analytical units into a single 375 g composite sample. Research has validated that up to fifteen 25 g analytical units of ground beef or trimmings can be composited without significant loss in *E. coli* O157:H7 detection sensitivity (Silliker & Nickelson, 1995). This permits testing at a more reasonable cost and makes it more practical to screen lots to detect those of higher prevalence. The statistical implications of the proposed sampling plan are discussed in the following section.

17.5.3.4 Disposition of Positive Lots

The definition of a lot, the amount of product in a lot, and means of disposition are important considerations in the disposition of a positive lot. In this example, a lot consists of all product produced on common equipment from cleanup to cleanup, including rework. Rework consists of deformed, broken, or underweight patties. Rework can be returned to the grinder or blender on a frequent basis during operation, with the portion remaining at the end of production being discarded or handled separately. None would be added into the next day's production. Possible risk management options for positive lots include diverting the ground beef for use in products to be cooked in a facility that has an effective control system (i.e., GHP, HACCP) to ensure proper handling and destruction of the pathogen. Cooking can provide a substantial margin of safety that *E. coli* O157:H7 will be killed. It is recommended, however, that such lots not be used for the manufacture of fermented meats or in high temperature-short time processes, such as for links, patties, or meatballs.

17.6 STATISTICAL IMPLICATIONS OF THE PROPOSED SAMPLING PLAN

One premise of this example is that sampling can be used to screen out some, but not all, lots that exceed the FSO. A major weakness in this premise is that contamination is

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more likely to be heterogeneous as illustrated in Figures 10–4b and c. The following discussion assumes, however, that contamination is randomly distributed (i.e., log-normal distribution and standard deviation of 0.8).

The proposed sampling plan involves analysis of 25 g from each of 30 sample units from a lot. A negative result provides 95% confidence that the concentration of *E. coli* O157:H7 in the lot is no more than 1 cell per 250 g (NAS-NRC, 1969; Foster, 1971). In addition, there is a 95% probability that if sampling and testing were extended indefinitely, the positive units found would not exceed 10%, equivalent to a lot average of fewer than one cell in 250 g (NAS-NRC, 1969). The underlying principles for relating the performance of a given sampling plan are explained in Legan *et al.* (2001) and in Chapter 7.

The sampling plans originally proposed by the NAS (NAS-NRC, 1969) and the Interagency-Industry Committee (Foster, 1971) were intended to be applied to suspect lots of food. They were to be used to supplement, not replace, existing routine tests being performed by companies to assess the acceptability of their products. Some companies, however, have adopted the sampling plans for end-product testing where the conditions of operation warranted concern. Similarly, the ICMSF has recommended the use of the appropriate sampling plans as a means to assess incoming lots of food at port-of-entry when no history of the conditions of production and storage is known.

Experience with the foregoing sampling plans has been favorable. Rarely has a food been found to cause foodborne illness, such as salmonellosis, when a lot was sampled and found acceptable. Conversely, foods implicated in foodborne illness normally have been found positive with the sampling plans. For about 30 years, sampling plans involving 15, 30, and 60 sample units from a lot have been accepted and applied by both industry and certain control authorities.

While it may be possible to rationalize the use of 30 sample units for determining the acceptability of a lot, the definition of a lot and the procedure for how and when samples should be collected from a lot are more difficult to justify on a statistical basis. Admittedly, the proposal provides for sampling frequencies that consider the need for procedures to be reasonable in a commercial operation. Confounding the issue is the uncertain distribution of *E. coli* O157:H7 throughout an operating day from cleanup to cleanup. The following from The Committee on *Salmonella* provides some guidance (NAS-NRC, 1969) on this concern.

The matter of *Salmonella* control is complicated by the question of distribution of salmonellae when they do occur in a food product. If this defect is random, it is not controlled by time; i.e., there is an equal opportunity for contamination to occur at any stage of the operation. If, on the other hand, contamination was limited to a certain segment of time during processing, then the defect would not be randomly distributed throughout the lot. If distribution is random, regular sampling procedures will detect the organism; if nonrandom, there is virtually no assurance of control unless the regulatory nature of time is known.

As previously indicated, in sampling a lot, random sampling is recommended. A random sample is one in which any individual tested is as likely to detect contamination as any other. This does not mean that one sample at one given time gives a measure of control of finished product, but rather that selecting samples at random during processing gives information on the lot. It is important that the lot be so defined that the foregoing requirements are met (NAS-NRC, 1969).

In conclusion, the sampling plan proposed above may be useful for screening out lots with a high prevalence of *E. coli* O157:H7 that exceed the FSO (-2.4 $\log_{10}$ cfu g^{-1}). How-

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Table 17–4 The Relationship between the Number of 25 g Samples That Would Need To Test Negative To Control a Given Concentration (See Also Figure 17–3)

No. of 25 g samples testing negative	*Prevalence (%) needed to detect positives at 95% probability*	*Log concentration per 25 g controlled at 95% probability*	*Concentration controlled at 95% probability*
60	4.8	−1.33	1/526 g
50	5.8	−1.26	1/455 g
40	7.2	−1.17	1/370 g
30	9.5	−1.05	1/278 g
25	11	−0.97	1/233 g
20	14	−0.87	1/185 g
15	18	−0.73	1/135 g
10	26	−0.52	1/83 g
5	45	−0.10	1/32 g
3	63	−0.27	1/13 g

ever, it is also true that the sampling plan will have a relatively poor ability to detect positive lots where the level of the hazard is not random or is much lower. Another way of explaining this is to consider the likely defect rates. When the mean count is around -2.4 $\log_{10}$ cfu g^{-1}, the defect rate would be around 10% (sigma 0.8). The current defect rate measured in the FSIS sampling program is closer to 1%, which would equate to a mean count of around -4.5 $\log_{10}$ cfu g^{-1}, assuming a similar standard deviation (see Table 17–4 and Figure 17–3). Hence, it is important to again reemphasize the limitations of testing

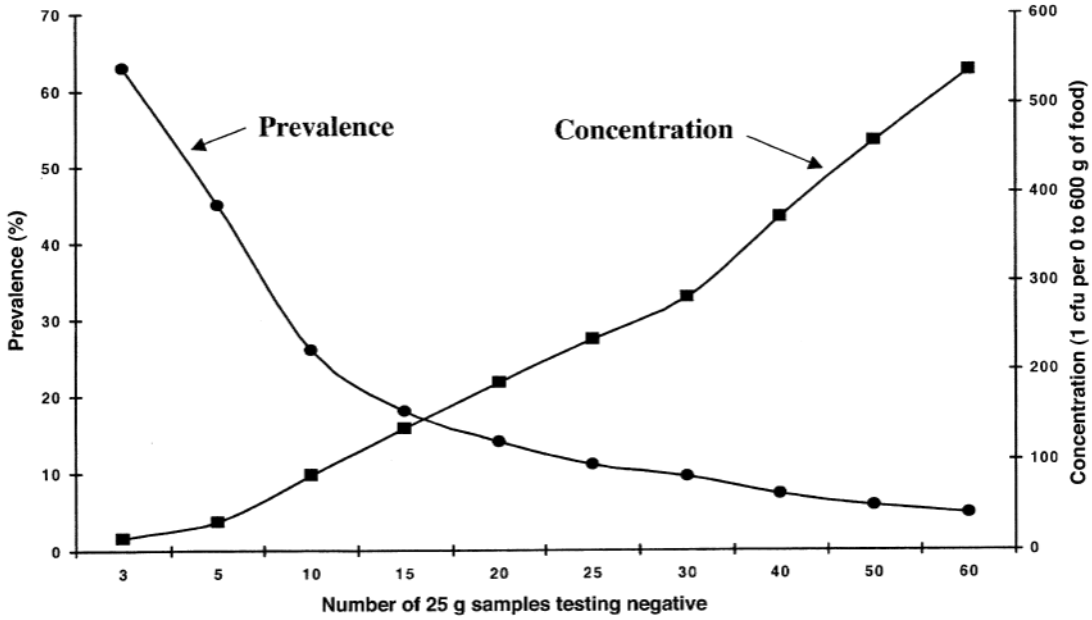


Figure 17–3 Relationship between the number of 25 g samples testing negative and the concentration and prevalence needed before the lot is rejected with 95% probability (assuming sigma = 0.8).

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food for the purpose of detecting and eliminating positive lots with a low defect rate. Even with the proposed sampling plan of $n = 30$, $c = 0$, there is a 74% probability of acceptance when the proportion of positive sample units is 1%.

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