Table 11–1 continued

Product	*Pathogen*	*Comments*	*Reference*
Canned meat	*S. typhi*	Use of nonpotable water for can cooling	Ash *et al.* (1964); Stersky *et al.* (1980)
Crabmeat	*S. aureus*	Contamination during manual picking of cooked meat	Bryan (1980)
Canned mushrooms	*S. aureus*	Possible growth of *S. aureus* in the brine bath before canning	Hardt-English *et al.* (1990)
Flavored yogurt	*E. coli* O157:H7	Pump previously used for raw milk	Morgan *et al.* (1993)
Pastry	*S. enteritidis* PT4	Equipment previously used for raw eggs or insufficiently cleaned piping and nozzles used for cream	Evans *et al.* (1996)
Yeasts	*S. münchen*	Contamination from the processing environment	Joseph *et al.* (1991)
Pasteurized milk	*S. typhimurium*	Possibly cross-connection between raw and pasteurized milk	Lecos (1986)
Pasteurized milk	*E. coli* O157:H7	Contamination from pipes and rubber seals of the bottling machine	Upton & Coia (1994)
Mexican type cheese	*L. monocytogenes*	Contamination from the processing environment	Linnan *et al.* (1988)

National Beef Ex. 1028
321

ditions that minimize cross-contamination during processing. The cleaning and disinfecting procedures normally are adequate to control the transient flora so that each day of operation is separate from the previous day. For example, the salmonellae serotypes present in one group of animals on day 1 may differ from those on day 2, and the resulting raw meat or poultry from each day will reflect the difference. Upon cooking, the salmonellae are killed and, should the products become contaminated, the serotypes will reflect those in the post-cooking environment. This concept applies generally to most commodities that receive a kill step during processing.

Resident microorganisms are introduced into the environment, become established, multiply, and persist for days or years. Normal cleaning and disinfecting procedures control their numbers but may not eliminate them from the environment. Commercial experience suggests the pathogens can be categorized as follows:

- extensive history of establishment—nontyphoid *Salmonella*, *L. monocytogenes*
- some history or the potential exists for establishment—*Staphylococcus aureus, E. coli O157:H7*, *Yersinia enterocolitica*, *Bacillus cereus*, *C. botulinum*, *C. perfringens*
- no history of establishment—*S. typhi*, shigellae, *Campylobacter jejuni*, viruses, parasites

Temperature is an important determinant of whether a pathogen can become established as a member of the resident flora of a food plant. If the environmental temperature is at, or near, the lower limit for growth, the pathogen would most likely remain transitory. For example, salmonellae and *E. coli* O157:H7 would not be expected to become established in a beef cutting, trimming, grinding, and packaging facility with a room temperature of 10 °C or below. The second most important factor is available moisture. Food processing environments that are *continuously* dry (e.g., no condensation or leaking roofs, no wet cleaning) would prevent establishment and growth of all the pathogens listed above.

Pathogens primarily of human origin (e.g., *S. typhi*, shigellae) have not been reported to become established in a modern manufacturing facility. Pathogens requiring a human host or living cells (e.g., viruses, parasites) cannot multiply in a food processing environment. The high temperature and microaerophilic conditions required for growth and sensitivity to dehydration preclude *C. jejuni* from becoming established in the processing environment.

Evidence for the establishment of spore-forming pathogens is sparse, but commercial experience with spore-forming spoilage organisms in a variety of foods is evidence for this possibility. For example, examination of spoiled canned foods in which spore-formers were acquired as post-retorting contaminants of leaking cans supports this possibility (Davidson *et al*., 1981; Matsuda *et al*., 1985; Lake *et al*., 1985a). A survey for mesophilic anaerobic spore-formers in three canning plants found significant numbers of these spores on various equipment and can conveyor tracks (Lake *et al*., 1985b). The recovery of spore-formers from spoiled low acid canned foods must be interpreted with caution (Segner, 1979). Post-process contamination of canned tuna fish and salmon with *C. botulinum* type E from within the canning factory has occurred (Johnston *et al*., 1963; Dack, 1964; Denny, 1982; Anonymous, 1984). There is evidence that *B. cereus* can adhere to dairy pipelines and lead to contamination of milk and milk products (Pirttijärvi *et al*., 1998). Furthermore, *B. cereus* isolates from one section of a dairy process differed from those of another, depending on the prevailing temperature (e.g., > 30 °C versus 2–4 °C).

While the potential theoretically exists for the establishment of *Y. enterocolitica,* this has not been demonstrated. For example, in pork slaughtering and cutting operations, the

primary source has been found to be the animals being processed (i.e., tonsil area of pigs and, thus, the intestinal tract and feces) and not *Y. enterocolitica* that have become established in the slaughtering environment (Nesbakken *et al.*, 1994; Fredriksson-Ahomaa *et al.*, 2000).

Despite the ambient temperatures during slaughtering, available evidence indicates that salmonellae and *E. coli* strains do not become established in the slaughtering environment (Magwood *et al.*, 1967; Bryan *et al.*, 1968; Cherrington *et al.*, 1988). Instead, the isolates detected on carcasses reflect those of the incoming animals.

A few examples will illustrate the importance of pathogens that do become established in the food processing environment. During the 1960s, nonfat dry milk was implicated as a source of salmonellosis (CDC, 1966). Subsequent investigations revealed that salmonellae could be detected in dried milk products and environmental samples from many different manufacturing plants (Ray *et al.*, 1971; Blackburn & Ellis, 1973). In Ray *et al.* (1971), the frequency of isolation was highest in the first product after start of operation (24.9%). The frequency then decreased during the production run (first acceptable bag of product, 9.0%; middle bag, 2.5%; last bag, 4.1%). Also, salmonellae were detected in 21.1% of the environmental samples from nine plants, with none of the plants testing negative. Such data led to considerable improvements in the processing conditions and the use of environmental sampling programs. More recently, an outbreak has been attributed to milk powder containing *Salmonella mbandanka*. Investigational sampling of the processing plant established a link between contamination of external areas of the buildings (roof) and the internal processing environment. Multiplication of the pathogen was shown to have occurred in a scrubber used to wash exhaust air and was an ideal source of contamination within the factory (Langfeldt *et al.*, 1988).

A major outbreak of salmonellosis attributed to a toasted oat breakfast cereal is another example of the importance of post-process contamination. While this contamination was originally thought to have involved the application of a solution of vitamins, this did not prove to be the case. Instead, the data indicated the pathogen had become established in the environment because it was recovered from the equipment on the production line, floor, and exhaust system. The contamination was limited to only one of 12 production lines in the manufacturing plant. Further evidence that *S. agona* had become a resident along that particular production line was recovery of *S. agona* from product produced on March 27 and recovery of the same strain from the equipment when the plant was investigated after May 28. It was estimated that as many as 16,000 cases of salmonellosis occurred before the problem was detected and the product was removed from the market (Breuer, 1999). These and similar experiences in other food operations (e.g., dried eggs, chocolate, peanut butter, soy protein, gelatin, cooked diced poultry, meat and bone meal, fish meal) demonstrate the ability of salmonellae to become established in processing environments that operate at ambient temperatures.

The number of reports of long-term persistence of specific DNA types of *L. monocytogenes* in food establishments continues to grow. One type was detected in an ice cream plant over a seven-year period (Miettinen *et al.*, 1999). In a cold-smoked fish operation, a specific randomly amplified polymorphic DNA (RAPD) type was isolated from products over a three-year period. This specific type was found in the slicing environment, whereas a variety of others were detected in the raw fish handling area (Fonnesbech-Vogel *et al.*, 2001b). The outbreak due to the soft cheese, Vacherin Mont d'Or, occurred between 1983 and 1987, indicating a period of long persistence (Bille, 1990). Other examples involving a variety of environments and foods have been reported (Cox *et al.*, 1989;

Lawrence & Gilmour, 1995; Salvat *et al.*, 1995; Nesbakken *et al.*, 1996; Loncarevic *et al.*, 1996; Unnerstad *et al.*, 1996; Ericsson *et al.*, 1997; Autio *et al.*, 1999; Fonnesbech-Vogel, 2001; Norton *et al.*, 2001).

S. aureus was found to colonize the rubber fingers of feather picking (plucking) machines and serve as the primary source for the strains found on raw chicken carcasses (Gibbs *et al.*, 1978; Notermans *et al.*, 1982; Adams & Mead, 1983; Dodd *et al.*, 1988; Mead & Dodd, 1990; Mead, 1992). The presence of a unique strain of enterotoxigenic (type B) *S. aureus*, phage type 94/96 with resistance to colistin, ampicillin, and penicillin, was recovered from Swiss cheese implicated in an outbreak. This strain was detected in cheese produced in one factory over a period of at least two years. Since the milk was pasteurized, this unique strain was introduced after pasteurization. The source was reportedly "contamination of the starter culture, probably through one of the company employees" (Todd *et al.*, 1981). In another outbreak involving 2,112 suspect vats of cheddar, Kuminost, and Monterey cheese, 59 vats were found to contain enterotoxin A. They had been produced between May and November during a time when process records showed the plant was having difficulty with slow and inactive starters. All the vats were phosphatase negative, indicating that the milk was contaminated after pasteurization (Zehren & Zehren, 1968). A survey of a whey powder plant concluded that *S. aureus* is infrequently found in the environment and is transient in nature, based on changing phage patterns. *S. saprophyticus* and *S. xylosus* were frequently isolated from the environment, each being associated with certain locations (Kleiss *et al.*, 1994).

It is very helpful to understand the difference between transient and resident microorganisms when designing a system to control in-process contamination. Furthermore, this distinction is essential when investigating and correcting a contamination problem (see section 11.6.8 and Chapter 9). A determination of whether pathogens are transient or resident can be made using a variety of traditional or more sophisticated DNA-based typing methods. Recovering the same isolates over a period of time is evidence that the pathogen is a resident of the environment.

The remainder of this chapter briefly considers factors that should be considered to minimize contamination. Some are applicable to both transient and resident pathogens.

11.5 MEASURES TO CONTROL PATHOGENS IN THE FOOD PROCESSING ENVIRONMENT

11.5.1 Minimizing Entry of Pathogens

It is not possible to prevent the introduction of pathogens into food processing facilities. This realization means GHP procedures should be targeted to control those pathogens most likely to be of concern for the food and conditions of operation. Among the sources of pathogens in food processing areas are:

- *Raw agricultural commodities* such as raw cocoa beans, raw milk, raw meat, poultry, fish or seafood, vegetables, fruit, nuts, and spices represent important sources of pathogens to food processing facilities. Physical separation of raw materials through plant design and layout is necessary to minimize entry of pathogens into processed product areas.
- *Food handlers and maintenance personnel* can be a source of food contamination. The effectiveness of gloves and other measures to minimize recontamination has been evaluated; the evidence suggests that training personnel in hygiene is more

National Beef Ex. 1028
324

important than the obligation to wear gloves (Fendler *et al.,* 1998a, b). Despite this finding, both hand washing and disposable gloves are common in many manufacturing facilities.

- *Personal clothing* and, in particular, shoes can transfer pathogens from one area to another. While preventive measures can be adopted, such as changing shoes or boots, it may be more effective to design the plant layout to direct the flow of personnel. The use of footbaths to control pathogens on shoes, boots, and equipment remains highly controversial. Some have found that maintaining clean, dry floors is more effective. Maintenance and management personnel and control authorities whose responsibilities require them to routinely visit and inspect all areas of the plant jeopardize control.
- *Air and water.* The role of air as a source of direct contamination of products is probably overestimated and not normally as important as other sources of contamination. Air quality is important in unique situations such as in the operation of aseptic packaging, air chilling, or drying and air conveying systems. The filters for compressed air can become a source of contamination if not properly maintained. Aerosols created during cleaning can disperse microorganisms throughout the processing environment. Water as it enters a manufacturing facility should be potable and in abundant supply. Water can become contaminated through use and serve as a vehicle for transmitting pathogens (e.g., water for washing, conveying, chilling) if not controlled.
- *Insects and other pests* such as flies, cockroaches, or rodents can serve as vectors for pathogens. Direct cross-contamination is assumed in one report where the spread of *Salmonella* in a household was investigated (Michanie *et al.*, 1987). In food processing facilities, however, insects rarely cause direct contamination, but can act as vehicles for transmission (Kopanic *et al.*, 1994; Olsen & Hammack, 2000; Urban & Broce, 2000).
- *Transport equipment,* such as racks, carts, trolleys, forklifts, or similar equipment, can be important vectors for transferring microorganisms throughout a facility. Some facilities color-code certain equipment and limit its use to specific areas.

11.5.2 Minimizing the Establishment of Pathogens in the Environment

Transport of pathogens into facilities is the first step in the process of pathogen contamination, but it is the establishment and multiplication that increase the risk that food will become contaminated. Establishment in the factory environment may be linked to one or more of the following factors:

- "macro" design
- "micro" design
- adherence and colonization of microorganisms.

11.5.2.1 Macro and Micro Design

Food processing rooms (e.g., walls, ceilings, floors, windows, doors, conduits or trays for electrical cable, overhead pipes) should be designed, constructed, and installed to minimize accumulation of dust and other material that can serve as sites potentially harboring microorganisms. They should be designed and installed to allow for efficient cleaning. Additional information is available (ICMSF, 1988; CAC, 1997; EHEDG, 1997).

Surfaces of equipment, floors, and walls may appear smooth to the naked human eye, but in reality microscopic examination of materials, such as stainless steel, rubber, or Teflon, shows characteristic rough structures where microorganisms can hide and that are conducive to adherence. Also, the microstructure of the materials of floors and walls is often rough or porous and may deteriorate due to exposure to water, heat, chemicals, and mechanical stress to form cracks and crevices.

Models are being developed that consider the hygiene aspects of food processing (Zwietering & Hasting, 1997a, b).

11.5.2.2 Adherence and Colonization: Biofilms and Niches

A range of factors contribute to microbial colonization of processing environments. When introduced into processing facilities, free or planktonic microorganisms may be on a carrier (e.g., dust, droplets, food particles) and, given the opportunity, tend to rapidly attach to equipment or environmental surfaces. This initial attachment phase is followed by adhesion. Adhesion depends on factors such as the type of surface (e.g., stainless steel, rubber), the type of microorganism and its physiological status, the physico-chemical status of the surface, and the existing microflora. Exposure to acid conditions or mild heat seems to predispose cells and trigger the phenomena of attachment and adhesion. Individual cells adhering to solid surfaces could already be classified as biofilms (nascent biofilm). This status confers ecological advantages such as increased resistance against dehydration, heat, and the effect of cleaning and disinfection agents (Norwood & Gilmour, 1999).

Biofilms. The most extreme forms of biofilms are found in systems with constant exposure to water, such as in pipes, flumes, air-cooling units, drains, and floors. The more developed and complex (mature) the biofilm, the stronger the protective effect against environmental stresses, the protection being enhanced by the presence of biopolymers and other material.

The formation of biofilms can occur in almost any environment with sufficient moisture and nutrients. Biofilms have been studied mainly on surfaces in contact with a liquid phase, such as pipes, cooling towers, and plate heat exchangers. Data from food processing environments, however, are limited (Taylor & Holah, 1996; Mettler & Carpentier, 1998). Detailed information on the formation, structure, and biochemistry of biofilms can be found in Wimpenny (1995) and Kumar & Anand (1999).

The microbial composition of biofilms depends on the food being processed and the operating conditions. In a survey of 17 different food environments, Gibson *et al.* (1995) found that pseudomonads and Enterobacteriaceae, in particular, were common colonizers of surfaces. The microflora in fish processing environments differs depending on the type of product being processed. Thus, the environment of smokehouses is dominated by pseudomonads, Enterobacteriaceae, and some yeasts (Bagge *et al.*, 2001). Similar types are found in processing facilities for semi-preserved fish, whereas factories manufacturing lumpfish roe harbor a flora consisting of *Vibrio* spp. and pseudomonads (Bagge *et al.*, 2001). Notably, these organisms can be easily recovered from many areas of the plant after cleaning and disinfection, indicating their ability to resist these regimens.

External stresses such as heat, acidity, and water activity can lead to increased resistance to adverse environmental conditions. In most of the cases, however, research on this topic has been restricted to the food matrixes and multi-hurdle systems. Reports of specific problems in food processing environments are very limited. Most have focused on resistance to disinfectants, particularly in biofilms (Carpentier & Cerf, 1993). Others have been devoted to the effect of environmental factors, such as moisture, drought, tem-

perature, etc., on microbial survival (Gundermann & Johannssen, 1970; Gundermann & Glück, 1971; Gundermann, 1972; Dickgiesser, 1978) and their behavior, for example, in aerosols (Marthi *et al.*, 1990; Walter *et al.*, 1990).

Niches. A niche is a site harboring microorganisms that, typically, is impossible to reach and clean with normal cleaning and sanitizing procedures. The processing environment may appear visually clean and acceptable. Examples include hollow rollers on conveyors, cracked tubular support rods on equipment, the space between close fitting metal-to-metal or metal-to-plastic parts, worn or cracked rubber seals around doors, saturated insulation, interfaces between floors and equipment, cracks, and crevices. Niches tend to be continually wet and hold residues of food and other material. Microorganisms, including pathogens, can become established and multiply within these sites, which then serve as a reservoir from which the pathogen is dispersed during operation and contaminates food contact surfaces and food. In a controlled environment, the niche usually affects only the food along one processing or packaging line and not the product on adjacent lines. Microbiological testing is necessary to detect a niche.

11.6 SAMPLING THE PROCESSING ENVIRONMENT

Environmental sampling is used to:

- assess the risk of product contamination
- establish a baseline for when the facility is considered under control
- assess whether the environment is under control
- investigate a source of contamination so corrective actions can be implemented.

11.6.1 Assessing the Risk of Product Contamination (Investigational Phase)

Biased, investigational sampling techniques (Chapter 9) are most appropriate for assessing whether a product can become contaminated with a pathogen. The purpose of this sampling is to find a target pathogen if it is present. The selection of samples will reflect the experience of the investigator and the process being investigated. In general, in-process food samples and/or sponge samples may be collected. Food samples should be collected from stages throughout the process that could permit contamination. Consideration should be given to collecting samples at different production times (e.g., first product, middle of production, end of production, following a break in production, after mechanical repairs, after a change in ingredients or packaging material). Product residues (e.g., shavings from slicing machines, sweepings from the floor of a dried milk plant) also can be collected since they serve as a type of composite sample from the process.

Environmental samples can be collected from product contact surfaces and the immediate surrounding. Sponges (Silliker & Gabis, 1975) or large cotton pads are suitable for this purpose. Since the intent is to detect the presence of a pathogen, if present, large areas should be sampled without regard to dimension. Environmental samples should also be collected at different times as just described for product samples.

Certain samples represent historical information measuring accumulation over time. Examples include residues from hollow bodies, cracks, and crevices; joints and seals between floor and equipment; water residues from siphons; drains; dust from vacuum cleaners; brooms, brushes, and mops; air filters; and wet or old insulation material.

In addition to analyzing for pathogens, samples may be tested for indicators (*E. coli*, listeriae) relevant to the pathogen(s) of concern. All the data should be organized in a

manner that a baseline can be established for what is considered normal when all GHP procedures are in control.

11.6.2 Establishing a Routine Environmental Sampling Program

Routine environmental sampling programs are usually focused on one pathogen and/or indicator organism and involve a limited sampling regime. The purpose of the routine sampling program is to detect increased risk of product contamination before it actually occurs. Data from the investigational phase are used to select sampling sites, times, frequencies, and types of samples that will most effectively fulfill that purpose. Some of the basic process control concepts described in Chapter 13 may be applicable in the design and implementation of an environmental sampling program. The use of trend analysis, in particular, is useful. Statistical analysis is seldom of value, however, if the program involves presence/absence testing for a pathogen, such as in a cooked product area. In this case, any positive sample is a warning that requires further investigation.

11.6.3 Sampling Locations: The Zone Concept

Some manufacturers design their environmental sampling programs around zones having different levels of risk (Figure 11–1). For example, the zone of highest risk (zone 1) includes product contact surfaces over or through which product passes during processing. The next zone (zone 2) consists of equipment and other items that are in close proximity to the product flow and may indirectly lead to product contamination. The third zone may include items or areas that are less likely to lead to product contamination, but nevertheless may hinder efforts to control pathogens and from which pathogens could potentially be transferred to zones 1 or 2. The fourth zone is outside the processing area and if not maintained at an acceptable level of cleanliness could increase the risk of introducing pathogens into zones 1–3. The concept of zones with differing levels of risk can be used to select sites for a routine environmental sampling program. In addition, the concept can be used as a teaching aid for plant personnel. It is important to recognize that the zone concept has not been standardized and the items designated for each zone differ among food operations, depending on accumulated data and experience.

The purpose of routine environmental sampling is to verify that the GHP procedures are controlling the risk of product contamination, with sampling locations selected according to the risk of product contamination. In the case of heat-processed foods, for example, attention should be on the zones after cooking and where the products are exposed (e.g., where cooked ham is sliced and packaged). Emphasis should be placed on sites in zone 1 and supplemented with sites from zone 2, as needed. Figure 11–2 illustrates possible sampling sites in four different food operations. Equipment located between when the food is smoked/heated and protected by wrapping or packaging is of greatest concern (designated by a shaded area). Samples from product contact surfaces of equipment (i.e., zone 1) that could be included in a routine sampling program have been identified. In addition, samples from floors or other sites in close proximity to the flow of the product (i.e., zone 2) have been indicated.

The types of samples collected for a routine sampling program should be determined from the investigational sampling data and experience as the program is implemented. Environmental sponge samples are more commonly collected for analysis than are product samples. Examples of sampling sites for the four food operations in Figure 11–2 are

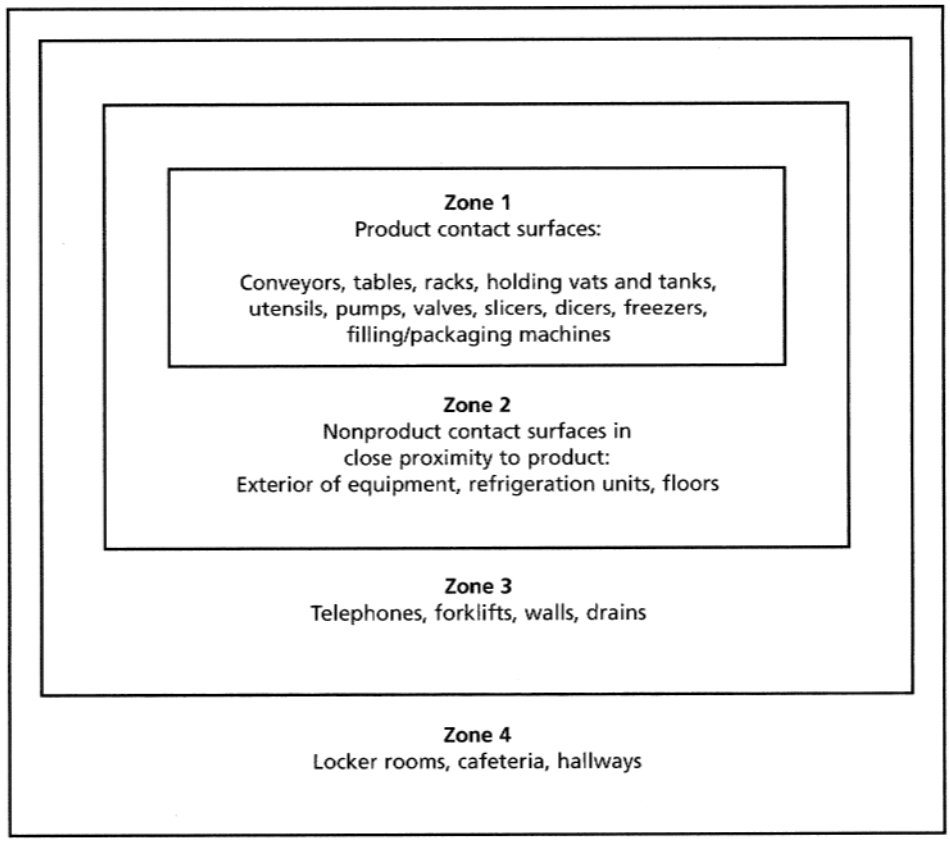

Figure 11–1 Zone concept to illustrate areas of highest risk (zone 1) to lowest risk (zone 4) for product contamination.

provided in Table 11–2. Environmental samples are intended to detect a microbial indicator or pathogen, if present. This may lead the sampler to collect from a large area from one piece of equipment and from a small area on another, relying on experience and previous results for guidance.

The sampling program could include in-process product samples where this may provide additional benefit over sponge sampling. The type of material sampled will depend on the type of product and processing line.

11.6.4 Number, Frequency, and Time of Sampling

Environmental sampling protocols are not statistically designed sampling plans. Instead, they are based on experience and knowledge of the sites most likely to detect a failure in GHP. The knowledge base continues to increase over time, enabling adjustments to further improve the system's sensitivity without increasing analytical costs unnecessarily.

The number of samples and frequency of sampling are normally determined by knowledge of the operation and its variability. Knowing when to collect samples may be more important than increasing the number or frequency of samplings. For example, it may be

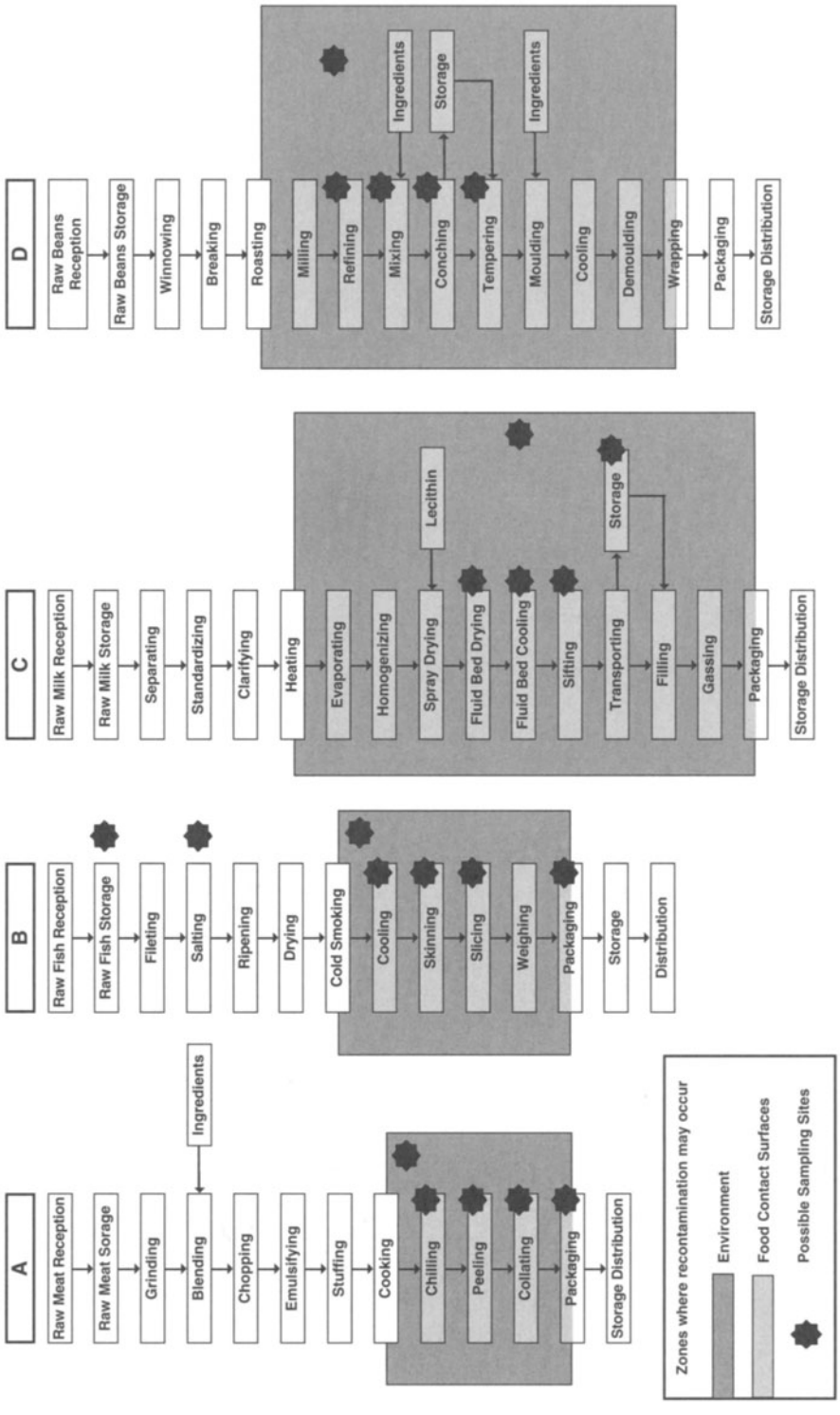

Figure 11–2 Flow diagram for 4 production lines and potential sampling sites in the environment and on product contact surfaces.
A: Frankfurter sausages; B: Cold smoked salmon; C: Milk powder; D: Chocolate

Table 11–2 Examples of Possible Sampling Sites in the Environment of Four Processing Systems

	Frankfurters	*Cold smoked salmon*	*Milk powder*	*Chocolate*
		Food contact surfaces (Sponge samples)		
Equipment	Brine chiller	Transport racks	After dryer	Mills
	Peeler table	Trays	After cooler	Refiner
	Casing removal system	Tables	Pipes	Conches
	Hopper after peeler	Skinner	Conveyor belts	Storage tanks
	Incline conveyor	Brine injector/tank for brining	Tote bins	Pipes
	Collator	Slicer	Silos	Mixers
	Containers/tubs/trays	Scale		Molds
	Final conveyor	Conveyor belts		Tempering equipment
	Packaging machine	Packaging machine		Transport trolleys
	Scale			Trays
Utensils	Knives	Knives	Brushes	Scrapers
	Tools	Tools	Scrapers	Paddles
		Scrapers		Spatulas
		Environmental sponge samples (Indirect contact but close to product)		
Equipment	Overhead piping	Overhead piping	Lids	Lids
	On/off switches/buttons	On/off switches/buttons	Covers	Covers
	Legs of equipment	Legs of equipment	External surfaces of silos	Piping above equipment
	Sides of equipment	Sides of equipment		Internal surface cooling tunnel
	Bottom of equipment	Bottom of equipment		
	Floor by packaging line	Floor near packaging line		
	Floor of holding cooler	Floor of chill room		
		Environmental sponge samples (Indirect contact)		
Environment	Floors	Floors	Floors	Floors
	Walls	Walls	Walls	Walls
	Refrigeration units	Refrigeration units	Pipes	Pipes
	Drains	Drains	Ducts	Ducts
	Doors	Doors	Overhead structures	Overhead structures
				Drains

National Beef Ex. 1028
331

known that sponge samples collected during operation will yield more useful information about whether the environment is under control than samples collected from cleaned equipment before the start of operation, which may provide misleading results. In another situation, the first product from the process may represent the highest risk. Other important factors to consider include ease of sampling, whether the integrity of the product being processed will be jeopardized and, in particular, safety for the person collecting the samples.

Environmental sampling plans normally involve an established, routine sampling plan with a defined minimum number of samples. The number, frequency, timing, and sampling sites may be increased when evidence indicates increased risk of contamination. Tables 11–3a,b,c, and d list examples of sampling sites, numbers and frequencies for the four operations described previously. In addition, the examples indicate a possible increased frequency when data indicate such a need.

When judged necessary, special samples can be collected, such as during construction, during major changes to equipment, or after unforeseen events such as heavy storms that could have caused contamination of the processing environment (e.g., leaking roof).

To minimize the analytical workload and cost, samples may be composited (i.e., bulked) before analysis (e.g., from the same sampling point over one week or from different sampling points along a processing line). Compositing should only be done when the quality of the information obtained is known to be unaffected.

11.6.5 Sample Collection

Meaningful information is gained only if appropriate tools are used to collect samples. Sampling materials (e.g., sponges or cotton pads, utensils, cups, bags) must be sterile to avoid contamination. Proper labeling and description of the samples are essential. Before analysis, samples should be stored under appropriate conditions according to the characteristics of the sample (see Chapter 12) so that numbers of the target organism(s) neither increase nor decrease.

The sampling tools need to be adapted to the type of sample to allow for efficient sampling. Spatulas and scrapers of different size are used to collect residues from surfaces, holes, crevices, etc. Paintbrushes can be used to collect dusty samples on surfaces of equipment or on the infrastructure (e.g., electric cables, control panels, etc). Cotton plugs, sponges, or gauze pads are useful for liquid or moist residues as well as surfaces with limited amounts of residues. Sponges must not contain antimicrobial substances (Daley *et al.*, 1995). Other sampling tools such as pipettes may be required, depending on the situation (i.e., flexibility is needed to adapt the means of taking samples to local situations).

The sampling tools must not introduce other nonmicrobiological hazards into the processing system. In most manufacturing plants, for example, a strict policy exists that glass cannot be introduced into the processing environment.

11.6.6 Sample Analysis

Samples taken for microbiological surveillance are analyzed for specific microorganisms, i.e., pathogens of concern (e.g., *Salmonella, L. monocytogenes*) and for indicators, such as *E. coli.* Usually, traditional microbiological methods are used, although some more rapid methods are gaining acceptance.

A routine testing program for a pathogen may be supplemented with tests for hygiene indicators. Pathogen testing is usually qualitative (presence/absence). Such analyses are, however, time consuming, costly, and cumbersome. Therefore, the potential presence of

pathogens may be assessed using indicators. Quantitative determination of Enterobacteriaceae, for example, allows the assessment of moisture control in environments intended to remain dry. The use of biochemical reactions to detect adenosine triphosphate (Flickinger, 1996) or residual proteins (Patrick & Bayliss, 1997) enlarges the concept of hygiene indicators. Although not directed toward the detection of viable microorganisms, the presence of residues of the manufactured products indicates the potential for growth and multiplication of microorganisms and thus for recontamination.

Of particular interest for environmental monitoring are sub-typing methods, which are used for pathogens and increasingly for other microorganisms. While biochemical methods are of limited use, serological, biological (phage-typing), and molecular methods, such as pulsed field gel electrophoresis, RAPD, ribotyping, and others, find more and more applications. These methods, and especially the molecular ones, are very powerful tools to trace microorganisms within processing facilities and establish links between isolates. Details on the technical aspects of these methods have been provided (Farber, 1996; Olive & Bean, 1999).

11.6.7 Managing the Data from Environmental Sampling

An environmental sampling program is useful only when data acquired are organized and frequently reviewed. In addition to reviewing the most recent data, it is also helpful to review data for the past quarter or year to detect weaknesses and trends that may not be otherwise evident. Normal, routine levels of sampling can be continued as long as the data are within the limits of acceptance. Detection of increased levels of indicators, such as Enterobacteriaceae, may not trigger immediate corrective actions but should serve as a warning. The response will depend on the type of product manufactured.

11.6.8 Investigational Sampling To Determine the Source of Contamination

When a trend or other information indicates an increased risk of contamination, the reason should be determined. This procedure involves a combination of increased sampling and biased, investigational sampling leading to collection of the data necessary to identify the source of contamination and to implement corrective actions. Tables 11–3a,b,c, and d provides examples of increased levels of sampling that could be used for the four processes introduced in Figure 11–2.

During investigational sampling to detect the source of contamination, it is important to assemble and review historical environmental sampling data that may reveal trends or suggest a particular cause. Of particular interest are data for finished product, ingredients, and the processing environment. These data may not include tests for the organism in question, but correlating data may provide insight into changes in the microbiology of the ingredients, process, or the environment. In addition, production records may associate the problem with specific events, such as construction, remodeling, mechanical repairs, or tests of new equipment or product formulations.

In addition, specialists with appropriate experience can conduct visual inspections of the cleaning and disinfecting procedures, the status of the equipment and operating conditions, employee practices and movement of equipment, product, and personnel.

The goal of the intensified sampling is to detect the contamination and its cause. Investigational sampling may identify biofilm formation, a niche supporting microbial growth in the equipment, or other causes. When pursuing a source such as a niche, it is

Table 11–3a Example of an Environmental Sampling Plan for *L. monocytogenes* in a Frankfurter Operation

	Frankfurters	
	Normal level	*Increased level*
Environmental sponge samples		
Floor in peeler area	1 x week	1–3 x day
Floor in vicinity of collating and packaging line	1 x week	1–3 x day
Equipment sponge samples, etc.		
Brine chill solution	1 x week	1–3 x day
Peeling table	1 x week	1–3 x day
Hopper/incline conveyor after peeler	1 x week	1–3 x day
Collator	1 x week	1–3 x day
Conveyor before packaging	1 x week	1–3 x day
Packaging machine	1 x week	1–3 x day
Finished product		
Finished product	1 x biweekly	1–3 x day

usually necessary to dismantle completely any suspected equipment. As this is being done, samples should be collected to confirm, if possible, that the source has been found. In such situations, care must be taken not to disseminate pathogens dislodged from their niche into the environment by careless handling of material and samples.

The increased frequency of sampling is normally not restricted to samples of the same type taken at the usual location. In reality, the source of the pathogen needs to be investigated to determine whether it is a transient or resident strain in the factory environment. Tracing, source detection, or troubleshooting can be a long and tedious process requiring repeated sampling campaigns, where attention is focused on sites in addition to those

Table 11–3b Example of a Sampling Plan for *L. monocytogenes* in a Smoked Fish Plant

	Cold smoked salmon	
	Normal level	*Increased level*
Environmental sponge samples		
Floor of chill room	1 x week	1–3 x day
Floor in vicinity of slicing line	1 x week	1–3 x day
Equipment sponge samples		
Brine injector/brine tank	1 x week	1–3 x day
Racks exiting chiller	1 x week	1–3 x day
Conveyor/table	1 x week	1–3 x day
Skinner	1 x week	1–3 x day
Slicer	1 x week	1–3 x day
Scale	1 x week	1–3 x day
Packaging machine	1 x week	1–3 x day
Finished product		
Finished product	1 x biweekly	1–3 x day

Table 11–3c Example of a Sampling Plan for *Salmonella* in a Dried Milk Powder Plant

	Milk powder	
	Normal level	*Increased level*
Environmental sponge samples		
Area zone drying tower	1 x week	Several points
Area lecithination room	1 x week	Several points
Area zone after dryer	1 x week	Several points
Area zone storage silos	1 x week	Several points
Area zone packaging	1 x week	Several points
Equipment sponge samples		
Star valve	1 x week	1 x day
Filters	1 x month	Several points
Cyclone residues	1 x week	1 x day
Sieves after cooler	1 x day	Several points
Silos	1 x week	1 x day
Packaging machine	First product	10 x day
Finished product		
Finished product	3 x batch	10 x batch

included in routine sampling. Preconceived ideas about the source should be viewed with caution and have been known to prolong the effort of detecting the true source. Sampling may be expanded to adjacent zones of lower concern to obtain a more complete picture of the extent of the contamination. Dismantling equipment, removing filters, etc. may be necessary to access sites that could harbor a niche.

Table 11–3d Example of a Sampling Plan for *Salmonella* in a Chocolate Plant

	Chocolate	
	Normal level	*Increased level*
Environmental sponge samples		
Area zone after roaster	1 x week	Several points
Area zone refining	1 x week	Several points
Area zone mixer	1 x week	Several points
Area storage rework	1 x week	Several points
Area storage ingredients	1 x week	Several points
Equipment sponge samples		
Mixer	1 x week	1 x day
Conches	1 x day (rotation)	1 x day (all)
Tempering equipment	1 x day (rotation)	1 x day (all)
Storage tanks	1 x day (rotation)	1 x day (all)
Molding	1 x week	Several points
Rework vessel	1 x week	1 x day
Finished product		
Finished product	3 x batch	10 x batch

Two examples of investigational sampling in problem solving are provided below.*

Listeria monocytogenes in *processed, cold-filled cheese*. In this example, *L. monocytogenes* was isolated from processed cold-filled cheese. Upon investigating the source, 80 finished product samples from a one-month period, as well as over 200 ingredient samples and 1230 environmental samples, were analyzed. *L. monocytogenes* was found in six finished product samples, three ingredient samples, and one environmental sample. The only ingredient found to be contaminated was butter from a particular supplier. The environmental sample that proved positive was the underside of an elevated electrical cable stand to a chopper.

All 14 isolates were of the same serotype (1/2 b), multilocus electrophoresis type (ET202), ribotype (DD0941), and phagetype (non-typable). The probability that all 14 isolates would, by chance, have the same pattern is extremely remote and indicates a common source of contamination. Most likely, the butter was the source of contamination for the processed cheese and the single environmental positive resulted from spilled butter or cheese during manufacture.

Since only one of the 1230 environmental sponge samples was positive, it can be concluded that the unique isolate was not a resident of the facility producing processed, cold-filled cheese.

This study illustrates how serotyping, electrophoresis, ribotyping, and phage typing can be used in investigations to detect the source of contamination.

Staphylococcus aureus in fermented sausage. High numbers (e.g. $> 10^5$ g^{-1}) of *S. aureus* detected during a routine verification at the end of fermentation for a dry fermented sausage could have been due to a variety of reasons. Initially, product produced before and after the suspect lot was analyzed to determine whether the problem was continuing or an isolated problem. Furthermore, individual lots produced during the period in question were sub-lotted into smaller batches by time of production. Review of the processing records for the incriminated lots helped to determine the origin of the problem. In addition, equipment and environmental sampling was conducted.

Since *S. aureus* numbers decline during the drying/aging step that follows fermentation, quantitative analysis for *S. aureus* might yield misleading information. Thus, the product was tested for heat stable thermonuclease as an indicator of high numbers of *S. aureus*. The samples were collected from the outer few mm of the sausages since this is where growth of *S. aureus* would occur. The accumulated information indicated that the products most likely to contain detectable levels of heat stable thermonuclease were the first batches produced after weekend shutdowns. Sponge samples from equipment initially failed to detect a source of contamination; however, the production records clearly suggested growth of *S. aureus* in the processing equipment during shutdown as the probable cause. Reexamination of the equipment revealed growth in an area of the equipment that was difficult to reach and clean (and sample). The investigation concluded that *S. aureus* multiplication occurred in a niche in the equip-

*Unpublished case studies.

ment during shutdown on weekends. Meat moving through the unclean equipment during start of production on Monday mornings became contaminated with relatively high numbers of *S. aureus*. As production continued, subsequent batches were contaminated with decreasing numbers of *S. aureus*. The lower levels did not reach unacceptable levels during fermentation.

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Chapter 12

Sampling, Sample Handling, and Sample Analysis

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

12.1 INTRODUCTION

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

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

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

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

National Beef Ex. 1028
342

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

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-

National Beef Ex. 1028
344

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.

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

National Beef Ex. 1028
346

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

National Beef Ex. 1028
347

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

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

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

National Beef Ex. 1028
350

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-

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 13

Process Control

13.1 Introduction
13.2 Knowledge of the Degree of Variability and the Factors That Influence Variability
13.3 Process Capability Study
13.4 Control during Production: Monitoring and Verifying a Single Lot of Food
13.5 Control during Production: Organizing Data from Across Multiple Lots of Food To Maintain or Improve Control
13.6 Use of Process Control Testing as a Regulatory Tool
13.7 Investigating and Learning from Previously Unrecognized Factors or Unforeseen Events
13.8 References

13.1 INTRODUCTION

This chapter discusses sampling and testing to assess whether food operations are under control (i.e., correct procedures are being followed and criteria are being met) and using the data to make the adjustments necessary to maintain control. Food operations must be controlled to produce foods of consistent quality and safety. A controlled process requires being proactive and informed of the factors that influence variability. Process control technology can be applied to the manufacture of a single lot of food produced on one day, or to multiple lots produced over days or years, and to both batch and continuous processes.

As described in Chapters 3, 4, and 5, the process or product criteria that are used to assess whether a process is under control can be based on a food safety objective or a performance criterion that is specific to the food, the process, and the microbial hazard(s). Product criteria can be standards, specifications, or whatever a control authority or processor considers necessary to ensure that a process is controlled and the food is safe when used as intended.

Since about 1990, there has been increasing interest in the food industry in quality enhancement, through programs that stress the use of structured quality systems, such as International Organization for Standardization (ISO) certification. The concept of continuous improvement has led to greater use of data in a more organized manner to improve quality and production efficiencies. This interest in quality systems occurred during a period when Hazard Analysis Critical Control Point (HACCP) planning was being more widely adopted throughout the industry. HACCP systems are, in essence, that portion of an establishment's overall process control system that focuses on food safety. Collectively, these programs have led to a greater awareness of the need and value of process control technology. Although food safety is emphasized below, the concepts described can be applied to ensuring microbiological quality.

National Beef Ex. 1028
354

Microbiological testing is conducted at various points along the food chain for a number of different purposes. Some of these are summarized in Table 1–2 and Exhibit 5–1. In Chapters 4 and 5, microbiological testing for evaluation of lots or consignments of food (raw materials and end products) in commerce is discussed. However, due to the time required for most microbiological analyses and the relative insensitivity of even the most stringent sampling plans, microbiological testing is of limited value for monitoring in quality and safety assurance programs (Chapters 6, 7, 9; NRC, 1985; ICMSF, 1988; NACMCF, 1997). More rapid tests must necessarily be used and will typically involve sensorial, chemical, and/or physical measurements such as time, temperature, acidity, pH, moisture, a_w, flow rates, and so on. The principles of process control technology described in this chapter can be applied to all such measurements. Thus, while the emphasis of the book is on microbiological testing, it should be kept in mind that other measurements will more commonly be used in process control systems (see Chapter 4).

HACCP is often referred to as a preventive system. However, from a statistical standpoint, HACCP would be more appropriately described as a means of minimizing the variability of a system. A statistical approach to safety can be effectively applied in HACCP systems. Control chart methods (see sections 13.3.1 and 13.4) provide an objective and statistically valid means to assess ongoing processes, and as such are particularly applicable to monitoring. Lot acceptance sampling can be used in verification and, to a very limited extent, in monitoring. These two statistical techniques for controlling quality and safety are well developed and documented in many textbooks, manuals, and periodicals (e.g., ASTM, 1951; BSI; Duncan, 1986; Grant & Leavenworth, 1972; ISO/TC 69; Massart *et al.*, 1978). The statistics of lot acceptance plans have been dealt with in detail in Chapters 6 and 7. Control chart methods with applications to food technology are treated in detail by Kramer & Twigg (1982).

This chapter is intended to serve as a brief introduction to the use of statistical methods for monitoring and verification. For more information on process-oriented control systems in the food industry and the statistical tools used for that purpose, other texts are recommended (Steiner, 1984; Hubbard, 1990; DeVor *et al.*, 1992; Smith, 1998; Juran *et al.*, 1999; Ledolter & Burrill, 1999).

Controlled food operations require:

1. *Knowledge of the significant hazards*
   The principles of HACCP should be considered for all food operations. At a minimum, a hazard analysis should be conducted. If the conclusion drawn is that significant hazards are not likely to occur, a HACCP plan would not be established.
2. *Knowledge of the factors that are necessary for control*
   If significant microbial hazards have been identified, it is necessary to investigate the conditions of processing that must be controlled to prevent, eliminate, or reduce their occurrence to acceptable levels and then to establish control measures. This will clarify the importance of certain steps in the process (i.e., critical control points (CCPs)) and establish specific Good Hygienic Practices (GHP) for controlling the identified hazards.
3. *Knowledge of the extent of variability and factors that influence variability*
   Understanding the factors that influence variability is an essential element of a process control system. Most food operators understand the factors that influence the cost of producing a food and strive to control each factor according to its relative impact on cost and profit. This same concept can be applied to producing safe

foods. Processes with a high degree of variability, particularly when that variability is not recognized or understood, are more likely to produce unacceptable, and possibly hazardous, food. Each process is unique, owing to differences in plant layout, equipment design and performance, equipment maintenance and cleanability, personnel, type of food being produced, and other factors. The conditions that influence variability at CCPs must be understood, as well as the extent of variability that can occur. The information should then be used to determine how this variability might be controlled within an acceptable range.

4. *Establishing criteria for the factors that must be controlled*
   The information from item 3 above should be used to establish operating parameters that take account of variability and ensure that critical limits are met. Through continuous improvement, variability can be reduced and result in improved safety, quality, and process efficiency. With limits having been established at critical steps in the operation, procedures must be developed to monitor those limits to ensure they are met during operation.
5. *Establishing monitoring procedures*
   A wide variety of measurements, such as sensorial, physical, chemical, and microbiological, are used for monitoring food processes. The method of choice will be the simplest, easiest, cheapest, and safest available that can provide, in a timely manner, the information needed to adjust the process and maintain control. Ideally, the measurements will be continuous with adjustments being made automatically. The measurements may include processing parameters (e.g., temperature, humidity, pH), food collected at different stages in processing, finished product, and/or environmental samples. A permanent record should be created for subsequent verification.
6. *Organizing and interpreting data*
   Considerable quantities of data are generated to evaluate current production, and if organized correctly, the data have great value. The data can be used to determine longer-term trends and to facilitate continuous process improvement. With the availability of powerful computers, data can be organized into databases that allow rapid interrogation and provide a cumulative record.
7. *Using the data to improve control and measure change*
   The value of an effective process control system is most evident when data are organized and used to further increase knowledge about a process and the factors affecting variability. The longer-term goal should be to use the data to reduce variability and achieve more tightly controlled processes. Properly organized, the data can be used to measure the effect of modifications to equipment and other factors in the process. In addition, the data can be used to detect trends over multiple lots of food that may indicate a gradual loss of control. When data are organized and routinely reviewed, adjustments can be made to maintain control during production of one lot or across multiple lots of food.
8. *Responding to the data*
   In the best situations, after a process has been modified, data will indicate reduced variability and improved control. This reaffirms the operator's understanding of the factors affecting variability and how the process may be further improved. Occasionally, trends may be unfavorable and indicate the need to determine which factor has changed and requires correcting. Many examples could be cited where failure to recognize change and to respond effectively has led to unsafe food and foodborne illness.

9. *Investigating and learning from previously unrecognized factors or unforeseen events*
   Occasionally, an unexpected change occurs in a process that results in loss of control. Upon investigation, the cause is determined and controls are implemented to prevent a similar event from occurring again. This could occur through equipment malfunction and lead to a new preventive maintenance procedure to avoid future malfunctions. Another possibility could involve weather changes (e.g., higher humidity in summer compared with winter and its effect on processes that involve drying) or power outages from storms or shortages of electricity in a region. Isolated events may result in no change to an existing process control system as long as the monitoring procedures and confidence in control are not affected, but more frequent occurrences may require a prepared plan of action.

Items 1 and 2 above are well known as the first two principles of HACCP (ICMSF, 1988; CAC, 1997; NACMCF, 1997) and are not discussed here.

13.2 KNOWLEDGE OF THE DEGREE OF VARIABILITY AND THE FACTORS THAT INFLUENCE VARIABILITY

13.2.1 Establishing a Baseline for a Process

Food manufacturers collect a variety of samples for microbiological analysis, including samples from equipment and the processing environment, ingredients, in-line food samples, and finished product. The selection of samples and choice of analyses are influenced by the type of food and food operation. The samples should generate data that can help the operator assess the degree of control and prevent problems. Since one or more days may elapse between sampling and obtaining a result from a microbiological analysis, the data provide a history of past performance.

While not always clearly articulated, the purpose of this sampling is often to provide a "microbiological history" of the food product and the processing conditions (Buchanan, 2000). By acquiring data over time on the microbial population, manufacturers establish a baseline for the level of control that is attainable when GHP procedures and the HACCP system are under control. Once established, subsequent analyses that differ from the baseline indicate a deviation from the norm due to a change in operating conditions. The baseline data also can be used to establish microbial specifications.

The extent of microbiological testing is typically limited and is not intended to provide assurance of any specific batch or lot of food. If sufficient data have been accumulated over multiple lots, statistical analysis can greatly enhance the usefulness of the data. This type of analysis, sometimes referred to as cross-batch testing, is similar to data analysis for a single batch, except that the data are collected over time and multiple batches are analyzed cumulatively. An underlying assumption is that when a process is under control, the between-batch variability is small.

13.2.2 Types of Microbiological Data for Baseline

Microbiological data can be collected from at least five sources that differ by location or time of sampling within the process (i.e., ingredients, in-line samples, end product, equipment/environmental samples, and shelf-life samples).

Ingredient data can be viewed as a type of in-line sample. Periodic microbiological testing of ingredients can be used to verify that a step in the process that is not directly under the control of the manufacturer is, in fact, under control. The microbiological limits for ingredients are typically defined through purchase specifications. Thus, periodic microbiological testing of ingredients by the purchaser is a means of verifying that the supplier is meeting the specifications. Ingredient testing is particularly important when microbial levels could be so large as to overwhelm the controls designed into the food safety system or when a sensitive ingredient is used in a process that has no kill step (e.g., blending dry ingredients for powdered infant formula or chocolate manufacture).

In-line sampling consists of collecting samples of the food at different steps in the process. The data provide information needed to understand the effects of each step on the microbial population. The time at which in-line samples are collected may be important (e.g., at start, middle, or end of production). Sampling before and after a critical step in a process can be used to validate the effectiveness and variability associated with control measures. Although end product testing is common, this may not provide the most useful information. Analysis of the finished product provides an integrated measure of all the steps that contributed to the total population, but if the product does not meet a specification, the results do not identify the cause of the problem. In-line samples may be necessary to identify the cause.

End product testing is generally not necessary on a routine basis when records indicate a process is in control. The merits and limitations of end product testing as a measure of process control are discussed in Chapters 4 through 7 and elsewhere in this book.

Equipment and environmental tests are used to measure the effectiveness of GHP within a food operation. Visual inspection is the most common method of assessing whether equipment has been adequately cleaned/sanitized between production runs. Many operators routinely supplement the visual inspection with sponge or swab samples collected from the equipment as a means of verification. The sampling sites and frequency of sampling are determined by experience and whether problems are detected. In other operations, an inspector decides when and where to collect microbiological samples, usually only when there is uncertainty or it is desirable to confirm an observation.

The value of environmental sampling for microbial control is discussed in Chapter 11. The correlation between the microbiological status of the environment and a food is highly dependent on the characteristics of the food manufacturing system. For example, an enclosed processing system has fewer opportunities to become contaminated from the surrounding environment; so the relationship between environmental samples and the product would be tenuous. Conversely, food production systems with a great deal of manipulation and exposure to the plant environment are more susceptible to environmental contamination and there is more likely a stronger correlation between microbes detected in environmental samples and the microbiological quality or safety of such a food.

Shelf-life sampling is a specialized form of end product testing that involves holding finished product for longer than the time specified on the package and then examining it for specific attributes. Shelf-life testing is most often used to establish code-dating practices based on quality attributes (e.g., time to spoilage). However, shelf-life testing can be used to evaluate whether certain pathogens can increase, not change, or decrease during the normal expected conditions of storage and handling. If the pathogen is initially present at levels for which detection by standard microbiological analytical methods is unlikely, holding the product under "market or use conditions" can offer one means of estimating consumers' potential exposure at the time of consumption.

13.2.3 Determining the Causes of Variability

Variability can occur for many reasons. There may be inherent differences in an ingredient from one lot to the next. Solid foods may vary in dimension or weight. Liquids and semi-liquids may differ in viscosity and other attributes. The variety, species, or age of an ingredient can introduce differences in composition, texture, and other properties of the food that is being processed. Equipment maintenance and performance can be a significant influence on variability. Facilities having the same equipment will likely experience different degrees of variability depending on equipment age, model, maintenance, etc. In certain regions, season and weather can significantly affect humidity and drying conditions, such as for dry cured hams, fermented sausages, and legumes and cereals during harvesting and subsequent storage. The composition of milk (e.g., fat content) varies with season and must be taken into account when manufacturing cheese. The factors having a significant impact on variability must be determined for each food operation and a decision made whether those factors are sufficiently important to be controlled. The food safety system must take account of variability when establishing limits and monitoring procedures and be sufficiently conservative to ensure that the food being produced will be acceptable and safe.

13.2.4 Desired Patterns of Variability in Foods and Food Processing Parameters

There are two distinct patterns of variability in food characteristics and in the control parameters of food processing systems. The first applies to intended characteristics, attributes, or process parameters, such as pH, temperature, or even the concentration of intentionally added microorganisms in fermented products. In this pattern, there is a *target value* and some degree of *variation* above and below that value. These intended characteristics are controlled by keeping the mean value for the process near the target value and minimizing the spread above and below the mean.

Intended characteristics or attributes of a food or a process are usually controlled in terms of the normal distribution, which is derived from a mathematical theorem called the "Law of Large Numbers." That theorem states, in simple terms, that a collection of mean values from a large number of samples, all from the same original distribution, will show the variation described by the normal distribution. Since the normal distribution applies to mean values, a great deal of control theory is based on the distributions of the mean values of multiple measurements. This has proven both reliable and valuable.

The means of even a small number of samples of a distribution are often nearly normally distributed. For many intended food characteristics or control parameters, such as pH and temperature, the distribution of individual values is essentially normal even when the number of samples being considered is relatively small, e.g., $n = 5$. In fact, for many characteristics or attributes in nature, such as the pH of an individual sample or the population density of bacteria in a growth medium, an individual sample can be viewed as the outcome of many small samplings (i.e., means). Therefore, if one can represent the attribute or characteristic in the correct units, the distribution of individual results will approximate to a normal distribution. In the case of bacteria cultured in an environment that supports non-steady state growth, such as occurs in a food, the increases in population density typically occur exponentially. Declines under adverse conditions are also roughly exponential. It has long been observed that "concentrations" of microorganisms have a log-normal distribution and consequently have a normal distribution when concentrations are expressed as logarithmic values.

The second pattern of variability applies to unintended and undesirable characteristics, such as concentrations of spoilage microorganisms or specific pathogens. In this instance, the goal is to keep the level as low as possible based on technological, economic, or public health considerations. In the case of infectious pathogens, the target value is often the absence of the biological agent by a specific microbiological assay in a given weight of product. However, even when the target value is absence in a specified quantity of food, there is some distribution of values inherent in the target. The rare chance occurrence of a pathogen is not necessarily indicative of the process being out of control. Typically, target values for indicators (e.g., coliforms) are as low as can be achieved with GHP and HACCP and, when indicator organisms are present, they are within levels considered acceptable for the process and food. Control of unintended characteristics such as microbiological contamination is usually achieved using two targets: a limit on the *fraction of product that shows contamination* and an upper limit on the *concentration of contaminants* that occurs in that fraction.

13.3 PROCESS CAPABILITY STUDY

Confidence in the performance and variability of individual processes, or entire systems, is based on data from past performance, often expressed as microbiological baselines. The use of historical, product development, and/or initial control chart data to set warning and action control limits is termed a process capability study (Bothe, 1997). Such studies can be considered part of the process validation activities that establish the efficacy of a new process or process step to control a microbial hazard. Like the HACCP plan itself, a process capability study is valid only for the process for which it was conducted. A change in the process that might alter product safety would require a new process capability study. Ideally, the process validation would be of sufficient duration to quantify all factors (e.g., seasonality, alternate sources of raw materials) that could influence process performance. However, this is seldom achieved, so subsequent verification activities are designed to acquire additional data to enhance the initial process capability study.

A process capability study has two parts:

- collecting data that show the distribution of microbial measurements when a process is operating as intended; and
- using the data to derive one or more limits on sequential patterns of data.

Whether for attribute (i.e., presence/absence) data or microbial numbers, the setting of microbiological limits will ultimately be a matter of judgment, experience, and considering the risk and consequences of failure. A process capability study of an attribute associated with a CCP often would be useful for establishing the critical limit, e.g., temperature of pasteurization. Similarly, a process capability study that examines the frequency and extent of contamination by a specific pathogen or indicator microorganism for a process could provide the basis for establishing criteria for use in verification. Several countries have acquired national microbiological baseline data on the levels of microorganisms in various food products. Those data can be viewed as industrywide process capability studies that provide a measure of the mean and variability of the foods produced by the industry. Such studies can be used to establish microbiological criteria and then, subsequently, to measure the effectiveness of industry performance and regulatory control policies and practices.

National Beef Ex. 1028
360

13.3.1 Establishing Criteria and Monitoring Procedures

Some of the general principles that determine which process evaluation techniques are most appropriate are introduced below.

Statistical process control (SPC) allows the processor to control the mean and minimize the variability of each important control parameter. If the process has achieved a predictable repeatability, the mean and the variability should remain relatively constant over time. In HACCP, SPC can be used to determine the capability of a process to maintain its mean and variability in relation to both the monitoring of CCPs and verification analyses. Data from HACCP records provide a temporal history of how well the system has been controlled. Such data are usually more useful when displayed graphically on charts called *control charts* (Figure 13–1 through Figure 13–5). First introduced in 1924 by Shewhart, control charts are the primary tool for visualizing, comparing, and analyzing process control data. Accordingly, statistical concepts related to SPC will be discussed mainly in terms of these charts.

The stringency of a process control system is established by *control values* for parameters in the process or product that require intervention to maintain control. In HACCP, the control values associated with CCPs are critical limits used to separate acceptability from unacceptability (CAC, 1997). Product produced during a process deviation would be unacceptable to release until it could be determined that the loss of control did not result in food of unacceptable, or uncertain, quality and safety.

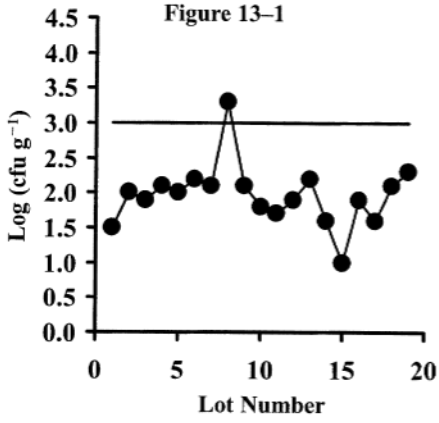
The stringency of a food safety system is a relative attribute, and establishment of a control value is a decision that requires consideration of risk and the consequences of a system failure. If a control value is set in such a way that even minor changes in the mean or the variability will violate the established value, then the system is very stringent. Conversely, if substantial deviation from the mean or a substantial increase in the variance is tolerable without an intervention, then the system is not stringent. Control values are set in different ways with different types of control charts, so examples of each will be discussed as various control charts are introduced. However, a discussion of some general concepts is often helpful in providing a framework within which the individual approaches can be interpreted.

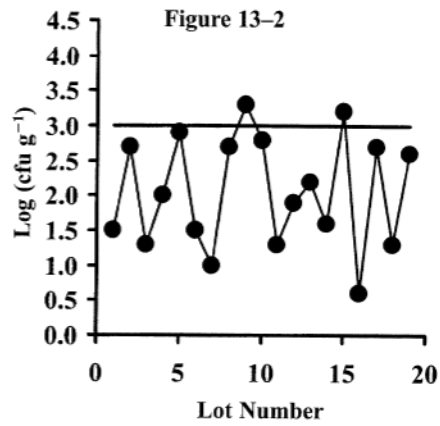
13.3.2 Types of Variability and Error

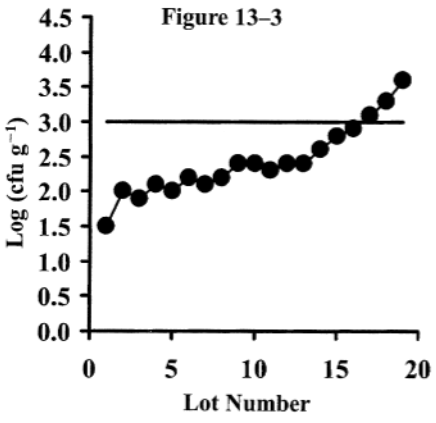
Two sources of variation are associated with food processes. The first is referred to as "common causes" of variation inherent in a process when it is operating as intended, i.e., in control. Reducing the common causes of variation would improve the process. The second source of variation is referred to as "special causes" (or assignable causes) of variation. This variability can occur when one or more steps in a process are no longer operating as intended (i.e., out of control). In this instance, identification and correction of the special cause of variation returns the system to the original degree of variability within which it was designed to operate.

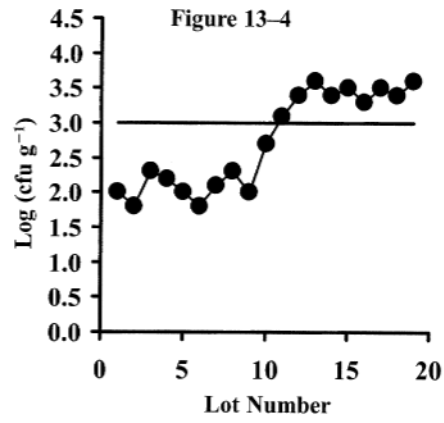
Differentiating common and special causes of variation is usually achieved by comparing series of measurements that indicate whether the products meet the specifications previously established when the process was under control. In their simplest form, the results of such a set of measurements fall into four categories:

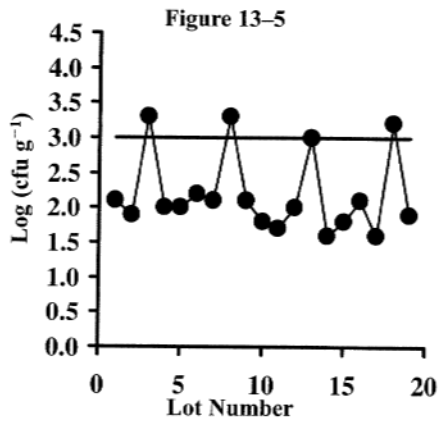
1. The measurement correctly identifies the system as in control.
2. The measurement correctly identifies the system as out of control.
3. The measurement incorrectly identifies the system as out of control.
4. The measurement incorrectly identifies the system as in control.











Figures 13–1 through 13–5 Hypothetical examples of using data from an assay for a microbiological indicator to verify the effectiveness of a food safety system. Examples depicted include the system under control (13–1), lack of control due to excess variability (13–2), loss of control due to gradual (13–3) and abrupt (13–4) process failures, and loss of control due to a reoccurring, transitory failure (13–5). The solid horizontal line depicts a hypothetical microbiological criterion above which a sample is considered only marginally acceptable. A criterion based on the presence of more than a specified number of marginal samples within a specified period of time would be the basis for determining if a process is out of control and requires corrective actions.

Categories 3 and 4 above are referred to as Type I and Type II errors, respectively, when the measurements lead to incorrect interpretations. A Type I error (category 3 above) indicates that a process is out of control when, in fact, it is still operating as intended. Such a Type I error can be viewed as a false alarm. Conversely, a Type II error (or missed signal error—category above) occurs when a series of measurements indicate that a system is in control when in fact it has an assignable source of variation. The stringency of the criteria that indicate the emergence of a special cause of variability is based on the consequences of making Type I versus Type II errors.

13.4 CONTROL DURING PRODUCTION: MONITORING AND VERIFYING A SINGLE LOT OF FOOD

Two types of data are collected during HACCP implementation, from monitoring CCPs and from verification. Control chart methodology is ideally suited to monitoring CCPs and other control points in an objective and consistent fashion. Decisions reached using control charts can be made with a measured degree of confidence. If the risk associated with a hazard is high, the control chart can be constructed to minimize the chances of the process going out of control. If the measurement is instantaneous, e.g., temperature, control is active, rather than passive, with control charts following the progress of a lot of food while it is being manufactured, detecting impending problems, and allowing adjustments to be made before control is lost.

The need for quick feedback as a food is being processed precludes the use of microbiological tests in control chart applications. Thus, control charts are normally used to record physical or chemical measurements. Control charts are easily produced when automatic in-line measuring is used (e.g., recording temperature during milk pasteurization). Control can be built into the system when a critical limit is not met, such as when milk is diverted by an automatic flow diversion valve to a balance tank, or holding tank, for repasteurization after the equipment has been adjusted to the required operating temperature.

Another example of control charting is in recording the internal temperature of beef roasts during cooking. To achieve a performance criterion of a 6 $\log_{10}$ reduction of salmonellae, it would be necessary to cook to an internal end point temperature of 62.8 °C and remain at this temperature or higher for a minimum of 4 minutes. Other time-temperature combinations could be used to meet the same performance criterion (6 $\log_{10}$ reduction of salmonellae). To ensure that the entire lot meets the performance criterion, internal temperature must be measured in the coldest area of the largest roasts in the lot. In addition, distribution of heat throughout the oven should be periodically verified (e.g., quarterly, monthly) to ensure that the roasts placed on the bottom, middle, and top layers of the cooking racks and in different areas of the oven meet the criterion.

Because continuous monitoring of the internal temperature of a number of roasts is even beyond the capabilities of many processors, many operators establish process criteria for the cooking conditions (i.e., oven temperature, humidity, time) as a simpler, cheaper, more convenient means to monitor the process. It is also common practice to limit the weight range and size of the roasts within the lot to avoid overcooking and yield loss among lighter weight roasts. Frequent measurements of the oven temperature can be collected automatically from thermocouples strategically placed throughout the oven. The data for the oven can be recorded automatically onto a computer-based chart logger that provides a visual record as the lot is being cooked. The oven operator can examine the chart to determine whether adjustments are necessary to meet the critical limit of

62.8 °C. When experience indicates that the roasts should be at, or above, the required temperature, a temperature probe is inserted into selected roasts to verify the criterion has been met. The lot would then be held for four minutes at that temperature before chilling would begin. Some operators may take account of the fact that the internal temperature will continue to rise several degrees even with no further application of heat.

An alternative approach to meeting the performance criterion could be based on the total lethality for salmonellae that occurs during the heating and chilling cycle. After placing thermocouples into the roasts and documenting the time the internal temperature is at 57, 58, 59, 60 °C, etc. during heating and chilling, the cooking procedure is calculated from the incremental lethality that occurs at the times and temperatures above 57 °C, the sum of which would meet the 6 $\log_{10}$ reduction performance standard for salmonellae. This approach should be continuously monitored with thermocouples placed in the largest roasts to provide a temperature profile that can be used for verification.

It is important to note that biased sampling is used in the selection of roasts to be monitored. Information about the largest roasts and, in some cases, the location of the roasts within an oven, is used to ensure all the roasts meet the criterion. If any of the selected roasts have not met the established criteria, then the entire lot must continue to be cooked. While information about variability is very important when establishing the cooking and monitoring procedures, information about both the mean and variability will determine the quality of the product and profitability of the process. In this example, and in many other food processes, a statistical evaluation is not used to decide when each individual lot is cooked. Statistical evaluation, however, can be an important tool in the design and validation of a process.

13.5 CONTROL DURING PRODUCTION: ORGANIZING DATA FROM ACROSS MULTIPLE LOTS OF FOOD TO MAINTAIN OR IMPROVE CONTROL

This section primarily considers the organization and interpretation of data collected from multiple lots produced over days, months, or even years to enhance control of production and to provide the information necessary for continuous improvement. However, the information in this section also applies to individual lots of food.

The emergence of process control programs such as HACCP has led to a substantial shift in the intent of microbiological testing programs. While testing of individual lots of some food still occurs, increasingly industry and control authorities are focusing their testing programs on verifying that food control systems are effective. While many of the microbiological assays employed for these two approaches are virtually identical, the statistical tools and assumptions that help in interpreting the results of monitoring and verification differ from those for batch testing. Particularly for verification testing, data from multiple batches, often over extended periods of time, are evaluated. Thus, unlike batch testing, this aspect of verification requires consideration of both within-batch and between-batch variability.

It is important to reemphasize that the purpose of such testing is not approval for release of batches or characterization of particular lots of product. In the case of HACCP systems, individual batches are characterized by monitoring CCPs, not verification testing. Instead, the purpose of such periodic testing is to provide:

- assurances that the conditions that enable a food process to produce safe products are being maintained.
- a basis for analyzing performance trends so that corrective actions can be taken before loss of control.

- insights into the cause for loss of control (e.g., periodicity of contamination).
- a warning that conditions have changed sufficiently such that the original HACCP plan may need to be reviewed.

One of the most important features of control charts is that they allow visual tracking of results over multiple lots. As trends develop, not only does it become apparent when action is required, but it is also possible, by careful study of the data, to determine approximately when the trend started. Cumulative sum (CUSUM) charts (Duncan, 1986), which use the same data as in the control charts but plot them in a cumulative fashion, provide a clearer visual display for this purpose. Moving sum (MOSUM) charts are another means of presenting cumulative data. Knowledge of when a nonrandom influence entered the process can yield valuable clues to the identification and elimination of the problem.

It is not good practice to wait until a control chart indicates an out-of-control situation before taking action. Some control charts have warning limits inside the control limits to signal impending loss of control. CUSUM charts are especially useful for early detection of trends. Many processes can gradually go out of control, providing plenty of warning as this occurs. Control devices may drift, mechanical components will wear (causing increasing variability as they do so), cleaning and disinfection procedures may be less stringently performed, or the microbiological quality of an ingredient may gradually worsen. Sometimes even an abrupt change in some procedure may result in a gradual change elsewhere.

In many situations, the need for both upper and lower control limits may not be readily apparent. One-sided control charts could be constructed for situations where the upper limit is of concern. Examples include the pH of an acidified food that is to be distributed at ambient temperature or the retort temperature for a canned food that must be kept above a certain minimum requirement, but too high a temperature is wasteful of resources. Frequently at CCPs in a food process, one limit controls product safety while the other limit represents economy of manufacture or some aesthetic feature of the food.

In addition to monitoring existing, well-established processes, control charts are useful in developing new processes. By their nature, control charts draw attention to unusual results that have occurred due to the influence of some nonrandom and controllable factor. Hence, in process design, control charts can be used to help isolate sources of process variation so that these sources can be traced and, if possible, eliminated.

Acquiring data in this manner can provide important insights concerning the performance of a food safety system and the types of problems being encountered. As a means of demonstrating this point, Figures 13–1 through 13–5 depict graphic examples of hypothetical microbiological data acquired to verify performance of a food safety system in relation to an established acceptance criterion.

Figure 13–1 depicts a system that is under control. Even with a well-controlled system, deviations characteristic of the system occasionally occur. Setting the criterion for what is or is not a failure is dependent on the level of performance that can be expected (i.e., the variability of a process) and achieved (i.e., technological capability), and the consequences of not meeting the acceptance criterion. Second, in a well-controlled processing system, the majority of data tend to cluster around a central value.

Figure 13–2 depicts the same system with greater variability. This is reflected in an increased number of samples above the acceptance criterion and an increase in the scatter of values that meet the acceptance criterion. Such a scenario could indicate one or more factors not being controlled.

Figure 13–3 depicts a situation where a component of the process is losing its effectiveness over time. It is apparent, through trend analysis of the data, that it might be possible to detect and correct this deficiency before the acceptance criterion is exceeded. This trend indicates a gradual loss of control at an important processing step. An example of this type of failure might be the buildup of cooked egg on the holding tubes of a liquid egg pasteurizer, leading to decreasing effectiveness of the heat treatment. By comparison, Figure 13–4 depicts an example of a loss of control such as when a key piece of equipment failed abruptly.

Finally, Figure 13–5 provides an example of a system with distinct periodicity. Such data indicate the existence of a recurring, intermittent problem. This pattern can occur with a seasonal effect, if the data were to represent a summary chart spanning years, or lots produced on Mondays due to microbial growth on inadequately cleaned equipment over the weekend.

While many food producers use microbiological testing to establish microbiological profiles for their products, these data are often not analyzed and evaluated in a rigorous manner. The knowledge that can be gained from these data can be greatly enhanced through use of the relatively simple statistical tools that are described in this chapter. Considering the cost incurred in conducting microbiological testing, the use of these statistical tools can be viewed as increasing the manufacturer's return on investment.

13.5.1 Variables Charts of Quantitative Data

As mentioned above, control charts are the primary means for arraying process control data for visualization and analysis. However, the type of control chart used will depend on the type of data being evaluated. As discussed in Chapter 7, microbiological analyses generate two types of data, *attribute* data (nonquantified, presence/absence assays) and *variable* data (quantitative, population density determinations), and there are different classes of control charts for evaluating those data. Attribute charts will be discussed in section 13.5.7.

13.5.2 General Principles of Variable Control Chart Construction

Control charts are plots of data over time (Ryan, 1989) and may represent data from one lot or from across multiple lots. The x-axis on the control chart is usually the time at which the sample or samples were collected during the process, and the y-axis is the value obtained for the measurement. The control chart consists of 3 parallel lines: a lower control limit (LCL), a center (or central) line, and an upper control limit (UCL) (Figure 13–6). In some instances where the LCL is below the lower limit of detection (i.e., absence), the LCL is assumed to be zero or some predesignated value below the lower limit of detection.

Each step in a process has a degree of inherent variability. When combined, the variability of each step contributes to the overall variability of the system. In a well-controlled system, data points tend to cluster around a central value. Traditional statistical measures of central tendency include the mean, median, and mode, and the measures of variability, such as the range, standard deviation, and standard error of the mean. The centerline in a control chart typically is a measure of central tendency, whereas the flanking lines are limits based on the expected degree of variability.

To appreciate how the LCL and UCL are often established, it is necessary to understand the standard deviation, or *sigma* (σ), concept as it applies to control charts. The σ

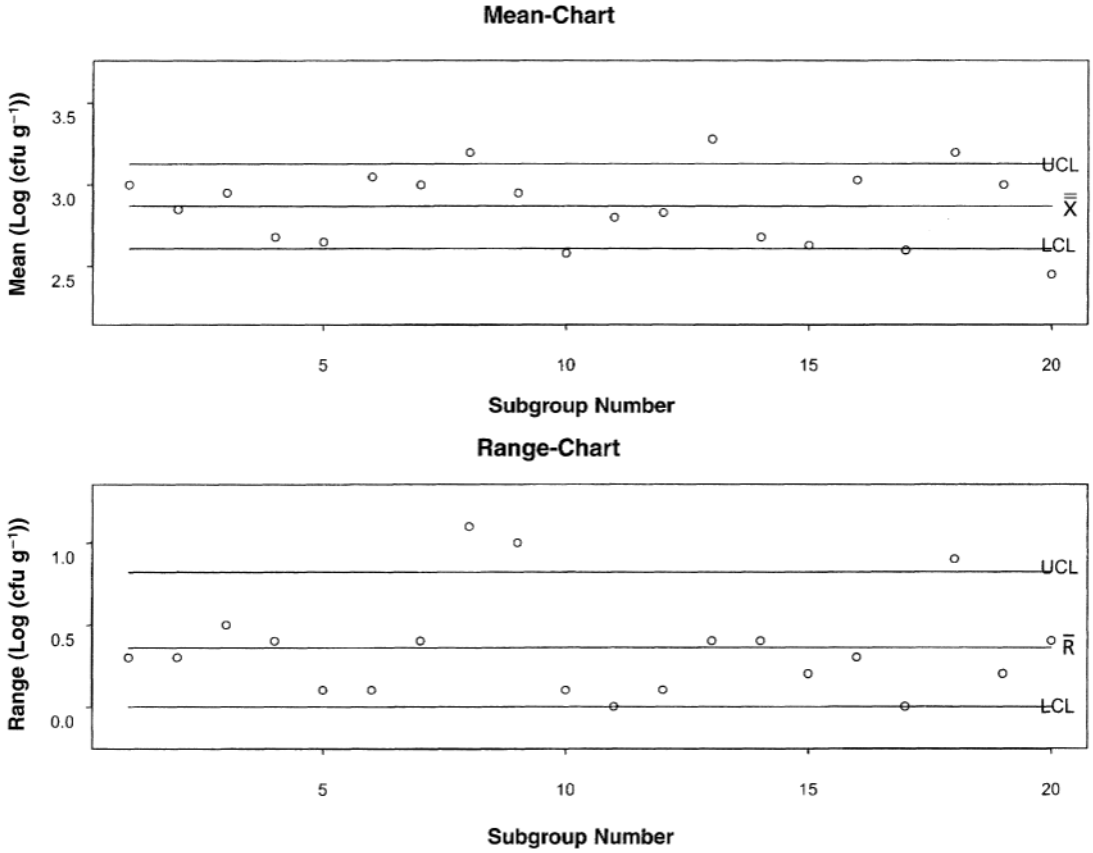


Figure 13–6 Hypothetical example of $\bar{\bar{X}}$ and R control charts developed using a process capability study that measured the "Total Aerobic Plate Count" for 20 data subgroups each with 4 replicates (Table 13–1). The middle solid line represents the "target value" ($\bar{\bar{X}}$ and $\bar{R}$} and the flanking dotted lines represent $\pm 3\sigma$.

concept is similar to that used in describing the variability of batches or lots. Control charting generally assumes that the distribution of data collected during the process is normal or approximately normal. Based on the normal distribution, approximately 68% of values will fall within plus or minus 1 standard deviation of the mean, approximately 95% within 2 standard deviations of the mean, and approximately 99.7% within 3 standard deviations of the mean (Figure 13–7). "One sigma" refers to one standard deviation from the mean; "2σ" to two standard deviations from the mean; and "3σ," to three standard deviations from the mean. Control limits are most commonly set at plus or minus 3σ from the mean. When 3σ control limits are used, the probability of any particular datum point being outside the control limits by chance alone is 0.3% when the process is actually *in control*. Thus, if the frequency at which values fall above or below 3σ is greater than 0.3%, then the process is deemed to be out of control.

In addition to determining when a process is out of control, control charts and their measures of central tendency and variation can be used to predict the frequency with which failures will occur despite a process being in control (i.e., frequency of Type I errors). For example, Peleg and co-workers (Nussinovitch *et al.*, 2000; Peleg *et al.*, 2000)

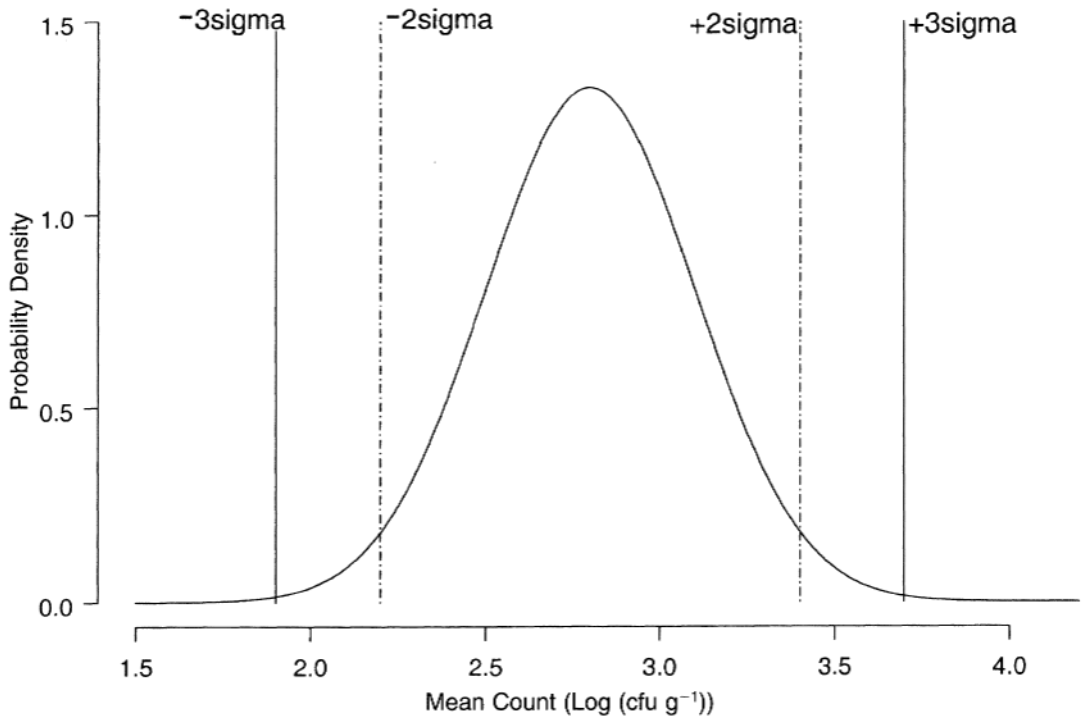


Figure 13–7 Density of a normal distribution.

used control charting in combination with a probabilistic model to predict the frequency of unusually elevated bacterial counts in foods.

As a means of introducing general concepts related to process control charting, the $\bar{X}R$ control chart will be used as an example. $\bar{X}R$ denotes the use of the mean ($\bar{X}$) of a subgroup of samples and the range (R) between the smallest and largest values in the subgroup of samples. It is commonly used in variables charting. The $\bar{X}R$ chart is actually two charts, one a measure of central tendency (i.e., the mean), and the other a measure of variability. It is based on the comparison of small subgroups, thus fulfilling the original intent or precision of the normal distribution, i.e., comparison of means. $\bar{X}$ and R are usually included on the same control chart to facilitate their use in tandem to establish whether the process is in control. The $\bar{X}R$ chart is one of the most useful from a practical standpoint because it is so simple to construct.

The first step in developing an $\bar{X}R$ chart is to define a subgroup, frequently a set of results from verification samples or monitoring data that are collected during a short time when the process is known to be under control. The variable of interest is measured for each item in the subgroup. Before the control chart is constructed, the general recommendation is to collect data on 20–30 subgroups, with each subgroup consisting of samples (n) of 4–5 units per subgroup. As an illustrative example, Table 13–1 shows a set of hypothetical total aerobic plate count data taken to verify the microbial quality of a ready-to-eat food when all manufacturing processes are in control. In this example, the control limits are based on 3σ and an equal number of items in each subgroup.

Table 13–1 Hypothetical Results of a Process Capability Study for a Ready-to-Eat Food Wherein Total Aerobic Plate Count Data (log_{10} cfu g^{-1}) Are Used To Verify the Acceptability of a Product

Subgroup	*Sample 1*	*Sample 2*	*Sample 3*	*Sample 4*	*Mean*	*Range*
1	3.1	3.0	2.8	3.1	3.00	0.3
2	2.7	2.9	3.0	2.8	2.85	0.3
3	2.8	3.3	2.8	2.9	2.95	0.5
4	2.9	2.5	2.6	2.7	2.68	0.4
5	2.6	2.7	2.7	2.6	2.65	0.1
6	3.1	3.0	3.0	3.1	3.05	0.1
7	3.2	2.9	2.8	3.1	3.00	0.4
8	3.8	2.8	2.7	3.5	3.20	1.1
9	3.5	3.0	2.5	2.8	2.95	1.0
10	2.6	2.5	2.6	2.6	2.58	0.1
11	2.8	2.8	2.8	2.8	2.80	0.0
12	2.9	2.9	2.7	2.8	2.83	0.1
13	3.0	3.3	3.4	3.4	3.28	0.4
14	2.9	2.5	2.7	2.6	2.68	0.4
15	2.5	2.6	2.7	2.7	2.63	0.2
16	3.1	2.8	3.1	3.1	3.03	0.3
17	2.6	2.6	2.6	2.6	2.60	0.0
18	2.8	3.4	3.7	2.9	3.20	0.9
19	2.9	3.0	3.1	3.0	3.00	0.2
20	2.4	2.3	2.7	2.4	2.45	0.4
				$\overline{\overline{X}}$	2.87	
				$\overline{R}$		0.36

1. Calculate the mean ($\overline{X} = [X_{1+} \ldots {}_{+}X_n]/n$) for each subgroup (Table 13–1). If evaluating microbiological population density data, the log_{10} of the individual values would be used, thus converting the log-normal distribution associated with microbial "concentrations" to a normal distribution of log values.
2. Calculate the range (R) for each subgroup (Table 13–1). The range is the difference between the maximum and minimum values of items in the subgroup.
3. Compute the mean range ($\overline{R}$) for all subgroups. The mean range is the sum of the ranges for all of the subgroups divided by the number of subgroups (n_{sg}). In the current example, the $\overline{R}$ value is 0.36.
4. Compute the grand mean for all subgroups. The grand mean ($\overline{\overline{X}}$) is the sum of the means of all the subgroups divided by the total number of subgroups ($\overline{\overline{X}} = [\Sigma\overline{X}]/n_{sg}$). In the current example, the $\overline{\overline{X}}$ value is 2.87.
5. Set the centerline of the $\overline{X}$ chart at $\overline{\overline{X}}$, and set the centerline of the R chart at $\overline{R}$ (Figure 13–6).
6. Calculate the 3-σ control limits around the target value for the $\overline{X}$ chart. The LCL is equal to $[\overline{\overline{X}} - (A_2 \cdot \overline{R})]$. The UCL is equal to $[\overline{\overline{X}} + (A_2 \cdot \overline{R})]$. The value of A_2 can be obtained from Table 13–2 for subgroups with sample size (n) of 2–10. In the current example, the LCL = 2.61 and the UCL = 3.13.
7. Calculate the control limits for the R chart. The LCL is equal to $D_3 \cdot \overline{R}$. The UCL is $D_4 \cdot \overline{R}$. The values of D_3 and D_4 can be obtained from Table 13–2 for subgroups with sample size (n) of 2–10. In the current example, the LCL = 0.00 and the UCL = 0.82.

Table 13–2 Parameters for Determining "3-σ" Control Limits for $\bar{X}$ and R Charts

Sample Size (n) per Subgroup	A_2	D_3	D_4
2	1.880	0	3.268
3	1.023	0	2.574
4	0.729	0	2.282
5	0.577	0	2.114
6	0.483	0	2.004
7	0.419	0.076	1.924
8	0.373	0.136	1.864
9	0.337	0.184	1.816
10	0.308	0.223	1.777

8. Plot the $\bar{X}$ values on the $\bar{X}$ chart and the R values on the R chart (Figure 13–6). Plotting the current example makes it apparent that there is a fair degree of variability associated with both the central tendency and dispersion in this hypothetical process.

The $\bar{X}$R chart is one of the simplest variable control charts. Detailed descriptions of the procedures and uses of the wide variety of other variable control charts are available from standard references such as ASTM (1990) and Duncan (1986).

13.5.3 Corrections When Data Are Autocorrelated

An underlying assumption in the development of most control charts is that as the individual data points are plotted, they are randomly distributed according to a normal ($\log_{10}$ values) or log-normal (arithmetic) distribution over time. However, in actual production settings, successive measurements of product parameters collected sequentially over time are often correlated with one another (i.e., the measurements are autocorrelated). Autocorrelation is especially likely when values are collected close to one another in time. Autocorrelation affects the pattern of data on control charts, with implications for setting control limits. Wheeler (1995) and other standard references provide in-depth coverage of autocorrelated data and control charts. Ryan (1989) notes that autocorrelation is primarily a problem for CUSUM procedures and acceptance, difference, median, and midrange charts.

13.5.4 Special Considerations for Charting Individual Counts of Microbial Population Density

Data on concentrations of pathogens or indicator organisms in individual sample units can be presented in simple variables charts such as the hypothetical example in Figure 13–8. This fictional example shows numerical estimates of concentrations of indicator organisms in samples of a finished product. This chart shows fairly normal fluctuations in viable counts up to week 40. After week 40, the same sort of fluctuations occur, but about a much lower midpoint. This could reflect a change in raw materials or equipment, a seasonal effect, a change in the analytical method, etc. Even though the change appears welcome, investigation is warranted since it could represent either an analytical problem or a means for identifying a factor that could consistently enhance the performance of the system.

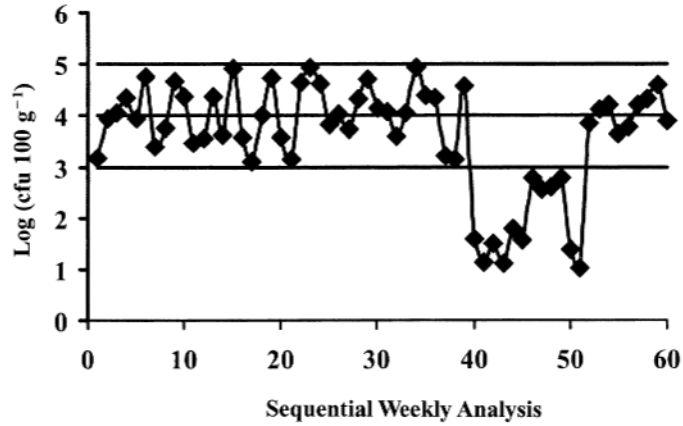


Figure 13–8 Hypothetical example of a "variables control chart" for a microbial indicator assay conducted weekly. The center horizontal line represents the mean value and the two flanking lines represent the upper and lower "warning" limits. An apparent period of significant improvement is depicted between weeks 40 and 51.

One of the most straightforward means of considering a set of quantitative microbiological data is to chart the individual test results. Such a control chart, commonly called an X chart, is simple and rarely misleading. Unusually high counts, or patterns of high counts, are typically the criteria used to designate loss of control and raise safety concerns. Conversely, unusually low counts, or patterns of low counts, may reflect a potential process improvement or a need to review analytical protocols. Obvious trends of either sort would merit attention. Subtle trends in the values for an indicator should not necessarily be viewed as trends at all, since some drifting up and down of indicator levels over weeks or months is to be expected. However, such trends may warrant a review of the process since they may give early warning of a gradual loss of process control (Figures 13–3 and 13–4), and thus allow corrective action before loss of control.

13.5.5 Selecting Limits for Variable Control Charts of Microbial Counts

Although professional judgments of food scientists, food microbiologists, and process managers are the most important factors in setting the upper and lower limits used for this type of variables control chart, there are SPC procedures that can help to select the initial choices of limits. The guidelines below will provide a reasonable starting point.

One of the key factors in selecting the limits is the test results for which no microorganism was detected, i.e., test value = 0. However, such a value is not directly useful for two reasons. First, the need to convert microbial population density data to $\log_{10}$ values results in the 0 values being undefined (and zero values are ignored by some software). Second, the inability to detect a microorganism in any specific sample reflects two possibilities: that the microorganism truly is not there, or that the microorganism is present, but at levels that the sampling protocol or analytical methods are incapable of detecting.

Consider first the situation in which at least 90% of the test results are expected to be quantifiable above the lower limit of detection for the method employed. Any results below the limit of detection (i.e., no microorganisms are detected) are assigned a value

of one-half of the limit of detection and transformed into a $\log_{10}$ basis. (This might slightly underestimate the standard error. That would make the resulting "warning rules" and "stopping rules" slightly overcautious, but this can be adjusted when a large number of test results is available.) The limit-setting study would begin with testing at a high frequency to acquire enough results to study their average and their variability. Typically at least 10 quantifiable results, and preferably 30 or more, would be acquired and charted. The mean ($\bar{X}$) and the standard error (σ) are computed, "warning limits" are set at $\bar{X} \pm 2\sigma$, and upper and lower "stopping limits" are set at $\bar{X} \pm 3\sigma$.

When more than 10% of the limit-setting data are zero values (i.e., too low to be quantified), it is better initially to derive the warning and stopping limits by comparing the gap between percentiles of the test data with that which would be predicted by the normal distribution.

If less than half of the results are below the lower limit of detection (i.e., zero), a simple approach is to find the difference between the $\log_{10}$ of the 50th percentile (i.e., 50% of the samples have a lower value) and the $\log_{10}$ of the 75th percentile. With a normal distribution, the difference between the $\log_{10}$ value at the 75th percentile minus the $\log_{10}$ at the 50th percentile (i.e., mean) should cover 67% of the σ. Thus, dividing the difference of these percentile $\log_{10}$ values by 0.67 provides an estimate of σ. For example, if the 50% value is 3.2 and the 75% value is 4.6, then $\sigma = (4.6 - 3.2)/0.67 = 2.1$. Thus, dividing the difference of these percentile $\log_{10}$ values by 0.67 provides an estimate of σ. The 50th percentile value is used in place of $\bar{X}$ drawing the limit lines. Using these estimates of $\bar{X}$ and σ, the limits for the control chart are drawn as described above.

If more than half of the results are below detection, the analyst should consider whether to proceed with a variables chart. It might be more appropriate to use an attributes (presence/absence) chart. If that is not the preferred option, however, any table of cumulative probabilities of the normal distribution will provide the 50th percentile and σ from the gaps in the percentiles of the data. For example, the gap between the 70th and 90th percentiles should be 76% of σ. As a rough rule of thumb, percentiles greater than the 90th should not be used for this purpose.

Once the initial limits have been established, the first test results acquired subsequently should be scrutinized carefully as a means of ensuring that the data used to set the initial limits were acquired when the process was in control. Ordinarily, one would hope not to find any values outside the upper and lower control limits at this stage. If deviations are observed, then the data should be studied for any trend, and any trend identified should be investigated. If any result is below $\bar{X} - 3\sigma$ or above $\bar{X} + 3\sigma$, or if three of any five successive results are below $\bar{X} - 2\sigma$ or above $\bar{X} + 2\sigma$, possible causes should be investigated, such as a change in the process or ingredients or a change in the sampling or analytical protocols. The process capability study will have to be rerun with the new data until the warning and stopping limit values stabilize. When the process capability study has been completed without any deviations or apparent trends, then these limits at $\bar{X} \pm 2\sigma$ and $\bar{X} \pm 3\sigma$ should be selected for initial control limits for verification tests.

As verification testing proceeds, if it appears that the limits selected are giving an unreasonable number of false alarms, then the limits should be compared against the likelihood of Type II errors (i.e., not catching a safety defect) and expanded if appropriate. Such an expansion of the limits should be done cautiously with continued or increased scrutiny until sufficient data are acquired such that the process can be adequately judged in relation to:

- how truly rare is deviation when the process is in control, and
- how frequently is control lost.

Two examples of reasons follow for why the initial limit-setting exercise might have proved too restrictive.

1. Seasonality might cause $\bar{X}$ or σ to shift in ways that make the limits too tight. If seasonality has no effect on $\bar{X}$, then it probably has no effect upon σ. If it does affect $\bar{X}$, then (a) the charts may need to be adjusted by drawing seasonal $\bar{X}$ lines on them, and (b) the data must be studied to see if the σ has also changed. The seasonal changes in $\bar{X}$ and in the control lines can define a series of zones with different straight lines or $\bar{X}$ can be fit by a curving line with σ-determined lines curving at fixed distances above and below $\bar{X}$. However, if seasonality produces an unacceptable risk to safety, the appropriate response would be to eliminate the variability due to seasonality and not to modify the control chart limits.
2. If the limit-setting study is done with a single source of raw materials and the process then proceeds to use a variety of sources, an additional source of variation has been added, and the initial limits will be too narrow. When more data, covering more sources of raw materials, are in hand, the $\bar{X}$ and σ should be recalculated. Again, if the additional sources of raw material represent an unacceptable risk to safety, the appropriate response would be to eliminate the unacceptable variability associated with the new source of raw material and not to modify the control chart limits.

13.5.6 Caution in Interpreting Certain Types of Variables Charts

Moving Range (R) charts are useful for process control monitoring of parameters such as pH and temperature. These charts track the absolute value of the difference between each result and the previous one. Declines and increases are treated the same. When dealing with levels of undesirable microorganisms, a significant decline in microbial concentration may not have the same significance as an increase. This is because downward trends are generally not a cause for alarm, and a low value preceding a high value looks significant as a range but doesn't make the high value any more important.

Standard procedures are described in general quality control texts for converting the average of the moving ranges into an estimate of σ. However, this is also not advisable for pathogens and indicator organisms. Weather, seasonal changes, shifting harvest zones, early or late crops, spot markets for ingredients, and other factors will force $\bar{X}$ up or down over periods of multiple tests in ways that cannot be predicted very precisely. This will induce a degree of autocorrelation. As described above, autocorrelation is the tendency for results to be more like those near them in test sequence and less like those farther away in sequence. For this reason, the distribution of differences between successive test results will usually underestimate the true variability of microbial levels in foods.

For similar reasons, CUSUMs of differences in the observed $\log_{10}$ microbial concentrations and some target log numbers are not appropriate for these variable charts. The mean, or target log number, is not truly a target value, since one does not intend to achieve a target value of pathogens. In this instance, the uncontrolled shifting of $\bar{X}$ results in an inability to set standard limits for a chart of the mean of the previous n observations. However, such charts, for small n, would still be useful as a means of illustrating shifting patterns, such as those that might occur as a result of seasonal variation. (Note that CUSUMs are useful for attribute charting.)

13.5.7 Attribute Charts of Presence/Absence Determinations

One of the simplest, most widely used microbiological tests for specific pathogens or indicator organisms is presence/absence testing. An analytical unit of known weight (mass) is tested by a standard method to determine whether the microorganism of interest is, or is not, detected in that food sample. Over time, this type of microbiological assay can be used to assess the maintenance of food safety control systems. However, interpretation of such tests is highly dependent on the method used to determine the presence of the microorganism, particularly its lower limit of detection. Consequently, it is generally not possible to compare or combine the results from one data set with those from another unless both data sets were acquired using the same standard method that has consistent analytical performance characteristics.

Consider a hypothetical example of a single microbiological presence/absence assay for *Salmonella* spp. performed once per day for a total of 50 successive days as a means of process verification. The microbiological test requires 48 hours to complete so there is an effective 2.5-day delay between the sample being taken and the results becoming available. Figure 13–9 depicts these data as a simple control chart. Let us assume that when the process being evaluated is under control, about 1 of every 4 assays is positive for *Salmonella*. Further, the stringency of the system has already been established based on the criterion that if *Salmonella* is detected on four successive days, this is considered a loss of control due to the introduction of an attributable source of variability. In this example, we have included an abrupt failure of a critical part of the process on day 29 that is not detectable by normal process control monitoring procedures but is detected by the supplemental verification testing. While evaluation of the control chart ultimately allows the user to estimate when the failure was likely to have taken place, it shows that there is a substantial delay before an action can be taken. Based on the established criterion of four consecutive positive assays, if the results were available instantaneously, the

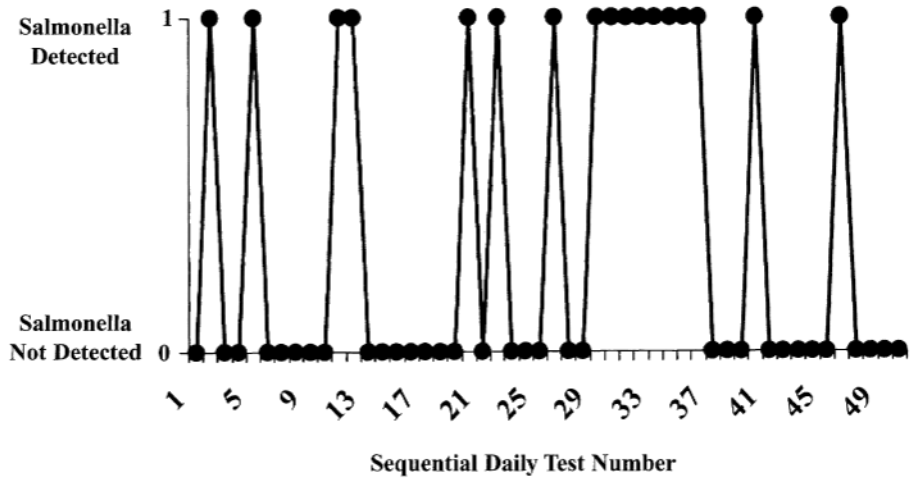


Figure 13–9 Hypothetical example of an "attributes control chart" for a single daily detection/nondetection assay for *Salmonella* spp. microbe where in-control detection rate is 0.25 (25%). In this example, control is lost on Day 29 and restored on Day 37.

chart would indicate a loss of control at day 33. However, because of the 2.5-day delay in acquiring the data, the actual day when the loss of control would be acted upon would be day 37. This is reflected in the control chart (Figure 13–9) when the values return to the normal pattern on day 37. It is apparent from this simple example that the response time associated with a control chart is based on the frequency of testing, the decision criterion established, and the time required to analyze the samples. For example, if the decision criterion had been three consecutive positive samples, the failure would have occurred a day earlier. However, this more restrictive criterion would increase the risk of Type I errors.

Presence/absence charts illustrate a sequential pattern of *yes/no* results. The usual assumption behind these charts is that when a process or system is operating under control there is some fraction p of sample units that will yield a *yes* result when analyzed. It is further assumed that successive test results are independent of each other. Analysis of charts shows whether these assumptions hold, and might lead to process improvements. For example, if the day shift has a higher p than the night shift at the same plant, this would suggest that there might be an additional source of variability during the day shift that may be reducible. Conversely, if one observes a longer than expected series of *no* results, the assumption of the independence of observations may not be true. This potentially could lead to identification of means for further process improvements.

If there is a change in a process that causes p to increase markedly, the frequency of detecting the microorganism of interest would be expected to increase (i.e., an increased frequency of *yes* results). Verifying that p is not changing is accomplished by tracking the sums of detection results over some interval of results. Starting from some beginning point in collection of data, one can determine for a number of future observations, n, an upper limit, denoted U(n), that should not be exceeded by the cumulative sum of the samples in which the microorganism is detected. Standard statistical software included in most spreadsheet programs will compute the probability of getting j positive isolations out of n tests when the probability of the presence of the microorganism is p. One needs only to decide the degree of rarity that will be tolerated before intervening in the process. Then that criterion will determine U(n) for every n. The process capability study that estimated p is followed by creation of a table of values of U(n). Then the process manager notes after each test the CUSUM and compares it with U(n). As long as CUSUM does not exceed U(n), the statistic has not shown a loss of control.

There are practical limits to the length of sequential results that are closely related to each other. The CUSUM is also fairly complex. For these reasons, another statistic is sometimes preferred. After the CUSUM has been followed up to a fairly large n, there is little loss of discrimination if the most recent (fixed) n results are summarized, without increasing n further. The number of times the presence of the microorganism was detected in the previous n results is called the moving sum, or MOSUM, because the count is derived from a zone that moves along the string of observations. The limit for this fixed MOSUM is of course the same as that for the nth step in the CUSUM. If one picks a small n for evaluating the MOSUM, one can achieve a rapid detection of any major shift in p. On the other hand, if p shifts slowly upward, the sum over a small n will not be sensitive enough to detect it. A larger n is more discriminating, and therefore is more sensitive to even gradual shifts in p, but a large n can delay the discovery of the shift. One of the MOSUM techniques that has been used extensively with microbial food safety verification testing is the Moving Window assay (see section 13.6).

This dilemma between quick response and high sensitivity can be overcome by using multiple MOSUMs. These are a compromise between a single MOSUM and the CUSUM. For many processes, a choice of two MOSUM charts, a short interval for responsiveness and a long interval for sensitivity, can be very useful. MOSUMS, by their nature, are highly correlated with their predecessors, so apparent long strings of MOSUMS moderately above the mean value have no special importance. Increased frequency of testing can be especially valuable when MOSUMS or CUSUMS get close to their action limits. When these limits are exceeded, the situation changes to one of investigational sampling (Chapter 9) and problem solving to determine the new source of attributable variability and return the process to an in-control state.

13.5.8 Advantages of Multiple Charting of Data Subsets

Verification sampling of the end product is an essential data set for effective control charting because it reflects the integration of all the process control steps and reveals failures due to problems that affect the entire production. However, additional verification sampling at key intermediate steps within the process can provide valuable information for isolating special causes of loss of control. In addition, plotting subsets of a single verification data set may more rapidly identify an emerging problem. Returning to an example discussed above, suppose a plant operated three shifts per day, and workers analyzed one verification sample per shift for *E. coli*. In addition to the chart of all of the test results, it would be beneficial to generate individual charts of the results for each shift. In this way, new sources of variability that were specifically associated with a single shift would be identified and isolated substantially faster.

13.5.9 Caution Regarding Control Chart Software

Control charting software is increasingly available and greatly simplifies the process of setting up a variety of control charts. Caution must be observed, however, in using any of the more sophisticated options that are included in most software packages for advanced applications. Food safety processing rarely satisfies all the assumptions that underlie many industrial quality control computations. Care must be taken to determine whether options selected are pertinent to the development of either attribute or variable control charts related to microorganisms or their metabolic products in foods. This is particularly important when designing variables control charts.

Caution should also be exercised in keeping control records only through software (and not on hardcopy). Some software products automatically readjust $\bar{X}$ and σ as the data are inputted. This will hide slow trends in $\bar{X}$ or σ by leaving no traces of the adjustments (Wise & Fair, 1997).

13.6 USE OF PROCESS CONTROL TESTING AS A REGULATORY TOOL

Food control agencies have traditionally relied on batch-by-batch testing for lot acceptance of foods, particularly in relation to international trade. However, with the increased emphasis on the adoption of food safety systems such as HACCP, there is likely to be increased focus on the validation, monitoring, and verification of process control measures as the means that food control agencies use to assess safety. An example of how these techniques can be used in a regulatory framework is the US Department of Agri-

culture Food Safety and Inspection Service's Pathogen Reduction/HACCP regulation (McNamara, 1995; FSIS, 1996). This regulation included two forms of microbiological testing as a means of verifying the effectiveness of HACCP programs required for the production of meat and poultry at slaughter. The first was testing of carcasses by industry for the presence of biotype I *Escherichia coli* as an indicator of fecal contamination. The second was testing for *Salmonella* spp. performed by USDA. Both were based on the application of a MOSUM control charting technique, the Moving Window.

In the case of *E. coli* testing, the technique was adapted for variables testing (i.e., consideration of quantitative data) using a limit that could not be exceeded (*M*-value) and a warning value (*m*-value) that could not be exceeded more than three times (*c*-value) in a moving window of 13 tests ($n = 13$). The *m*-value and *M*-value were based on national baseline surveys of the various types of meat and poultry and are specific for that commodity (FSIS, 1996). The rate of sampling (one sample taken for a set number of animals slaughtered) is also commodity-specific.

The method for evaluating the carcasses for *Salmonella* was limited to presence/absence testing. Accordingly, the moving window technique was adapted for attribute testing. Again, the limits that indicated that the HACCP system was no longer under control were based on national baseline survey results for each commodity. The number of samples within the window and the number of positive isolations of *Salmonella* wherein the system is still considered in control varies among the different types of meat and poultry (Table 13–3).

13.7 INVESTIGATING AND LEARNING FROM PREVIOUSLY UNRECOGNIZED FACTORS OR UNFORESEEN EVENTS

In part, HACCP verification can be viewed as additional testing conducted for the purposes of ensuring that the conditions and requirements identified in the hazard analysis upon which a HACCP program was developed are still valid. As an example, consider pasteurization of liquid egg products. An obvious CCP for production of pasteurized liquid egg products is the time/temperature conditions of the heating step. This CCP is effectively monitored on a real-time basis by recording temperature and duration of heating. Parameters for this processing step were developed using data acquired from inoculated pack studies with *Salmonella* and have been highly successful for controlling this

Table 13–3 *Salmonella* Performance Standards Associated with the USDA Pathogen Reduction/HACCP Regulation

Class of Products	*Performance Standard (% positive for* Salmonella*)*	*Number of Samples Tested (n)*	*Maximum Number of Positives to Achieve Standard (c)*
Steers/Heifers	1.0	82	1
Cows/Bulls	2.7	58	2
Ground Beef	7.5	53	5
Broilers	20.0	51	12
Hogs	8.7	55	6
Ground Turkey	49.9	53	29
Ground Chicken	44.6	53	26

pathogen in these products. However, these studies were originally conducted at a time when most eggs used to make liquid egg products were eggs left over from the marketing of shell eggs. These eggs were typically several days to weeks old. When initially laid, the white of an egg has a pH of between 7–8, but within several days pH rises to between 10–11. This elevated pH substantially decreases the heat resistance of *Salmonella*. Since development of the original heat resistance data for *Salmonella* in egg products, there has been a substantial change in the demand for liquid egg products and an increasing portion of the eggs used come directly from the egg producer. The time between laying and pasteurization has decreased to the point where the pH of egg white is still in the range of pH 7–8. At this pH, *Salmonella* is substantially more heat resistant. Monitoring the CCP for temperature and duration of heating alone would not detect this gradual shift in a key characteristic of the egg. Instead, the egg industry was alerted by an increased incidence of salmonellae observed in periodic samples taken as part of effective verification programs.

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Chapter 14

Aflatoxins in Peanuts

14.1 Introduction
14.2 Risk Assessment
14.3 Risk Management
14.4 Acceptance Criteria for Final Product
14.5 References

14.1 INTRODUCTION

Aflatoxins are the most potent liver carcinogens known, capable of causing cancer in all animal species studied, including birds, fish, and humans. Aflatoxins are also acute, chronic, genotoxic, and immunosuppressive poisons, but these effects are usually considered to be less important.

The primary sources of aflatoxins are the common fungi *Aspergillus flavus* and the closely related species *A. parasiticus*. *A. flavus* is very common in tropical and subtropical regions of the world and is particularly associated with peanuts and other nuts, and with maize and other oilseeds. *A. parasiticus* is primarily associated with peanuts and has a more restricted distribution.

The four major naturally produced aflatoxins are known as B_1, B_2, G_1, and G_2. "B" and "G" refer to the blue and green fluorescent colors produced under UV light on thin layer chromatography plates, while the subscript numbers 1 and 2 indicate major and minor compounds, respectively. *A. flavus* produces only B aflatoxins and only about 40% of isolates from nature are toxin producers. *A. parasiticus* produces both B and G aflatoxins, and virtually all natural isolates are toxin producers (Klich & Pitt, 1988).

Because aflatoxins are extremely toxic, regulatory levels in many countries are very low, ranging from 1 to 25 $\mu g\ kg^{-1}$ (van Egmond, 1989). The Codex Committee on Food Additives and Contaminants (CCFAC) of the Codex Alimentarius Commission (Codex) has recommended to Codex that the limit for foods in international trade be set at 15 $\mu g\ kg^{-1}$ total aflatoxins.

Aflatoxins are readily detected by a very strong fluorescence in ultraviolet light. Analyses may be performed using thin layer chromatography, high performance liquid chromatography, or immunoassays (AOAC, 1984; Nesheim & Trucksess, 1986).

In commodities, especially peanuts, aflatoxins are very irregularly distributed; consequently, aflatoxin determinations must be based on large and representative samples obtained using carefully constructed sampling plans. Samples must be ground or finely divided, then subsampled for assay. Assays based on small samples are unreliable. In more homogeneous products, e.g., peanut butter, this is less of a problem.

14.2 RISK ASSESSMENT

Aflatoxins are among the most potent mutagenic and carcinogenic substances known. Extensive experimental evidence has shown that aflatoxins are capable of inducing liver cancer in most animal species. However, translating that information to human risks has

National Beef Ex. 1028
380

proven to be extremely difficult. Some evidence suggests that humans are at substantially lower risk of liver cancer from aflatoxins than other species. Some epidemiologic studies have suggested that intake of aflatoxin alone poses a detectable risk, but others show that the presence of other factors, such as hepatitis B virus (HBV), are necessary to induce liver cancer.

The identification of hepatitis C virus (HCV) is an important recent advance in understanding the etiology of liver cancer. Epidemiologic studies are largely consistent in showing a strong association between antigens to HCV and the occurrence of liver cancer. The risk linked to HCV is independent of HBV and other risk factors. HCV is likely to be the major cause of liver cancer in countries at low to intermediate risk of liver cancer, such as the US, Europe, and Australia. The epidemiologic evidence of the carcinogenicity of HCV has been reviewed and endorsed by an international group under the leadership of the International Agency for Research on Cancer (IARC, 1994).

Viral hepatitis is a major worldwide public health problem. It is estimated that over 300 million individuals are chronically infected with HBV and perhaps 100 million with HCV. Although the evidence remains inconclusive, it is estimated that 50–100% of liver cancer cases worldwide are associated with persistent infection with HBV and/or HCV. HBV is prevalent in the developing parts of the world, and HCV is emerging as a major cause of hepatocellular cancer in Japan and western societies (Bosch, 1995).

The Joint FAO/WHO Expert Committee on Food Additives (JECFA; [Food and Agricultural Organization/World Health Organization]), which is responsible for assessing the toxicology of chemicals in foods, has summarized this position.

> Risks from specific exposures to aflatoxins are difficult to estimate and predict, despite extensive information available from epidemiological studies, mutagenicity tests, animal bioassays, in vitro and in vivo metabolic studies, and p53 mutation studies. Many questions remain regarding the independence of aflatoxin as a human carcinogen, the extent to which hepatitis B, hepatitis C, and other factors modify the effect of aflatoxin, how findings from countries with high liver cancer rates and high prevalence of hepatitis B may be compared to those from countries with low rates, how to deal with the wide range of susceptibility to aflatoxin carcinogenesis among experimental animals, and how to describe the dose–response curve over the wide range of aflatoxin exposure found worldwide (JECFA, 1997).

JECFA has also reviewed dose–response analyses performed on aflatoxins. All of these analyses suffer limitations, of which three are especially important. First, all of the epidemiologic data from which a dose response relationship can be developed are confounded by concurrent HBV infection. The epidemiologic data are from geographic areas where both the prevalence of HBV positive individuals and aflatoxins is high; the relationship between these risk factors in areas of low aflatoxin contamination and low HBV prevalence is unknown. Second, the reliability and precision of the estimates of aflatoxin exposure in the relevant study populations are unknown. In particular, the biological markers currently used to indicate aflatoxin intake by humans do not reflect long-term aflatoxin intake, and in most cases, analyses of crops for aflatoxins do not take account of reduction in levels of aflatoxins consumed in foods after processing. Third, the shape of the dose–response relationship is unknown, which introduces an additional element of uncertainty when choosing mathematical models for interpolation.

Observations concerning the interaction of HBV and aflatoxins suggest that two separate aflatoxin potencies exist, one in populations in which chronic hepatitis infections are common and the second where such infections are rare. In consequence, JECFA divided potency estimates for analyses based on toxicologic and epidemiologic data into two basic groups, applicable to individuals with and without HBV infection. Despite differences in mathematical models, JECFA found these estimates useful, as a broad range of possible values is covered. Epidemiologic data for which HBV infection status was unknown and for which potencies were calculated were also reviewed and found to be in the range of potencies for HBV infected or uninfected individuals. The recent JECFA review also looked at the extrapolation of animal data to estimate potency in humans; these also generally fell within the range of the potency estimates derived from the epidemiologic data.

To permit discussion of population risks from the intake of aflatoxins, specific potency estimates must be developed and used. JECFA has reviewed the potencies of the aflatoxins estimated from the positive epidemiologic studies and chosen separate central tendency estimated potencies and ranges for HBV positive and HBV negative individuals. Potency values were chosen, in positive individuals, of 0.3 cancers per year per 100,000 population per ng aflatoxin ingested per kg body weight per day with an uncertainty range of 0.05 to 0.5. In negative individuals, potency values were 0.01 cancers per year per 100,000 population per ng aflatoxin per kg body weight per day with an uncertainty range of 0.002 to 0.03.

The fraction of the incidence of liver cancer in a population attributable to intake of aflatoxins is derived by combining aflatoxin potency estimates (risk per unit dose) and estimates of aflatoxin intake (dose per person). In one calculation, JECFA assumed a population with a European diet, from which all samples containing over 20 $\mu g\ kg^{-1}$ aflatoxin had been removed. The mean aflatoxin intake for this population was 19 ng per person per day. Assuming a 60 kg person, the mean cancer risk for that population was 0.004 cancers per 100,000 population per annum.

At the other end of the scale, these figures should be contrasted with those of Lubulwa & Davis (1994), who estimated deaths from aflatoxins in Indonesia, a country of high risk. They used data of Pitt & Hocking (1996) and Pitt *et al.* (1998) on the incidence of aflatoxin in Southeast Asian commodities, and estimated that the liver cancer rate from aflatoxins in Indonesia was 10 per 100,000 population per annum, a rate 1000 times higher than the JECFA estimate for European populations. Peanuts accounted for most of the ingested aflatoxins. Given that the Indonesian population approaches 200 million people, those figures indicate 20,000 deaths per annum from liver cancer due to aflatoxins in Indonesia.

Because some data on contamination levels used in these examples were derived from nonrandom samples (which might be biased upward because studies focused on contaminated lots of commodity) and some data are not likely to be based on current Codex sampling recommendations for aflatoxins, these figures must be used with caution.

14.3 RISK MANAGEMENT

Because aflatoxin is a well known chemical hazard (albeit from a microbial source), risk management has taken a different path from that expected for bacteria or bacterial toxins. In the years following the discovery of aflatoxins, the limits set for aflatoxins in

foods at first amounted to the limit of detection by chemical assay. In importing countries, this was at first 5 $\mu g\ kg^{-1}$, then in some cases reduced to as low as 1 $\mu g\ kg^{-1}$ (van Egmond, 1989). However, it soon became clear that producing countries could not meet such limits: the US set 25 $\mu g\ kg^{-1}$ and Australia 15 $\mu g\ kg^{-1}$ as practical limits that would reduce aflatoxin ingestion as far as possible without destroying the peanut industries in those countries.

Epidemiologic and animal studies that followed established that aflatoxins were genotoxic carcinogens, lacking an apparent no-effect level. Equations for cancer incidence in relation to aflatoxin consumption were developed, based of necessity on epidemiologic evidence, but were not universally accepted because of the probable interaction with HBV and HCV, as outlined above. In consequence, limits continued to be set more on the basis of perceived risk in importing countries or on attainable levels in developed exporting countries. Limits established in other producing countries were seldom met in practice.

14.3.1 Tolerable Level of Risk

As noted above, the development of a tolerable level of risk (TLR) for aflatoxins has proven very difficult, both because a no-effect level has not been established, and because of the interaction, or even synergy, of aflatoxins with HBV and HCV. On the one hand, it could be argued that a TLR logically should be at the limit of detection of cancer in man, say the amount of aflatoxin in the total diet that would induce one case of liver cancer per 10^6 population per annum. On the other, it can be argued that any level of cancer from aflatoxins is too high, so that the TLR might just as logically be lower than that by a factor of 1000. The calculations above made by JECFA, based on European intakes of aflatoxins, would suggest that in practice the actual risk, 0.04 cancers per 10^6 population per annum, lies near the mean of those two hypothetical figures. In contrast, the figures calculated for Indonesian people, 100 cancers per 10^6 population per annum, clearly imply an unacceptable level of risk from aflatoxins.

14.3.2 Food Safety Objective

In the case of a chemical toxin such as aflatoxin, the limits set by a country for aflatoxins in foods can be logically considered also to have the status of a food safety objective (FSO). If it is accepted that a maximum permitted level established within a country is equivalent to an FSO, then by 1990 each major country importing or exporting peanuts had established a *de facto* FSO set, not on the basis of risk analysis, but on more pragmatic approaches.

During the mid-1990s, JECFA and CCFAC carried out a thorough reexamination of the toxicity of aflatoxins, especially in the light of newer evidence on the influence of hepatitis viruses on carcinogenicity of aflatoxins. After protracted discussion, CCFAC recommended to Codex that the maximum permitted level for total aflatoxins in foods in international trade should be 15 $\mu g\ kg^{-1}$. If Codex adopts this recommendation, and it is accepted that the FSO in this case is equal to that limit, then an FSO of 15 $\mu g\ kg^{-1}$ has been established for peanuts in international trade.

This FSO is based on a number of factors. First, statistical analyses of detailed surveys of the levels of aflatoxins occurring in European foods have shown that reduction of the permitted limit to 10 or even 5 $\mu g\ kg^{-1}$ has only a marginal effect on the risk asso-

ciated with aflatoxin consumption in importing countries. Second, it is difficult for producing countries to reliably supply nuts below 15 $\mu g\ kg^{-1}$ to consuming countries. Third, JECFA has stated that the evidence that aflatoxin is a confirmed Class I carcinogen in humans *in the absence of hepatitis B virus* remains inconclusive. The FSO may have to be adjusted downward if conclusive evidence becomes available. The FSO is considered to be technologically achievable by major exporting countries, including the US, China, and Australia, but is currently out of reach of a number of producing countries in the tropics.

14.3.3 Control Measures

Controlling aflatoxins in peanuts is not easy. Under the drought stress conditions that often prevail when peanuts are grown as a dry culture crop, aflatoxins may be produced before the nuts are pulled from the ground. Under these conditions, it is clear that control of aflatoxin formation by measures taken after that point cannot be totally effective. Aflatoxins are also quite resistant to normal food processing, including heating (ICMSF, 1996), so processes used to reduce bacterial or fungal contamination cannot be relied on to remove aflatoxins.

14.3.3.1 Good Hygienic Practices (GHP)/Hazard Analysis Critical Control Point (HACCP) System

Quantitative aflatoxin assays. The primary control measure taken to limit aflatoxins in peanuts in developed producing countries, i.e., the US and Australia, is quantitative assay of representative samples. Although not considered a conventional control point as defined in bacteriological terms, this step is indeed the critical control point (CCP) in controlling aflatoxins in peanuts.

Sampling peanuts for the presence of aflatoxins is very difficult because usually only a small proportion of nuts is infected with *A. flavus*. However, such nuts may contain high concentrations of toxin, so it is essential to use well devised sampling plans to ensure that assayed nuts are representative. In consequence, the primary concern in the implementation of this HACCP strategy is the adequacy of the sampling plan used. Sampling accounts for more than 90% of the variability associated with aflatoxin assays (Whitaker *et al.*, 1994a).

A variety of mathematical expressions have been put forward for suitability for accurate assessment of the distribution of aflatoxins in peanuts (e.g., Jewers *et al.*, 1989; Whitaker & Dickens, 1989; Giesbrecht & Whitaker, 1998), and a variety of sampling plans developed (e.g., Brown, 1982, 1984; Coker, 1989; Read, 1989; Whitaker *et al.*, 1994b). Sampling plans in current use include 3×8 kg samples, 3×21.8 kg, 4×7.5 kg, and 1×10 kg, usually per 10 ton lot (Read, 1989; Whitaker *et al.*, 1995).

The probability of acceptance of lots failing to meet specifications is quite high and inherent in sampling plans (Brown, 1982, 1984; Whitaker *et al.*, 1994a), so it is strongly recommended that all lots of peanuts accepted by manufacturing companies or importers be again assayed on receipt, using appropriate sampling plans. This provides a much higher measure of assurance of acceptability.

Producing countries that export peanuts either carry out their own assays or rely on importing authorities for testing. However, in many producing countries, nuts destined for domestic consumption are sold and consumed at the village level, so testing or regulation of aflatoxin levels is often poor to nonexistent.

National Beef Ex. 1028
384

Color sorting. The second major control measure used for aflatoxin reduction in peanuts in developed countries is color sorting, carried out before the assay step. In this procedure, nuts are inspected individually by electronic or laser sorting systems, and discolored nuts removed. The rationale for aflatoxin reduction by color sorting is that the growth of a fungus in a peanut results in discoloration, so removal of discolored nuts sorts out those containing aflatoxins as well. In the United States and Australia, it is standard commercial practice that every individual shelled peanut entering commercial streams has been color sorted at least once and in some cases up to five times. Color sorting is not a CCP, but rather a major feature of Good Hygienic Practices (GHP).

The color sorting process is sometimes ineffective. This occurs when severe drought stress causes peanuts to commence drying in the soil before harvest, under which conditions growth of *A. flavus* may occur between shrinking cotyledons. Discoloration (and aflatoxin production) is often invisible to color sorters inspecting intact shelled nuts. When this problem occurs, it is common practice to blanch to remove skins, then roast and color sort again. This accentuates the darkening process and facilitates color sorting.

Total control of aflatoxins in peanuts is possible by these combined processes: color sort, assay, blanch and roast, then assay again. However, this procedure is expensive and results in nuts with a limited storage or shelf–life, requiring packaging under inert atmospheres. Much effort continues to be expended on techniques for limiting aflatoxin by other means.

Basic approaches to aflatoxin control include biocontrol by competitive exclusion and genetic engineering, such as incorporation of the gene that codes for chitinase and other enzymes in peanut plants to inhibit fungal infection.

14.3.3.2 Performance Criteria

The performance criterion is to use color sorting and other procedures as necessary to reduce the levels of aflatoxins in peanuts so that assays on representative samples indicate that an acceptable level of < 15 μg kg^{-1} has been achieved consistently.

14.3.3.3 Process Criteria

Farm management practice. Farm management practice has an important role in limiting aflatoxin in peanuts. Management of drought stress by irrigation is the best preventive measure, but most of the world's peanuts are grown under dry culture conditions where irrigation is expensive or impractical. Factors such as weed control, increased plant and row spacing, and any other technique increasing water holding capacity in soils is important. Cultivars of reduced susceptibility to *A. flavus* infection have long been sought, but with little success.

Improved drying practice, especially the use of improved pulling techniques, rapid threshing, and mechanical drying are all valuable measures, but applicability varies widely with farm type. Adequate storage on-farm is essential, a minor problem in developed economies such as the US and Australia, but very difficult to implement in subsistence agriculture in the humid tropics.

Total control of aflatoxin formation in peanuts is impossible with current knowledge, primarily because in bad seasons, i.e., seasons with severe drought stress in the 2–3 weeks before harvest, aflatoxin forms in nuts before pulling from the soil. In regions where dry land farming is practiced and irrigation impossible, good farm management cannot overcome this problem. Hence GHP practiced on-farm can assist in aflatoxin reduction, but not in complete prevention.

The most important good farm practices are:

- maintaining soil moisture by weed control and other appropriate measures;
- harvesting as early as possible to reduce the time and severity of drought stress;
- drying to safe moisture contents (0.70 a_w, equivalent to 8% moisture) as rapidly as possible, either in the field or by mechanical means;
- storing at constant temperatures, in well designed bins, in shade, and with good moisture control, preferably with monitoring of humidity;
- cleaning before transport to shellers to remove shriveled and damaged nuts more likely to contain high levels of aflatoxins.

Shelling plants, which generally control commercial peanut handling after harvest, have good storage practice for shelled peanuts in developed countries, but practice in developing countries often leaves much to be desired. Transport of peanuts also may cause problems due to moisture migration.

Major GHP measures in the shelling plant include:

- random sampling at intake to assess moisture and aflatoxin content. Loads with excess moisture should be rejected and returned to the farm for drying; loads with excess aflatoxin should be segregated.
- careful storage on-farm, with control of insects, temperature gradients, and moisture;
- color sorting after shelling, preferably using instruments that can distinguish more than one color and that can be set to segregate larger or smaller proportions of the nuts, depending on the aflatoxin status of the raw material;
- aflatoxin assays carried out with an adequate sampling plan, preferably on a continual basis;
- as necessary, roasting and blanching before color sorting and reassay;
- storage of acceptable product under carefully controlled conditions until shipped.

14.3.3.4 Monitoring

Monitoring consists of aflatoxin assays to ensure that the processing system in the shelling plant can consistently deliver sorted nuts with $< 15\ \mu g\ kg^{-1}$ total aflatoxins. This system, which provides validation for the GHP/HACCP processes, would be carried out on color-sorted nuts, either continuously from the process stream or using a recognized sampling plan.

14.4 ACCEPTANCE CRITERIA FOR FINAL PRODUCT

14.4.1 Organoleptic

Peanuts are graded on size and color for distribution in international trade. No other organoleptic criteria are currently used for peanut acceptance.

14.4.2 Chemical and Physical

The primary criterion for acceptance of peanuts in international trade is certification of total aflatoxins as $< 15\ \mu g\ kg^{-1}$. No specific assay methods have been internationally approved, but the methods of the Association of Official Analytical Chemists (AOAC, 1984) are a de facto standard. No sampling plans have been internationally recognized to date.

14.4.3 Microbiological

Although well standardized procedures exist for the examination of peanuts for the presence of *A. flavus* (e.g., Pitt & Hocking, 1997), these are unlikely to find a place in international trade.

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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

National Beef Ex. 1028
389

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),

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.

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

National Beef Ex. 1028
392

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., $H_0 = 0$).

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

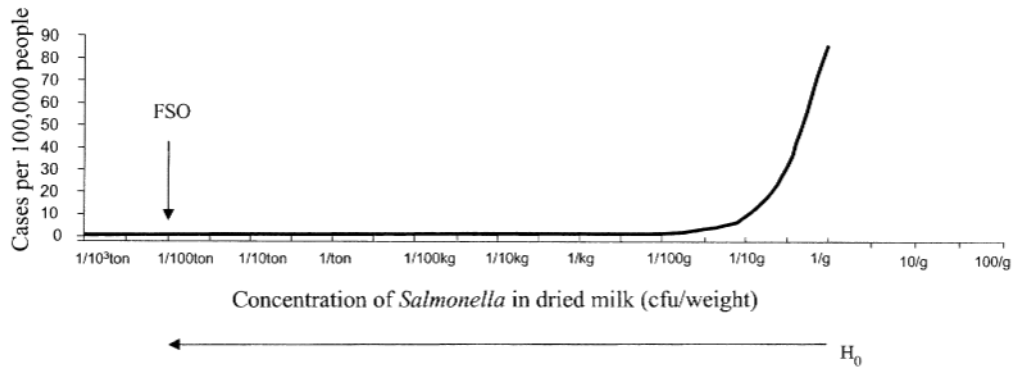

Figure 15–1 Pasteurization of raw milk and preventing recontamination in the factory.

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

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

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.

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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Chapter 16

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,

National Beef Ex. 1028
401