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Industrial Food Microbiology

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KRISHNA NAGAR, DELHI

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CHAPTER 1

FOOD MICROBIOLOGY

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It's your first day of work at a neighbourhood restaurant's kitchen. Only the cooks are dressed in aprons, you note, while the majority of the staff are dressed in casual attire. The manager shakes your hand, washes his hands on his jeans, and requests that you assist to bring in a food delivery to begin. As you can see, there is a lot of fish. Deliveries of poultry, veggies, and dairy goods were left heaped in the scorching heat outside the rear door. You transfer everything to the workspace's big counter. Some of the fruits and vegetables are not even packed, and many of the boxes lack labels. A dog is dozing in the corner of the restroom at the rear entrance. You find there aren't any paper towels in the dispenser after washing your hands. You pat the friendly dog and your jeans dry after washing your hands. Putting the food away is the next task you have. You put the food away wherever you can find room on the refrigerator's shelves, imitating other workers. The sous chef stops by to pick up items for the house specialty, chicken with different veggies, while you cram the broccoli behind a shelf with raw beef. When she pleads for your assistance, you seize the chance.

She motions to a cutting board where she has just finished deboning several birds and asks you to chop some veggies. While someone is warning you about the need of avoiding using the same knife for meat and vegetables, you turn to look around and see that others are wiping their knives on their clothing. One worker sneezes nonstop and keeps wiping her nose with her sleeves. You agree when a chef offers you a sample of his spaghetti sauce. The spoon he used to taste the sauce is the one he gave you. The sauce is mouthwatering. You start to believe that you'll like your career. The folks are welcoming, the ambience is laid-back, and the cuisine is delectable. You start when you wake up and realize that this was all a dream. As you begin to unwind once again, you repeat your most recent dream while applying everything you've learned about food microbiology to it. You gently go back to sleep, reentering the dream as you consider this restaurant and all the live beings you are unable to see –.

Your dirty hands spread faecal germs to the broccoli by touching the dog fur. The unpasteurized apple juice from a nearby farm that you brought in from the food delivery is home to Escherichia coli O157:H7, a faecal bacterium strain that may produce toxins. The undercooked chicken the sous chef used to make the house speciality is developing Salmonella and Campylobacter. You witnessed the dog running around outdoors, people picking up apples off the ground or from an orchard shared with cows, and chicken corpses being washed in blood in your dream, so you know these microorganisms are there. Around the room, fungus and mould are developing between the surfaces, and the black smudges in the corner were made by a mouse that was racing down the wall.

You become conscious of how much you have studied and how much more knowledgeable you are about the unseen world of microbes when your alarm clock awakens you for work.

There are many invisible bacteria all around you, some of which might be harmful disease-causing. If the environmental circumstances become ideal best for these bacteria, they will expand swiftly. There are invisible germs everywhere, including on your hands and clothing,

the room's utensils, the worktops, the floor, the ceiling, the water you drink, and the air you breathe. The study of microbes is referred to as microbiology. Microorganisms, often known as microbes, are creatures that need a microscope to be seen as you might have guessed. Algae, bacteria, fungi, viruses, parasites made up of protozoa and worms, and fungi are all examples of microorganisms. Over 10 trillion human cells and over 100 trillion microorganisms make up the body of an adult person. These microorganisms help us digest our food, share it with others, and recycle nutrients from our waste. While some of these microorganisms defend us, others that may harm us are pathogenic. Our hands, lips, nose, and stomach are all home to germs. Bacteria will settle down wherever they find a welcoming habitat. Our bodies not only contain billions of bacteria, but also many viruses and fungi.

You start when you wake up and realise that this was all a dream. As you begin to unwind once again, you repeat your most recent dream while applying everything you've learned about food microbiology to it. You gently go back to asleep, reentering the dream as you consider this restaurant and all the live beings you are unable to see. The undercooked chicken the sous chef used to make the house speciality is developing Salmonella and Campylobacter.

You witnessed the dog running around outdoors, people picking up apples off the ground or from an orchard shared with cows, and chicken corpses being washed in blood in your dream, so you know these microorganisms are there. Around the room, fungus and mould are developing between the surfaces, and the black smudges in the corner were made by a mouse that was racing down the wall. You become conscious of how much you have studied and how much more knowledgeable you are about the unseen world of microbes when your alarm clock awakens you for work.

Food Microbes

The study of microbes is referred to as microbiology. Microorganisms, often known as microbes, are creatures that need a microscope to be seen as you might have guessed. Algae, bacteria, fungi, viruses, parasites made up of protozoa and worms, and fungi are all examples of microorganisms. There are many invisible bacteria all around you, some of which might be harmful disease-causing. If the environmental circumstances become ideal best for these bacteria, they will expand swiftly. There are invisible germs everywhere, including on your hands and clothing, the room's utensils, the worktops, the floor, the ceiling, the water you drink, and the air you breathe.

View the brief simulation at the following link to get a sense of how the size of microorganisms stacks up against that of other objects and living things. Enter the website and utilise the bottom scroll bar to zoom in and out; be careful since some people report feeling dizzy while viewing. Over 10 trillion human cells and over 100 trillion microorganisms make up the body of an adult person. These microorganisms help us digest our food, share it with others, and recycle nutrients from our waste. While some of these microorganisms defend us, others that may harm us are pathogenic. Our hands, lips, nose, and stomach are all home to germs. Bacteria will settle down wherever they find a welcoming habitat. Our bodies not only contain billions of bacteria, but also many viruses and fungi. A microbiome is a group of bacteria that live in a certain habitat –.

Every individual has a different microbiome, just as each of us has a unique fingerprint. People that live near to one another, such as parents and their kids, will have microbiomes that are more similar but not identical. The people we interact with, the locations we travel through, and the habitats the food itself has been through on its route from farm to fork all have an impact on the composition of our microbiomes.

People may respond differently to medication treatments and some may be more vulnerable to illness than others for reasons related to variances in their microbiomes. There are numerous microorganisms that may infect humans and degrade food, but there are also many more that are helpful and even necessary for the environments and our body's health. Scientists used technological advancements to catalogue the microorganisms that live within our bodies for this study. We learned more from this investigation about the microbial populations that dwell within and on top of us. For instance, these microbes increase the estimated 19 000 human protein-coding genes in the human body by more than 1000 genes. The way we see ourselves in the world has altered as a result of these discoveries.

Currently, antibiotics are often used to combat dangerous germs. Antibiotics, however, may also kill out the healthy bacteria in your body along with the bad, much like spraying an insecticide-filled garden with helpful insects that are then eliminated. If the microbiota can heal is a question that scientists are still trying to answer. Once the antibiotics have done their work, certain bacteria often referred to as opportunistic pathogens move in and may lead to life-threatening disease. Bacteria like Clostridium difficile, for instance, may enter the digestive system after antibiotic therapy difficult is hard to eradicate and may lead to serious intestinal issues.

Probiotics are microorganisms that benefit a person's health. These beneficial bacteria are found in probiotic foods like yoghurt, which contains live Lactobacillus and Bifidobacterium bacteria. It is healthier for human health to have a microbiome with these bacteria than one with germs like difficile, which are known to cause sickness –.

The subject of microbiology is constantly changing, and new findings not only advance our knowledge of the unseen world we live in but might also one day help us control it for the benefit of human health. Scientists may one day be able to modify a person's microbiome to promote health, treat illness, and even battle obesity. Researchers at the University of British Columbia have discovered connections between growing newborns' microbiomes and conditions including obesity and asthma. They have also connected the microbiome's makeup to several neurological conditions, such as multiple sclerosis, Parkinson's disease, and Alzheimer's disease.

GRAS, Microbial Safety

The Federal Food, Drug, and Cosmetic Act (FDCA) were amended to include the term "Generally Recognized as Safe" (GRAS) as a foundation for US regulatory compliance in 1958. A food additive is any material that might potentially end up in food or that alters how food behaves in some other way. The U.S. Food and Drug Administration (FDA) requires premarket clearance for food additives unless the chemical is deemed GRAS by specialists qualified by scientific training and experience to assess the substance's safety in the intended usage. The GRAS assessment depends on the substance's safety being sufficiently shown by scientific methods or by experience based on widespread usage in food before 1958, in the circumstances of its planned use. The terms "qualified expert" and "general recognition" are crucial parts of this regulatory text. A technical foundation for concluding that the technical evidence of safety is widely recognised and accepted is required to declare a substance GRAS. The facts and information used to determine safety must be publicly accessible, and competent experts must agree that the chemical is safe for usage as intended.

A total of 16 stomacher homogenates (SH) were made as ten-fold serial dilutions in BPW, followed by quantitative bacterial isolation in duplo: Total aerobic bacteria were isolated following ISO 4833 part 2 on Plate Count Agar; total anaerobic bacteria were isolated following ISO 15214 on De Man, Rogosa, and Sharpe medium (MRS, 3564244, Bio-RAD),

incubated at 30°C for 48 h; and lactic acid bacteria (LAB) were isolated following ISO 15214 on PCA, as described above, but, on PCA, colonies having a bacterial growth of between 30-300 CFU/plate and between 15-150 CFU on all other plates were enumerated. A portion of each colony was taken from all PCA and other selective isolation plates chosen for counting, pure-cultured on Trypticase Soy Agar (TSA, CM0131, OXOID), or MRS, and incubated as previously.

The leftover bacterial growths after picking on the PCA plates, as well as on the equivalent PCA plates infected with one dilution higher, were recovered using a plate washing approach to identify the bacterial population with a previous culture by 16S rRNA amplicon sequencing. For this, the remaining bacterial mass on the three PCA plates (aerobic 7°C, 30°C, and anaerobic, each condition including two plates) as well as the total biomass from the same PCA plates but one dilution higher (PW+1), if available, were harvested from the PCA plates of the eight selected chicken carcasses (C1 to C8). To do the subsequent 16S rRNA amplicon sequencing 5 ml of BPW was applied to each agar surface, the biomass collected from 2 plates was resuspended using a cell scraper, and each sample was pooled into a single centrifuge tube.

After the Food Additive Amendment of the FDCA was passed in 1958, the FDA published a list of GRAS ingredients, which is now codified in the Code of Federal Regulations Part 182, and in 1972 it established, by regulation, a GRAS affirmation process, which allowed the industry to request the agency to make a GRAS determination. According to the regulation, a company may submit a GRAS Notice to the Agency, and after evaluation, the FDA will publicly post its answer in a letter. The complete list of GRAS Notices that the FDA's Center for Food Safety and Applied Nutrition (CFSAN) has received is available online. The GRAS Notice Inventory is the name of the list kept by CFSAN. 5 Since the FDA received its first GRAS notification in 1998, information regarding GRAS Notices submitted since that time is available in the inventory of GRAS notices. The content of the FDA's response letter is made accessible as part of the record for a GRAS Notice after FDA has responded to it. FDA occasionally updates this data throughout the year. Through 2015, CFSAN received over 600 GRAS Notices, with over 14% of the notices being on food enzymes. The graph shows that food enzyme GRAS Notices regularly fulfil the GRAS standards and that there are very few instances in which the FDA finds the notifier's GRAS assessments to be unsupported or the notifier withdraws them.

Factors That Influence Microbial Growth

When determining whether a food or category of foods requires time/temperature control during storage, distribution, sale and handling at retail and in food service to ensure consumer protection, the factors discussed in this section serve as an inclusive, rather than exclusive, list of intrinsic, extrinsic, and other factors that may be taken into account. When determining if a given meal requires time/temperature control for safety, several considerations must be taken into account. Intrinsic and extrinsic influences may be distinguished among these. Extrinsic variables are those that relate to the environment around the meal, while intrinsic factors are those that are characteristics of the food itself. The need for time/temperature management is mainly based on the risk of contamination with pathogenic microorganisms of concern the risk of future growth and/or toxin generation.

The majority of authorities are likely to classify foods into one of three groups based on their assessment of the following factors: those that do not require time/temperature control for the protection of consumer safety; those that do require time/temperature control; and those whose exact status is ambiguous. Additional scientific data, such as simulations of microbial

development or demise or real-world studies of microbiological challenges, may be used to support the conclusion in the case of dubious items.

Internal Variables

To proliferate in food goods, microorganisms need water in an accessible form. One of the first used preservation techniques is the regulation of the moisture level of foods. The water activity (aw) of the food or environment is a common way that food microbiologists characterise the water needs of bacteria. Water activity is defined as the difference between the water vapours pressure of the food substrate and the water vapour pressure of pure water at the same temperature aw = p/po, where p is the solution's vapour pressure and po is the solvent's vapour pressure usually water.

A dehydrated meal has an aw of 0.00 whereas pure water has a value of 1.00. The equilibrium relative humidity above the food is connected to the aw of a meal on this scale from 0.00 to 1.00, which ranges from 0 to 100%. Equilibrium Relative Humidity (ERH) = aw x 100, as a result. The AW of a food indicates how much water is "bound" in the food, how much water is available for chemical and metabolic processes, and how much water is available to support microbial development.

The majority of fresh foods, including fresh meat, vegetables, and fruits, have aw-values that are rather near to the threshold required for most microbes to flourish (0.97 - 0.99). Foods may have their water content changed in a variety of ways, for as by adding solutes like salt or sugar, physically removing water by drying or baking, or binding water to different macromolecular components. These dietary elements will lose weight for weight in the sequence shown below: Ionic compounds are preferred over sugars, polyhydric alcohols, amino acids, and other low-molecular-weight substances. High-molecular-weight substances like cellulose, protein, and starch are also not preferred.

Depending on a variety of variables, microorganisms react to aw in various ways. The generation of microbial metabolites and, in certain situations, microbial growth may be highly sensitive to changes in aw. Depending on other growth factors in their surroundings, microorganisms often have optimal and minimal amounts of aw for development. The taxonomic categorization of the bacteria is one sign of their reaction. Gram (-) bacteria, for instance, are often more susceptible to low aw than Gram (+) bacteria.

It is important to note that only aureus can thrive and manufacture toxins at water activity below aw 0.90, whereas numerous bacterial pathogens are controlled at levels considerably above 0.86. The capacity of different solutes to inhibit microorganisms at the same aw value makes it important to stress that these are just approximations. For instance, the lower aw limit for the development of Clostridium botulinum type A was discovered to be 0.94 with NaCl as the solute vs 0.92 with Glycerol as the solute. Microbiological challenge testing may be used when producing foods that utilise aw as the main pathogen control mechanism to ensure that the lowered aw is still effective even when the target aw is close to the organism of concern's growth limit , .

Other methods may provide more accurate moisture monitoring for certain items since aw limits vary with various solutes or humectants. For instance, it is known that variables other than aw regulate the antibotulinum effects of pasteurised processed cheese spreads. To inhibit infections in certain food items, aw may also be employed in conjunction with other elements. Analyzing meals with many components requires caution since accurate measurements of aw could not accurately capture the value at the interface between the components or in a microenvironment. In these situations, it is important to evaluate the aw at the food's interface regions as well as in any relevant microenvironments.

Survival and Decline in Foods

Because survival and fertility are assumed to increase when food is more plentiful, food availability is a crucial component in population dynamics and life-history development in wild populations. Because food abundance interacts with other ecological parameters, the precise processes by which it affects survival and reproduction are yet unknown. Because animals are often more susceptible while foraging and a high food supply allow for a decrease in the time spent foraging, for instance, an increase in food availability is likely to lessen the danger of famine but also influences exposure to predators. As a result, an increase in food supply might have a significant impact on survival via an impact on the rate of predation, with the rate of famine having a minor impact.

As an alternative, increasing the food supply may raise the local density of conspecifics, which would lead to a rise in the number of predators and a consequent rise in the per capita predation rate. The discovery that dietary restriction often promotes survival and longevity in experimental animals supports the idea that the relationship between food availability and survival is complicated. A recent meta-analysis found that food supplementation in wild populations had no discernible impact on survival, perhaps as a result of a combination of these factors. Thus, at least within the spectrum of food availability where animals typically exist, it is unclear to what degree food abundance in isolation influences survival in wild populations. It is regrettable since, in our rapidly changing environment, knowledge of the processes mediating demographic consequences of food availability may be necessary to forecast such effects. To address this problem, experiments must be conducted. To that end, we provide the findings of a lengthy, large-scale study in which we examined the impact of food availability on longevity and ageing in Taeniopygia guttata zebra finches. We changed food availability in a manner that resembles natural variation in food availability, namely by adjusting foraging costs, which are here defined as the effort necessary to collect a unit of food. This is a key component of our strategy. Animals could, as they do in the wild, increase their foraging efforts in response to less food being available. The effects of food availability on adult survival may be directly ascribed to food availability since there were no predators and controlled densities in the foraging cost manipulation in adulthood.

Interesting demographic effects may result from individual variation and individual heterogeneity in sensitivity to environmental influences like food quantity. All ages are affected by environmental variables, but environmental circumstances during development are regarded to be especially significant in defining individual variation in lifespan and, more broadly, adult health. When the consequences rely on adult environmental situations, the relationship between developmental conditions and longevity might be complicated. According to the "silver spoon theory", for instance, phenotypes from benign prenatal environments may produce high-quality phenotypes that survive hard conditions in adulthood better than phenotypes from harsh developmental situations.

The match-mismatch theory, on the other hand, contends that environmental difficulties encountered during development may help people deal with comparable difficulties encountered throughout maturity, but a mismatch may lead to health issues. Last but not least, some stresses encountered during development may aid the emergence of adult tolerance to similar stresses. Since animals in a (homogeneous) environment or just high-quality adult environmental settings such as standard laboratory conditions have been examined in experimental investigations of developmental impacts on longevity, it is impossible to differentiate between the two. Such a test necessitates the independent management of the environment both throughout development and in maturity in a crossover design, which often calls for a laboratory setting. There aren't many of these tests and the

ones that do have mostly been unsuccessful. However, all of these research used species with cyclical growth and/or developmental periods. In ways that are not possible for animals with species with deterministic growth, such as birds and humans, such developmental patterns boost the chance to offset the consequences of severe developmental environments. Thus, for animals with determinate growth, it is uncertain how much longevity is influenced by match-mismatch effects as opposed to silver spoon effects.

Here, we provide the findings of an investigation into the impact of foraging costs on longevity and senescence both during development and as adults. The zebra finch is an appropriate species because of its predictable growth and because developmental circumstances have been shown to have an impact on the phenotypic in ways that are crucial for adult health and longevity. Cross-fostering chicks to either small or big brood allowed us to control foraging costs throughout development, which in a sense raises "foraging costs" since chicks must be more for each piece of food reward. In maturity, we raised the flight costs per food reward experimentally, and individuals were kept under these circumstances until they died naturally.

A change in the (age-independent) baseline mortality rate vulnerability to the ageing process and/or a change in the age-dependent mortality rate (actuarial senescence or "ageing rate" may lead to variations in longevity when the age at death follows a Gompertz distribution. Given that these impacts are probably due to several biological processes, it is instructive to determine which parameter is altered when there is a change in a lifetime. So, we divided our data analysis into two parts. First, we used Cox proportional hazard (CPH) analysis to identify treatment effects on longevity. Next, by fitting the Gompertz mortality function, we assessed the contributions of variations in age-independent and age-dependent mortality to the observed longevity differences. We investigated the impact of foraging costs on longevity and the variables describing the mortality trajectory throughout development and maturity.

Randomly paired birds were kept indoors on a 14 L: 10 D schedule in a cage (L H D: 80 40 40 cm) with a nest box and nesting material at a temperature and humidity of around 25°C (hay). There was unlimited access to drinking water, sepia, and a commercial tropical seed blend. To prevent potential diet variation between birds raised in large and small broods, no "eggfood" was given during the nestling phase. Instead, a teaspoon of fortified canary food, or "eggfood," manufactured by Bogena, Hedel, the Netherlands, was given three times a week until the first chick hatched. All of the chicks in a brood were cross-fostered at random to small and big broods when the oldest chick was around four to five days old. We experimentally produced small broods (89% had two chicks and 11% had three) and big broods (80% had six, 7% had five, 9% had seven, and 4% had eight). These brood sizes fall within the range that has been noted in the wild.

The effort needed per unit of food was greater in big broods as compared to small broods, according to behavioural observations, which revealed that birds in large broods had to beg the parents for more food during each feeding session. Young birds were placed in bigger indoor cages with up to 40 additional young of the same sex and two male and two female adults for sexual imprinting from the age of 35 days until roughly 120 days after the birds were ringed.

In line with other studies, growing up in a big brood resulted in 1.4 g (12%) reduced mass at the age of 15 days, or right before fledging. Estimates of the long-term impacts of developmental circumstances may be skewed by the selective disappearance of low-quality individuals, which reduces variability, although survival between cross-fostering and age 120 days was high and unaffected by the number of siblings after cross-fostering.

Quality and Safety indicators and microbiological Criteria

Microbiological standards have been established in cases when risk analysis has shown a relatively high risk of food-borne disease connected with the intake of a particular item and a standard might help minimise the hazards. When a standard's rationale could not be identified, guideline criteria were created. These requirements for guidelines are meant to supplement existing risk-management tactics used by the government and businesses. These requirements must be met to identify undesirable microbiological contamination of food and to start corrective action when the limits are exceeded. Failure to achieve recommended levels often indicates a flaw in the process or hygiene measures, necessitating action to find the source and fix the issue.

Microbiological Standards

A criterion is a benchmark that may be used to make a judgement or conclusion. A microbiological criterion will state that a particular kind of microorganism, a particular group of microorganisms, or a toxin produced by a microorganism must either not be present at all, be present in a small number of samples, or be present in a quantity of food or food ingredient below a certain threshold.

Elements of a Microbiological Standard

The following criteria need to be included in a microbiological standard:

1. A description of the food's composition
2. A description of the potential contamination, such as the microorganisms or collection of microorganisms, toxin, or another agent
3. The analytical technique to be utilised for the pollutant of concern's detection, counting, or quantification
4. A sampling strategy
5. The microbiological tolerances were deemed suitable for the product and consistent with the sampling strategy.

There is a certain amount that the meal is not allowed to go above. Food that does not fit the criteria must be put through some kind of action, such as being rejected by the buyer, being destroyed, being reprocessed, being marketed as lower quality, or being redirected to a usage where contamination is not an issue. If limitations are continuously exceeded by certain necessary criteria, the licence to prepare food may be lost. Advisory criteria often act as a warning sign for processing, distribution, storage, or marketing errors. Although they are not required, they allow judgement to be used when the restrictions are not satisfied.

An administrative rule, statute, or ordinance that includes microbiological criteria is known as a microbiological standard. A standard is a condition that must be met. It is a breach of the law, ordinance, or regulation and will be dealt with following the enforcement guidelines established by the regulatory body with authority.

The food sector or a regulatory body often utilises microbiological criteria to keep an eye on a production process. To indicate if the microbiological conditions present at crucial control points or in the completed product are within the usual range, guidelines serve as alarm mechanisms. They are thus utilised to evaluate processing effectiveness at GMP-compliant key control points. Advisory criteria, a microbiological guideline prevents a specific lot of food from being pulled off the market or even downgraded if it exceeds the limit for non-pathogenic organisms. However, guidelines may be required in the sense that management of

food companies and regulatory bodies may demand that the circumstances causing recurrent microbiological inadequacies to be immediately remedied.

A microbiological specification is a microbiological criterion that is employed as a purchasing requirement, making compliance with it a necessity for the sale of a product or ingredient between the customer and seller. Microbiological specifications might be required or recommended. Microbiological Standards for Sample Acceptance or Rejection The food that must adhere to the microbiological standards established for that food, the microorganism or group of microorganisms of concern, the number of sample units to be collected and tested, the level of microorganisms deemed acceptable, marginally acceptable, or critical (depending on the sampling plan specified), and the number of samples that must adhere to these standards are all listed.

Sampling strategy and interpretation: The following words are defined and used in the creation of FSSAI standards for various milk and milk products following the International Commission on Microbiological Specifications for Foods (ICMSF) is the number of sample units from a batch or lot of food that must be inspected to meet the specifications of a certain sampling plan. The maximum permitted number of flawed sample units is given by c. This is the maximum number of sample units allowed before reaching the m-specified microbiological limit. If the findings did not go above M's limit, they are regarded as slightly acceptable –. The sampling strategy rejects the lot if there are more than this number discovered.

In terms of the sample design, values above m = Represent an acceptable level and are only marginally acceptable = A microbiological parameter that distinguishes between subpar/possibly dangerous quality and barely acceptable quality. Following the sampling strategy, values over M are undesirable, and the lot would be rejected if one or more samples were found to have values above M.

No more than 2 units (c = 2) from a lot or consignment should exceed the maximum tolerance (m) for microbiological levels stated in the reference criteria, and no more than 1 unit should exceed the level for the maximum tolerance when 5 or more units of the same variety from a lot or consignment are analysed (n = 5). Indicators of microbiology and their interpretation: Based on the total plate count, the levels of indicator organisms (Coliform count, yeast & mould count), and the quantity or presence of pathogenic bacteria, three categories of microbiological quality have been designated in the standard. These are acceptable, unacceptable, and maybe dangerous. A maximum c/n value between m and M, together with the other values observed being m, is considered satisfactory. This indicates that the findings are within the bounds of acceptable microbiological quality and that no further action is necessary.

Unacceptable: Results outside acceptable microbiological limits linked with hygiene indicators Total plate count, Coliform count, and Yeast and mould count) and are indicative of poor hygiene or poor handling practises if one or more values observed are >M or more than c/n values are between m and M. Under these circumstances, the facilities producing such subpar goods must cease production until thorough investigations are conducted for nonconformity/noncompliance during manufacture. Only once the HACCP/GMP audit of the premises has been cleared by the food safety authorities and the fresh product has complied with the required limitations can the manufacture of such a product be restarted –.

Potentially dangerous: If one or more of the observed values are >M or more than c/n values are between m and M, this indicates that the results are outside of acceptable microbiological limits linked to pathogenic bacteria and that immediate corrective action

should be taken. The relevant food safety authorities will take enforcement or legal action in response to such findings. Removing any food that is still on the market or in circulation, and, if necessary, issuing a recall. To start taking corrective action, an inquiry into food manufacturing or handling techniques must be conducted to identify the possible problem's source or origin. A thorough risk analysis must be completed as well. When an officer has cause to think that a product is infected with harmful germs, the owner must either stop manufacturing it or remove it from the sale or recall it after being asked to do so.

CHAPTER 2

FOODBORNE PATHOGENS

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Consuming food contaminated by bacteria and/or their toxins, parasites, viruses, chemicals, or other agents may result in foodborne disease, often known as food poisoning. The federal government estimates that there are around 48 million instances of foodborne disease each year, even though the American food supply is among the safest in the world. According to this projection, 1 in 6 Americans will become sick from eating tainted food, which would cause 128,000 hospital admissions and 3,000 fatalities.

People become sick by eating or drinking things that are contaminated with germs, chemicals, or poisons. Age and a weaker immune system are only two of the variables that might affect the severity of food poisoning symptoms. The FDA's Coordinated Epidemic Response and Evaluation (CORE) Network closely collaborate with local, state, and federal partners as well as the Centers for Disease Control to determine the source of the outbreak and stop the spread of disease. When required, the FDA collaborates with food companies to assist in voluntary recalls of potentially contaminated items; the FDA Food Safety Modernization Act also gives the agency statutory recall powers (FSMA).

In the current investigation, the bacterial diversity was evaluated in addition to the degree of bacterial contamination. Pseudomonas consistently emerged as the dominating genus in TAB, regardless of the incubation temperature used, and this was also validated using a method that was independent of culture. Pseudomonas has already been described as a prevalent genus on chicken products, however, the findings of the current investigation call into question the added utility of searching for potential Pseudomonas species on selective plates. Despite the intricate and unclear taxonomy of the genus, pseudomonas is thought to be an indicator bacterium for rotting.

As seen in the current work, unambiguous species identification is still challenging even with the use of MALDI-TOF MS and 16S rRNA gene sequencing. According to MALDI-TOF MS results, the species Pseudomonas extremorientalis, Pseudomonas fluorescens, and Pseudomonas fragi are generally regarded as having credible identifications (score > 2.3) and are consistent with earlier determinations. In the research, in which chicken drumsticks were inspected and identification was carried out using V1-V3 amplicon sequencing, Brochothrix, the second most prevalent species, was reported as the most dominant genus. Intriguingly, Brochothrix was discovered to be the dominating genus in the current study's stomacher homogenate sample group analysis utilising the Qiagen extraction kit, even though this species was less prevalent on isolation plates than Pseudomonas. These results suggest that culture techniques, DNA extraction, and molecular analysis all appear to affect the outcome. Pseudomonas and Brochothrix surpassed more than 80% of the total bacterial isolated growth, independent of the incubation temperature, making it difficult to distinguish between "mesophilic" and "psychrophilic" bacterial flora using present methods. Only PCA plates with an incubation temperature of 7°C showed Psychrobacter in a small percentage of samples. It needs extra 16S rRNA gene sequencing for the MALDI-TOF MS to identify the 14 PCA plates that were treated at 7°C using 16S rRNA amplicon sequencing. There are just

two strains of Psychrobacter in the Bruker commercial library (Psychrobacter lutiphocae and Psychrobacter sp.), and this library does not seem to be able to recognise the broiler isolates, not even at the genus level.

There have been reports that the Lipid and Acyl structure, which is the outer membrane of certain Psychrobacter species, might vary depending on the incubation temperature, perhaps leading to a changed spectrum, as a possible explanation for this. The Bruker database indicates that incubation at 37°C was used, as opposed to 30°C in the current work. Although 16S rRNA amplicon sequencing revealed that 0.7% of the total sequences found using the analytical methodologies we used might be attributable to this species, the genus Photobacterium was also not identified by MALDI-TOF MS. The contamination level of C1 to C4 is at least 1 log lower than C5 to C8, taking into account the association between initial contamination level and bacterial diversity. Even when lower dilutions were considered using plate-washing amplicon sequencing, the predominant bacteria and overall diversity was comparable throughout the samples. In terms of food safety, only Yersinia enterocolitica, a possible foodborne pathogen, was isolated on the general counting plates and was also discovered by 16S rRNA amplicon sequencing analysis; further virulence investigation was not carried out.

Toxigenic Molds

While mould is almost always present and generally not hazardous, there are several varieties of mould or fungus that, if present in your house, may seriously endanger the health of you and your family. Here are some of the most typical types of poisonous mould that may develop inside houses anywhere there are moist areas or water damage, along with some advice on what to do if you suspect your house may have dangerous mould.

Stachybotrys

Stachybotrys, sometimes known as "black mould," is one of the most harmful species of mould and may lead to severe respiratory problems, flu-like symptoms, diarrhoea, migraines, and memory loss. Children are much more in danger of acquiring health issues from exposure to black mould since their lungs are still growing. Black mould is as dark as its name would imply, yet it may also be grey or dark green. It is generally found in very moist sections of houses and other structures and has a strong musty or mildewy stench –.

Chaetomium

Chaetomium flourishes in moist, dark settings like drywall, wallpaper, baseboards, and carpets, hence it is most often seen in houses with water damage. This kind of mould resembles black mould, and in certain cases, they even live in the same surroundings. Chaetomium spores may result in brain damage and several autoimmune illnesses in addition to the typical symptoms of allergies including red, watery eyes and breathing difficulties.

Aspergillus

Although aspergillus spores may be found in the air that many of us breathe daily, those who have compromised immune systems may be at risk from this form of mould. Lung infections, allergic responses, and the particular illness known as aspergillosis are all possible side effects of aspergillus exposure.

Penicillium

Similar to aspergillus, penicillium is widespread in many common places, but if it becomes trapped inside your house, it may lead to respiratory issues including bronchitis and other symptoms of asthma.

Fusarium

The mould fungus known as fusarium is most often found in plant waste and soil, but it may also be found in other wet locations of your house, including under carpeting, within drywall or foam insulation, and in humidifier pans and HVAC units. Exposure to fusarium spores has been linked, albeit rarely, to a severe eye illness called fusarium keratitis –.

Alternaria

Alternaria, a well-known mould that causes allergies, is prevalent in large numbers in various temperate zones throughout the spring and summer. Although this sort of mould is often not found in construction materials, it may be present in indoor air as well as in cardboard, canvas, and other fabrics, as well as in electrical wires.

If you suspect that your house may contain hazardous mould, you must get expert assistance since toxic mould exposure poses a major health risk. Have your house properly tested for mould as the first step. This should include a thorough examination of your property to look for any water damage or mould development, as well as an air quality test to look for airborne spores. Usually, it takes an hour or less to complete this procedure.

To ascertain if there are dangerous mould species in your house, samples will next be submitted to an environmental testing laboratory. If necessary, you may next contact a mould remediation business to get rid of the hazardous mould in your house and identify any undiscovered sources of water damage. A clearing air test may be carried out after the mould has been removed to make sure the mould levels in your house are safe.

Control of Microorganisms in Food

Humans have utilised numerous physical microbial control techniques for food preservation for thousands of years. High temperatures, radiation, filtration, and desiccation (drying) are a few examples of common control techniques. By rupturing membranes, altering membrane permeability, or destroying proteins and nucleic acids via denaturation, degradation, or chemical modification, many of these techniques kill cells in an unintended manner. This section describes many physical techniques for microbial control.

Heat

One of the most popular and traditional methods of germ control is heating. It is used in straightforward processes like canning and frying. By changing microbial membranes and denaturing proteins, heat may kill microorganisms. The lowest temperature at which all microorganisms perish after a 10-minute exposure is known as a microorganism's thermal death point (TDP). Some microbes are more heat tolerant than others when it comes to how they react to high temperatures. The time required to kill every microbe in a sample at a certain temperature is referred to as the thermal death time (TDT), which is a comparable metric. When describing sterilising processes that make use of high heat, such as autoclaving, these characteristics are often employed. One of the first moist-heat management techniques for microorganisms is boiling, which normally kills vegetative cells and certain viruses rather well. Boiling, however, does not destroy endospores; certain endospores may withstand boiling for up to 20 hours. Additionally, boiling can be less successful at higher elevations because water has a lower boiling point and requires a longer boil time to kill germs. Boiling is not regarded as an effective sterilising method in a laboratory or clinical context due to these factors –.

In the laboratory or clinic, a wide variety of heating protocols may be employed for sterilisation. These protocols can be divided into two primary groups: moist-heat sterilisation and dry-heat sterilisation. In the laboratory, an aseptic method often entails some direct

application of high heat for dry-heat sterilisation techniques, such as sterilising inoculating loops. All microbes are destroyed by incinerating at very high temperatures. A dry-heat steriliser, such as an oven, may also be used to apply dry heat for relatively long periods at least two hours at temperatures as high as 170 °C. However, since moist heat penetrates cells more effectively than dry heat, moist heat sterilisation is often the most successful method.

Autoclaves

In autoclaves, moist heat sterilisation is used. To sterilise goods like surgical equipment from vegetative cells, viruses, and notably endospores, which are known to withstand boiling temperatures, they are heated above the boiling point of water. The modern autoclave was created in 1879 by Charles Chamberland (1851–1908) when he was a researcher in Louis Pasteur's lab. The autoclave is still regarded as the most efficient way to sterilise things. Broad industrial autoclaves called retorts enable moist-heat sterilisation on a large scale outside of laboratory and clinical settings.

As more and more steam is trapped within the autoclave's enclosed chamber instead of the evacuated air, the internal pressure rises and the temperature rises over the boiling point of water. The method used to remove air from the chamber varies between the two primary kinds of autoclaves. Steam is supplied into the chamber from the top or sides in gravity displacement autoclaves. Since air is heavier than steam, it sinks to the chamber's bottom and is then blown out via a vent. Longer cycles may be necessary for such weights since complete air displacement is difficult, particularly for bigger loads. Before adding steam to the chamber, the air is evacuated from it using a high-speed vacuum in pre-vacuum sterilisers. The steam may more readily permeate wrapped goods since air is removed more thoroughly. Many autoclaves can perform both gravity and prevacuum cycles; the former is used for waste decontamination, media sterilisation, and unwrapped glassware treatment, while the latter is used for packed instrument sterilisation.

A representation of an autoclave. A big metal cylinder contains a pressure gauge. Steam from the jacket may enter the chamber via an operational valve, and a safety valve is also present. An ejector valve, a waste line, an exhaust valve, a steam supply line, and a pressure regulator are all connected to the main chamber a picture of an autoclave, which is a large metal box that stands as tall as the person operating it. Depending on the amount and kind of material being sterilised, the exposure period will vary, but it will usually be 20 minutes or greater. Larger volumes will need longer exposure durations to provide enough heat transmission to the items being sterilised. Instruments are wrapped loosely in paper or foil and containers are left partially closed because the steam must come into direct contact with the liquids or dry items that need to be sterilised. To completely sterilise items using an autoclave, the temperature has to be high enough to destroy endospores.

Quality control is crucial since sterilisation is crucial to safe medical and laboratory practices. Recorders for keeping track of the pressures and temperatures reached during each run may be included in autoclaves. Along with the items to be sterilised, internal indicators of different kinds should also be autoclaved to guarantee that the correct sterilisation temperature has been attained. The use of heat-sensitive autoclave tape, which has white stripes that become black when the proper temperature is reached after a successful autoclave run, is one frequent kind of indication. This kind of indication may be utilised during every run and is reasonably priced. Autoclave tape, however, does not indicate the duration of exposure, hence it cannot be used as a sterility indicator.

To ascertain if the endospores of Geobacillus stearothermophilus are destroyed by the procedure, a biological indicator spore test employs either a strip of paper or a liquid solution

of the endospores. The gold standard utilised for this purpose is the endospores of the obligatory thermophilic bacteria stearothermophilus due to its great heat tolerance. Additionally, employing stearothermophilus, Bacillus atrophaeus, subtilis, or pumilus spores, biological spore indicators may assess the efficacy of additional sterilisation techniques such ethylene oxide, dry heat, formaldehyde, gamma radiation, and hydrogen peroxide plasma sterilisation. The endospores are incubated after autoclaving in the case of verifying autoclave function to make sure no live endospores are left.

Biological indicator spore assays that look for acid metabolites or fluorescence generated by enzymes originating from live stearothermophilus may be used to track bacterial development after endospore germination. The Diack tube, a glass ampule holding a temperature-sensitive pellet that melts at the right sterilising temperature, is a third kind of autoclave indication. Spore strips or Diack tubes are used on a spore-by-spore basis to check the autoclave's operation.

Useful Microorganisms in the Food Industry

Microorganisms are crucial to the food business. They are utilised in the manufacturing of a variety of food items and are also in charge of food spoiling, which results in infections and intoxication, as was previously covered in the preceding chapter Contributions of Microbiology in the Food Industry. The majority of the time, food items get contaminated by microbes while being processed, stored, transported, or distributed, or even just before consumption microbial development and food spoilage. The growing conditions offered by various food sources vary for microorganisms. Inherent variables including nutrients, pH, moisture content, and the physical makeup of the food, as well as external elements like temperature, relative humidity, and gases, regulate microbial development (CO_2, O_2).

Thus, given the ideal environments created by the internal and external elements, microorganisms flourish and cause the food product to deteriorate and degrade, resulting in a sour, rancid-smelling, or fungus-covered mass that is inedible. Additionally, microbial development in food may result in outward modifications including colour changes, the deposit of powdery growth, effervescences on the food surface, etc. Food may get contaminated by microbes at any stage of the manufacturing process, including growing, harvesting, transit, storage, and final preparation. Foods that are not kept correctly may also get spoiled. Protein- and fat-rich meat and dairy products provide a perfect habitat for microbial deterioration, which leads to proteolysis and putrefaction of the food items. Fruits and vegetables deteriorate in a different way than meat and dairy products because they contain significantly less protein and fat.

The Food Industry's Uses of Microorganisms

There are already around 3500 traditionally fermented foods available worldwide. They are a staple of our everyday lives and may be either animal or vegetable based. Not only are alcoholic beverages fermented; but following harvest, cocoa beans, coffee grains, and tea leaves are also fermented to produce their distinctive flavour characteristics.

Bacteria

The majority of unicellular microbes are bacteria. Cocci, spherical cells, bacilli, cylindrical or rod-shaped cells, and spiral or curved forms are the three categories into which the morphologies of medically significant bacteria are categorised. Though most pathogenic or disease-causing bacteria are gram-negative, three gram-positive rods have been linked to food poisoning, including Clostridium botulinum and C. difficile.

Other common bacteria that cause food to spoil, infect people, and cause disease include Acinetobacter, Aeromonas, Escherichia coli, Proteus, Alcaligenes, Flavobacterium, Pseudomonas, Arcobacter, Salmonella, Lactococcus, Serratia, Campylobacter, Shigella, Citrobacter, Listeria, Staphylococcus, Micrococcus, Corynebacterium. Additionally, diverse food and dairy products are produced using various bacterial strains. Erwinia, Lactobacillus, Bifidobacterium, and Streptococcus strains, among others, are used in the creation of dairy and fermented food items. Yoghurt is made using Streptococcus thermophilus and Lactobacillus bulgaricus.

Food and Agriculture Analytical Bacteriology

Microorganisms are studied, applied to, and used in analytical microbiology as reagents for the quantitative analysis of specific chemical substances. These processes are dependent on how a certain microbe responds to its surroundings. This analytical technique for the quantitative assessment of the substance may be developed to meet the needs of food culture, fermentation, or preservation if a microbe responds to a specific chemical entity with a measurable reaction and produces an acceptable result.

Detection of Food Borne Pathogens and Toxins

Foodborne illnesses have considerably grown in frequency over the last 20 years, and as a result, they have emerged as a serious global public health issue. Although it is difficult to quantify the worldwide prevalence of foodborne infections due to some cases being under-reported, particularly in poorer nations, the incidence of these illnesses has been on the rise in many regions of the globe. For instance, in Taiwan, the incidence of a foodborne illness outbreak climbed quickly from 121 in 1995 to 177 in 1996, and it has continued to rise since then. Despite having the world's healthiest food supply, the Centers for Disease Control and Prevention (CDC) reports that every year in the United States, 48 million people become sick, 128,000 people are hospitalised, and 3000 people pass away due to foodborne infections. Additionally, the risk of contracting a foodborne illness is now increased for nearly a fifth of the population.

In most cases, consuming food or water that has been contaminated with bacteria or toxins is what causes foodborne illnesses. Foodborne pathogens include bacteria, viruses, fungi, and parasites and are pathogens that cause foodborne illnesses. In the United States, 31 foodborne pathogens have been discovered, and it is believed that although bacteria are the main cause of hospitalisations and fatalities, viruses are mostly responsible for the disease. Listeria monocytogenes, Escherichia coli O157:H7, Staphylococcus aureus, Salmonella enterica, Bacillus cereus, Vibrio spp., Campylobacter jejuni, Clostridium perfringens, and Shiga toxin-producing Escherichia coli (STEC) are the common foodborne pathogens that cause the majority of foodborne disease outbreaks.

The desire for minimally processed ready-to-eat items and the volume of street food available have started to worry public health organisations about ensuring food safety. Foodborne viruses may be found in a variety of foods, including ready-to-eat items like fruits, vegetables, and prepared dishes. If food safety concerns are not taken into account, this might result in foodborne illnesses. Additionally, eating raw or undercooked foods including shellfish, pork, and poultry is often linked to the development of foodborne illnesses. To provide a secure food supply and reduce the incidence of foodborne illnesses, it is crucial to examine the food for the presence of foodborne pathogens.

The traditional techniques for identifying the presence of foodborne bacterial pathogens in food include cultivating the germs on agar plates, followed by conventional biochemical identifications. However, since they rely on the microbes' capacity to proliferate in various

culture media, including pre-enrichment media, selective enrichment media, and selective plating media, conventional approaches may be time-consuming. Typically, traditional procedures take two to three days for preliminary identification and more than a week for confirmation of the infection's species. Conventional approaches are time-consuming since they include preparing culture medium, inoculating plates, and colony counting. Additionally, the poor sensitivity of traditional approaches may be a limitation. Pathogens that are viable but non-culturable (VBNC) may lead to false negative findings. The danger of disease transmission would rise if foodborne pathogens were not detected.

To get over the limits of traditional techniques for the detection and diagnosis of foodborne pathogens, many fast approaches with high sensitivity and specificity have recently been devised. Additionally, scientists continue to create new techniques that are faster, more sensitive, more specific, and better suited for in situ examination for identifying live cells. Rapid detection techniques are crucial because they can quickly identify the presence of pathogens in both raw and processed foods, which is significant for the food business. Rapid techniques are sensitive enough to find infections in small quantities in the food. Sensitivity is crucial since even one virus found in food has the potential to infect people. Rapid techniques save time, and money, and are less prone to human mistakes. However, each quick approach has pros and disadvantages of its own. Rapid detection techniques are often divided into three categories: nucleic acid-based, biosensor-based, and immunological-based techniques. This study looks at the uses of these modern quick detection techniques for finding foodborne bacterial infections, as well as their benefits and drawbacks.

Using Nucleic Acids as a Base

Specific DNA or RNA sequences in the target pathogen are found using nucleic acid-based approaches. This is accomplished by hybridising a synthetic oligonucleotide (probes or primers) with the target nucleic acid sequence, which is complementary to the target sequence. Numerous bacterial infections, including Escherichia coli O157, Vibrio cholerae, Staphylococcus aureus, and Clostridium botulinum, generated toxins that led to foodborne illnesses. Nucleic acid-based techniques may be used to identify the toxin-related genes in these infections. Furthermore, nucleic acid-based techniques may be used to identify and confirm pathogens with equivocal phenotypic traits, such as isolates of Campylobacter jejuni that are hippurate negative. Methods based on nucleic acids find the target infections' particular genes, avoiding outcomes that are unclear or misinterpreted. Simple polymerase chain reaction (PCR), multiplex polymerase chain reaction (mPCR), real-time/quantitative polymerase chain reaction (qPCR), nucleic acid sequence-based amplification (NASBA), loop-mediated isothermal amplification (LAMP), and microarray technology are some of the more recent nucleic acid-based techniques described.

Chain Reaction of Polymerase (PCR)

Polymerase chain reaction is one of the most widely utilised molecular-based methods for the identification of foodborne bacterial infections (PCR). About 30 years ago, the PCR technique was developed, allowing for the identification of a single bacterial pathogen present in food by identifying a particular target DNA sequence. A particular target DNA sequence is amplified by PCR in a cyclic three-step procedure. First, at high temperatures, the target double-stranded DNA is denatured into single-stranded DNA. The forward and reverse primers, which are two single-stranded synthetic oligonucleotides or particular primers, will then anneal to the DNA strands. After that, the primers complementary to the single-stranded DNA are extended during the polymerization process using deoxyribonucleotides and a thermostable DNA polymerase.

PCR in Multiplex (mPCR)

By simultaneously amplifying many gene targets, multiplex PCR provides a faster detection than standard PCR. mPCR operates on the same fundamental tenet as traditional PCR. In contrast to traditional PCR assays, which only utilise one set of particular primers, mPCR assays employ multiple sets of unique primers. The advancement of mPCR depends heavily on primer design since effective mPCR assays need primer sets with identical annealing temperatures. Additionally, the concentration of the primers plays a crucial role in mPCR. This is due to the possibility of interaction between different primer sets in mPCR, which might lead to primer dimers; as a consequence, the primer concentration may need to be changed to assure the synthesis of trustworthy PCR products.

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CHAPTER 3

MICROBIOLOGICAL HAZARDS AND THEIR CONTROL

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The aim of this book is to provide the reader with an introduction to a systematic method for managing food safety, which includes creating management objectives for food safety management systems, sampling, and microbiological testing. This organised method links operational management efforts at certain stages of or across a food supply chain with governmental food safety management in the context of protecting public health. The structured approach's foundation on an appreciation of risk rather than the possible existence of a hazard is an essential component. The framework of risk analysis offers national responsible authorities a systematic method for managing food safety management decisions. The operational foundation of general food safety management is strongly reliant on precursor programmes including good manufacturing practises, good agricultural practises, and good hygienic practises. Prerequisite programmes provide assistance for systems that are based on the Hazard Analysis Critical Control Point (HACCP) principles that particularly manage food safety for a certain food company activity. If the suite of good practise programmes is applicable, "prerequisite programmes" is utilised as a collective phrase throughout this for the sake of brevity.

The adoption of new risk management measures known as the Food Safety Objective, Performance Objective, and Performance Criterion was advised by the International Committee on Microbiological Standards for Foods. Together, these metrics were developed and approved by the Codex Aliment Arius Committee to bridge the gap between current food safety standards and the idea of an appropriate level of protection as it is described in the Sanitary and Phytosanitary Agreement of the World Trade Organization. How the different measures theoretically relate to one another. The level of the danger entering the step has to be modified in a controlled manner, and this change is known as the PC. With the employment of FSO and levels are fulfilled in the food supply chain, the PO. The "food safety management system" for a certain phase is the collection of controls that can give the PC for that process, and it is implemented utilising prerequisite programmes and HACCP methods. Such control measures may relate to a food's pH or water activity, which are controlled by so-called product criteria; they may also relate to a specific treatment to be used, such pasteurisation, for which the controlling factors are known as process criteria. Since testing foods for a particular microbe might be a useful method of ensuring that these objectives are satisfied, a microbiological criteria may be established on the basis of a PO or an FSO.

The phrase "food safety management system" is used widely in this book to refer to any single or combination of control methods employed in the production, manufacturing, and distribution of a food or food component up to the point of final preparation at any point in the food chain. Codex uses the phrase "food safety control systems" as a synonym. A crucial element of the RA paradigm emerging in Codex Alimentarius is the capacity to explain the anticipated performance of a food safety management system in terms of the essential management of public health hazards. According to this paradigm, the FSOsSPS agreement) stipulated that foods might be freely imported as long as they wouldn't jeopardise a country's

ALOP with regard to protecting consumer health. The same agreement established risk assessment as the method to determine whether or not a meal might damage the ALOP. The agreement didn't go into detail on how an ALOP should be stated or completed. The Codex Commission Committee for Food Hygiene began developing a methodology for microbiological risk assessment at that time. The General Principles of Food Hygiene, which include an appendix on HACCP, and the Principles for the Establishment and Implementation of Microbiological Criteria for Foods were additional articles in the Codex system. The ICMSF acknowledged that if the ALOP of an importing country was stated in words like "the number of diseases per 100,000 of a population caused by a hazardhazard combination," it would be difficult for the food sector to demonstrate that a product would fulfil that ALOP. The Commission believed that these achievement levels would serve as the foundation for a specific ALOP. To put it another way, an ALOP or the portion of it relevant to a certain food or concentration of a danger deemed acceptable for consumer protection. The FSO could then be converted into the performance of a food process that would ensure that the level of risk in a food at the moment of consumption would not be higher than the value established in the FSO. The risk categorization may be articulated for a nation as "the projected number of illnesses per year" or other phrases comparable to the ALOP that reflect a country's public health objectives for foodborne hazards, therefore risk assessment was seen to be beneficial in achieving the FSO. To derive an FSO from the ALOP, information from hazard characterization, such as dose-response relationships, is essential, and exposure assessment data may assist identify control actions that would ensure the FSO would be satisfied.

As a result, the ICMSF advised the Codex secretariat in 1996 to utilise a step-by-step procedure to control viruses in foods. Doing a microbiologic risk assessment would be the first step, and creating an FSO would be the next. The third step should verify that the FSO is technically feasible via the use of prerequisite programmes and HACCP. Where necessary, step four included the establishment of microbiological criteria, and step five involved developing additional acceptability standards for goods traded internationally.

Between stages one and two, a new step—the application of the recently developed concept of risk management—was added in 1997. With the addition of this stage, it was realised that the creation of an FSO was not just a social choice that the many stakeholders should be involved in, but also an exercise in scientific risk assessment. The phrase "food safety goal," which was intended to address the results of all sanitary measures, was also proposed in the Codex Committee on Import and Export at the same time that the FSO idea was brought up for consideration in the Codex Committee on Food Hygiene. The nature of FSOs, the reasons for their need, the goals they should pursue, etc. were all unclear as a result of this circumstance. The Codex Alimentarius Commission ruled in 2000 that the Codex Committee on Food Hygiene would be the only entity authorised to use the word FSO for its intended purposes.

The Commission opted to incorporate the FSO idea as the foundation for establishing a food safety management system while working on a revision of ICMSF Book 2, stressing that FSOs should be wider than only transforming an ALOP into a degree of danger in a product. It was also acknowledged that, in many instances, an expert panel and a less thorough risk review would be sufficient instead of conducting a comprehensive risk assessment in accordance with Codex protocols to identify what controls would be necessary to fulfil an FSO. However, it was obvious that operationally, HACCP and prerequisite programmes would be the systems to implement in order to implement adequate product and process designs that ensure meeting an FSO for a specific hazard in addition to controlling all other relevant hazards for the particular operation, and these would be accomplished following

Codex guidelines. Initially, the WTOWHO's description of the ALOP idea and the FSO concept were connected. This circumstance has generated considerable confusion. On the one hand, a nation cannot demand of a nation that exports food that the exported food satisfy standards higher than those that are already met by the industry in the nation that imports the food. FSOs used to manage foods in international commerce, on the other hand, may be viewed independently from FSOs employed in a country's internal programme to improve food safety. In the first edition of ICMSF Book 7, the ICMSF acknowledged this circumstance and recommended that FSOs be utilised for both purposes in order to inform industry of the upper limit of a hazard in a food that must not be exceeded. As mentioned above, a risk assessment was seen to be beneficial in establishing the FSO, but the Commission advised that FSOs also take into account the epidemiological data that shows that a certain level of a hazard in a product does not result in an unacceptable public health issue. Lower limits should be established if they can be achieved by control methods that are technically feasible with affordable costs if there is proof that a certain level of a hazard in a food is in fact unacceple. There were broad principles provided, but the specifics of how FSOs should be created for the different objectives may vary depending on the circumstance.

Since it is through their combined efforts that efficient food safety management systems are established and validated, the different responsibilities of industry and government are outlined. A process is laid out for a scenario in which the existing systems for food safety are impacted by an improvement in the management of a risk in a specific food. Government risk managers employ epidemiological data to estimate the degree of human sickness linked to a microbiological agent and, if feasible, a dietary source. In reaction to a public health problem, they may also form an opinion about the severity of a disease. Risk managers may decide to establish an expert panel to develop recommendations, ask risk assessors to compile a "rough risk assessment" or "risk profile," or ask risk assessors to elaborate a more detailed risk assessment depending on the urgency, severity, risk, likelihood of further occurrences, the hazard-food combination, and other factors. These methods may assist the accountable risk manager in selecting the appropriate course of action for making timely risk management decisions given the problem with food safety and the overall circumstance. Risk managers may take fresh information and data about the hazard into account when ordering an expert elicitation, a risk profile, or a risk assessment. A new strategy for goods that are sold locally or via food imports is required in the latter scenario. To better comprehend the risk management choices available for reducing or concentrating the relevant micro-biological hazard in the specific meal at the moment of consumption, further information may be needed. The government risk manager would be sure that the country's ALOP is achieved or contributed to as anticipated when this FSO is fulfilled by the industry engaged in managing the relevant food supply chain. The risk food combinations to the overall ALOP on the basis of epidemiological data, risk assessment, or other accessible information may be significant for monitoring and reviewing the ALOP and related FSOs when satisfying a given FSO results in a certain ALOP.

As previously stated, the applicable competent authority, legislative body, or regulatory body is responsible for making the decision to approve an ALOP on behalf of society. The appropriate course of action for risk managers in government to guarantee that the present burden of sickness in a nation is not expanding or that additional measures should be put in place for reduction depends on the society as a whole. Earlier stages of the food supply chain than the point of consumption may include verification. This point is known as the performance objective, and it defines the maximum frequency and FBOs involved is then needed to determine the suitability of the entire food safety management throughout the food supply chain at hand. As previously mentioned, the food safety management system is made

up of one or more control measures that together exert the necessary degree of control over the dangers entering that phase, such as via ingredients, raw materials, or the environment of the operation. So-called "system thinking" may enhance coordination of proper management of product safety at both the individual and cross-step levels.

The food safety management system set up for a single or several stages should be able to consistently satisfy the PO, taking into consideration potential variations in the danger level entering the food at the step. The so-called Performance Criteria is the difference in risk level between the starting point of the phase and the PO for that step. In actuality, the PC is the end result of the many control measures used by a food safety management system at a given point in the food supply chain to establish control over a certain danger. By the use of precondition programmes and HACCP, the selected food safety management system is operationally implemented. Procedures that enable monitoring of operational parameters to confirm process control while accounting for process variability should be included in these. Inspection procedures and monitoring of key parameters should be ensured to verify the adequacy of operations to control microbiological, chemical, and physical hazards relevant for the food and food operation and to meet a PO set for a microbiological hazard when specific knowledge about the ability of a food safety management system to actually meet the PO for a specific step is lacking, for example when the underlying control measures cannot be based on sound science A competent body may, as necessary, issue a microbiological criteria related to a PO for the purposes of monitoring and verification by the government. If governments use these MCs, they do so primarily as risk-based criteria for lot acceptance or process control that highlight lots or processes that don't adhere to the current federal PO standards. Only in situations where there is a clear need and where application is feasible can MCs be developed and used. ICMSF book 6 contains information for a variety of commodities that may be used to assess if achieving an MC for a certain disease is relevant. Zwietering et al., which is a special volume of Food Control on the "Development of Microbiological Criteria for Food," provides examples of how MCs may be achieved to operationalize a PO or FSO utilising a risk-based approach. Industry may also produce MCs for evaluating the final product's acceptability or for verification of continuous control of their functioning at a particular point in the food supply chain. Monitoring the microbiological state of the environment or the product throughout processing may benefit from additional microbiological testing. To achieve the desired level of public health protection, the industry should consider implementing new technologies or other operational capabilities that are not yet in place so that the FSOor process design of the food product is such that the hazard is sufficiently controlled, and the competent authority should impose a market ban on the product until new technologies, process capabilities, or alternative options are available. World Health Organization, but did so on purpose to highlight how food management systems were taking the place of the previous strategy of end-product testing and providing better control of microbiological hazards. This strategy excluded consideration of sampling plans and microbiological criteria.

The first version of which is the present book, the progress of management systems proceeded. Book 7 attempts to underline once again that end-product testing is only one of several factors that, when combined, contribute to ensuring food safety. Since they are designed to be utilised when seeking to identify a problem and its cause, many kinds of sample plans—some more intense than the "attributes plans" often employed at ports of entry—are taken into consideration. As manufacturing and processing techniques, technological advancements, and new information are always emerging, risk managers in subsidiary bodies must make judgements when creating standards, codes of practise, or guidelines. In 1999, when it held its 32ndsession, the CCFH identified 21 pathogen-and-goods

combinations that pose serious risks to the public's health. CCFH prioritized these according to criteria such as the significance of the public health problem, the extent of the problem in relation to geographic distribution and international trade and the availability of data and other information with which to conduct a risk assessment. CCFH advised that in order to provide guidance on microbiological risk assessment, FAO and WHO hold ad hoc expert discussions. As a consequence, the Joint FAOWHO 2015 was founded.

JEMRA has contributed to a significant amount of the work done by CCFH since 2000, either via their international risk assessments or expert consultations, as well as input from specialised observer organisations like ICMSF. Principles and Guidelines for the Conduct of Microbiological Risk Management, Code of Hygienic Practice for Eggs and Egg Products, Guidelines on the Application of General Principles of Food Hygiene to the Control of Listeria monocytogenes in Ready-to-Eat Foods, Code of Hygienic Practice for Powdered Formulae for Infants and Young Children, and Guidelines on the Application of General Principles of Food Hygiene to the Control of Listeria monocytogenes in Ready-to-Eat Food ICMSF has actively participated in the creation of these global standards as a "international non-governmental observer" to CCFH.

Foodborne Illness: Bacterial Contaminants and Etiologic Agents

According to reports from those who have conducted thorough analyses of the number of illnesses, hospitalisations, and fatalities caused by contaminated foods, norovirus, Salmonella enterica, Campylobacter spp., Clostridium perfringens, and Staphylococcus aureus are the most common causes of foodborne illness in industrialised nations. *L. monocytogenes* has also gained widespread attention as a reason for worry due to its high case fatality rate. According to Scallan et al., non-typhoidal Salmonella was the most common cause of hospitalisations and fatalities in the US, whereas norovirus accounted for the majority of foodborne illnesses. 90% of all domestically acquired instances of foodborne illnesses, hospitalizations, and fatalities in the U.S. were caused by Campylobacter spp., non-typhoidal salmonellae, Clostridium perfringens, L. monocytogenes, and E. coli O157, norovirus, and Toxoplasma gondii combined. Cronobacter spp. has also received attention since it may be connected to powdered newborn formula and has been related to neonatal deaths and sequelae.

Just a small percentage of diseases may be linked to specific diets. The common contributing components are quite comparable across nations where that has been feasible. For instance, huge Christmas turkeys sometimes required insufficient thawing before cooking. Salmonellosis resulted from the following cooking failing to eradicate salmonellae in the core of the partly frozen poultry. Attempting to cool large amounts of heated food that cannot be cooled quickly, resulting in the growth of surviving bacteria during the slow cooling, and consuming without reheating above ca. 63°C to kill the vegetative cells of, for example, Bacillus cereus in rice or Clostridium perfringens in meat or gravy, are examples of inadequate temperature control after cooking. Another significant cause of foodborne disease, such as during barbecues, is the contamination of a cooked, safe dish with bacteria from raw food.

Salmonellae are thought to be responsible for 94 million instances of non-typhoidal gastroenteritis annually, making them the most significant foodborne pathogenic bacterium in the world. Salmonella Enteritidis and Salmonella Typhimurium have been the most often mentioned salmonella in many different nations. Salmonellosis outbreaks have been linked to a variety of different meals and dietary components. Meat and poultry, eggs and egg products, milk and milk products, fresh fruit, unpasteurized liquids, and spices are common carriers of salmonella. S. Enteritidis outbreaks decreased in the UK from 1996–1997 and then

continued to fall until 2012, despite the fact that the prevalence of this virus, notably in poultry and eggs, grew significantly in several countries from the mid–1980s to become a serious concern.

The prevalence of illnesses brought on by thermophilic Campylobacter spp. has sharply risen in several developed nations in recent years. For instance, Campylobacter is the main bacterium responsible for infectious intestinal sickness in the U.K. and the second most prevalent bacterial cause of hospitalisation related to foodborne illness in the U.S. Campylobacteriosis outbreaks are uncommon; the majority of cases are sporadic and are often attributable to cross-contamination from raw chicken or undercooked poultry. Raw milk, untreated water, and other foods have also been connected. Vibrio parahaemolyticus illness is common in nations where raw seafood consumption is significant. Western nations sometimes have outbreaks, although there the source of transmission is often processed seafood as opposed to raw seafood. Consuming raw oysters infected with Vibrio vulnificus can, however, offer a serious risk to those with impaired immune systems. In many tropical nations, Vibrio cholerae is an endemic disease, and water is a significant factor in cholera epidemiology.

Moreover, Shigella spp. are a substantial public health issue in many underdeveloped nations and are responsible for serious sickness in wealthy nations. Shigellosis cases are often linked to travel, food handlers, and daycare facilities in affluent nations. Shigella spp. can only be found in people, hence human carriers may spread the virus via tainted food or drink. Worldwide, Yersinia enterocolitica and Yersinia pseudotuberculosis-related illness is common and has been predominantly linked to eating undercooked or raw meat and food that has been stored at low temperatures for a lengthy period of time.

Escherichia coli strains are a typical component of an animal's healthy microbial flora, including humans. The majority of strains don't hurt you, but some do. As eating of even little amounts of these organisms carries a risk for life-threatening sickness, strains with highly virulent characteristics have become a severe menace. Enterohaemorrhagic E. coli, which produces verocytotoxins, commonly known as Shiga toxins, has become a significant foodborne danger during the last three decades. VTs or STs are produced by several distinct serotypes of E. coli. While serogroup O111 is the most frequent cause of sickness in Australia, E. coli O157:H7 was initially to blame for the majority of human outbreaks of EHEC. Many E. coli serotypes that produce verocytotoxin are now known to cause human sickness, with serotypes O157, O26, O45, O103, O111, O121, and O145 being responsible for almost 90% of infections. Initially, intake of undercooked ground beef and, infrequently, unpasteurized milk was linked to human instances of EHEC. Fresh produce tainted with EHECs is being linked to an increasing number of illnesses, too. Since no food source, besides powdered infant formula, has been epidemiologically linked to a disease caused by Cronobacter spp., the focus of food safety management has been on the possibility that these pathogens are present in the facilities that process infant formula, raw materials, and finished goods.

Because of the potentially fatal nature of the illness and the negative effects on commerce of the product type in question, botulism is a severe worry despite its rarity. For a long time, home-canned or home-cooked foods were more commonly impacted than commercially processed goods, but more recently, a number of commercially produced items have been linked to contamination. Rotavirus, erroneous processing, and Norwalk-like viruses are known to cause foodborne disease. NoV has been acknowledged increasingly often as the most significant cause of non-bacterial gastroenteritis worldwide as detection techniques have improved and governmental activities on monitoring and ascertainment have strengthened.

While viruses are sometimes engaged in significant outbreaks, their real significance and scope in the development of foodborne disease have not been well studied. Mead et al. assessed that viruses are more significant than bacteria and protozoa as a cause of foodborne disease during their study of the burden of foodborne illnesses in the USA. Recent statistics showing that norovirus alone is responsible for 58% of illnesses, as opposed to 30% of illnesses overall being caused by the top three bacterial pathogens, verified this. NoV was shown to be the second most common reason for hospitalisation and the fourth most common cause of mortality, in addition to being the main cause of foodborne disease. Data from the UK's surveillance programme for infectious gastrointestinal diseases indicate that rotavirus, sapovirus, and norovirus are the main culprits there. NoV was shown to be the primary cause of foodborne disease in Australia, accounting for 30% of infections. Live bivalve molluscs are often linked to outbreaks of viral foodborne illness. An examination of NoV-related food- and waterborne outbreak occurrences between 2000 and 2007 found that 42.5% of the time, the epidemic was started by a food handler, who then infected water, bivalve shellfish, fresh vegetables, sandwiches, and catered meals. NoV and HAV are the most commonly involved in foodborne viral infections, according to a review of the global burden of disease and of specific viruses of concern. Identification and removal of contaminated products from the market, correction of poor food-preparation practises in food-service establishments, processing plants, and homes, identification and appropriate treatment of human carriers of foodborne pathogens, and potential detection of new agents of foodborne infection are also important. Nevertheless, the Foodborne Disease Burden Epidemiology Reference Group initiative of the WHO is attempting to determine the real worldwide burden of foodborne disease. According to the current information, foodborne disease is a global public health issue that is impacted by demography, industrialization and the centralization of food supply chains, commerce and travel, and microbial evolution and adaptability. Scallan et al. estimate 38.4 million instances of foodborne illness in the USA alone, along with 9.4 million cases of illness brought on by 31 other recognised pathogens, including viruses and protozoa.

Evaluation of product and process design, intended user/consumer, potential unintended usage, etc., as well as confirmation that relevant food safety requirements in legislation or industry practise will be fulfilled consistently are required to determine what food safety management system needs to be put in place. This all-encompassing "systems thinking" will influence the choice of effective control methods and the zeal with which they are to be implemented. Control measures are procedures and practises that either avoid or get rid of a risk to food safety or get it down to a manageable level. To ensure that a food is safe to eat, one or more control measures may be required at each point along the food chain. Several diverse control methods, such as raw material selection, regulated storage, hygienic handling before to a process, proper application of the process, and post-process contamination prevention, are included in effective food safety management systems. The HACCP systems and precursor programmes handle the set of control measures. Critical control points in ICMSF Book 4 are separated into CCP1 (control measures that completely remove the risk) and CCP2 (control measures that may minimise the risk but not completely avoid or eliminate it). It would be easier to comprehend many HACCP designs if the distinction was brought back.

Considerable steps should be taken to control Campylobacter and Salmonella at the pertinent points in the broiler supply chain, including the use of ASC in online reproduction, crust freezing, and high oxygen content during chilled storage. Moreover, forced air chilling and air chilling utilised to lower carcass temperatures will be useful in lowering the danger of campylobacter. In order to limit the prevalence of Salmonella in eggs, vaccination of laying

hens has been found to be an effective control method. This has helped to reduce the number of salmonellosis cases associated with eggs in the UK.

Because to the subsequent survival and FDA 2001; FAOg, many food items are more susceptible to microbial contamination from primary manufacturing. Key control procedures include monitoring and regulating the temperatures in refrigerated storage. The product shouldn't be heated over 6 °C. The shelf life of the product could be shortened as a consequence of temperature abuse that might favour the development of L. monocytogenes.

Another significant aspect of the danger connected to foods that promote the development of *L. monocytogenes* is the shelf life. Since that *L. monocytogenes* may develop at refrigeration temperatures, the shelf-life of such goods should be in line with the need to restrict the growth of *L. monocytogenes.* Even the proper low temperatures may not be kept up to the point of consumption throughout the whole food chain. Vulnerable individuals, such as the elderly and immunocompromised, should only ingest eggs that have been cooked until the yolk is solid, or use a professionally pasteurised product, to avoid domestic salmonellosis from S. Enteritidis in eggs. Many incidences of salmonellosis have been connected to using raw eggs in homemade mayonnaise and tiramisu. In the kitchen, handling raw chicken may transfer thermophilic Campylobacter spp. to adjacent work surfaces. When handling raw poultry, hands should be thoroughly and regularly cleansed.

All business sectors should consider the meals meant for vulnerable customers.

FSO in the Management of Food Safety Would POFSO in the Management of Food Safety. Politicians and risk managers of the responsible authority or authorities overseeing food safety in a nation decide what defines safe food. They must take public health effect, technical viability, economic ramifications, and other dangers in daily life into consideration. In essence, a meal's or food category's degree of risk must be "acceptable" or "tolerable" in the nation in question for that food to be considered safe. Food that has "no danger at all" or "zero risk" does not always mean it is safe. Risk, on the other hand, is an estimation of the likelihood and gravity of the unfavorable impacts that an exposed population could experience as a result of a food hazard. Even if there is a very little possibility that a susceptible group would be exposed to a certain danger, the risk may nonetheless exist. The degree of acceptance might be expressed as an ALOP, or the number of cases per unit of population in a nation, which represents the amount of public health protection that has been accomplished or is within reach.

An ALOP aim, such as "there must be no more than 20 domestic instances of a given foodborne disease per 100,000 people per year in a nation," cannot be used by food operators to define the acceptable level of risk. Despite the fact that this objective may be beneficial, it calls for the cooperation of several parties. The microbiological, chemical, and physical risks potentially linked to the food raw material or product that food operators handle or manufacture at the specific phase in the value chain that they are engaged in are, in theory, the only ones over which they may exert control. Although every operator along the food chain must be aware of their position and conduct their business to eventually satisfy an FSO, they cannot be held accountable for the acts of every other operator. To successfully establish a suitable performance objective for the step that can be controlled and derive the performance criteria to satisfy the PO, it is crucial that each FSO expresses the degree of risk that is deemed bearable at the point of consumption in plain terms. The communication of obligations down the food chain is notably strengthened when FSOs and POs are quantifiable and verifiable. This is not always possible, though, because there is a lack of information on, for example, the relevant characteristics of the risk, the causes of unfavorable public health effects, the conditions required to control the risk, and/or the physical characteristics of a

food that deliver or contribute to delivering the overall PC by restricting the growth of a pathogen or aiding in its inactivation. Product criteria may also be used to analyse the accepility of a food. The relationship between the microbial reaction in foods and their composition and environment is becoming more well understood and accepted. As pH, water activity, temperature, and gas atmosphere are the primary parameters impacting food safety, measuring them provides a quicker way to assess the safety of certain foods. For instance, if it has been proven that a certain pH or water activity guarantees that the meal would fulfil an FSO for development of a disease, the food may be deemed acceptable.

Default criteria are conservative standards created to guarantee the food or process safety. Default values may be used if there aren't enough resources to do the necessary study to come up with precise process or product requirements. An example of a default setting is heating extended shelf-life ready-to-eat refrigerated meals for 10 minutes at 90 °C internal temperature to eradicate non-proteolytic C. botulinum. The majority of the time, control authorities or advisory groups have created default settings. These principles outline the minimal requirements that must be fulfilled in order to guarantee the production of safe food.

FSO and FSO and may be used to determine if a process is under control based on the data supplied in risk-based metrics. When proper processes are followed and established criteria are satisfied, a process is said to be under control. It is ideal for the techniques used to evaluate and modify a process's control to be founded on the same ideas as those used to choose the control measures. When validating, monitoring, and verifying control over a process, statistical process control may be required.

Acceptance Standards

Acceptance criteria are descriptions of requirements that set apart accredited from unaccredited food businesses. Acceptance criteria should specify ancillary information, such as the number of samples to be collected, how and where the samples are collected and held prior to analysis, the analytical unit, the method of analysis, and the range of values considered acceptable. Acceptance criteria may be sensory, chemical, physical, or microbiological. Each with a distinct goal, the examination may be carried out by a control authority, a client, or even an independent auditor hired by the food operator. Moreover, acceptance criteria are employed to rate the acceptability of certain food lots or consignments.

CHAPTER 4

MICROBIOLOGICAL TESTING AND CRITERIA

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To assess the microbiological quality and safety of foods, microbiological testing is widely employed. The findings of such tests are sometimes perceived as absolute, although they should never be taken as such owing to simple statistical effects and typically significant variation in the distribution of microbes in food. There are several uses for microbiological testing. It is crucial to take into account the reason for the utilisation of microbiological tests. The goal dictates the sort of test, the technique, the sample, the interpretation of the result, and actions to be performed.

Assessing Risks and Achieving Performance Goals for Food Safety

Seldom has a structured method been used to establish the amount of consumer protection that a society or nation would deem necessary in the case of a foodborne microbiological danger. Yet, while creating and putting into practice policies and strategies for the management of microbiological risks, governmental risk managers keep such objectives in mind. The foundation for the present effective food safety management systems is a long history of implicit or instinctively choosing public health protection solutions.

This amount of protection may not be stated clearly, but it can be inferred from statistics on the incidences of diseases that occur domestically. The following are the results of a survey conducted by the National Institute of Standards and Technology (NIST) on the use of the ANSI/IEC 61508 standard. In 2010, the total case rate for listeriosis in Europe was 0.33 per 100,000 people, with the highest rates reported by Finland, Denmark, and Sweden, while rates in other Member States were below 0.6 per 100,000 people. The incidence of listeriosis was predicted to be 0.53 per 100,000 people in 2011, according to comprehensive estimates of foodborne diseases provided by the US CDC. Since 2007, the Foodborne Disease Burden Epidemiology Reference Group has carried out foodborne illness burden research under the direction of the World Health Organization. FERG estimated estimates of listeriosis cases at the worldwide level in 2010 to have been 23,150, 5463 fatalities, and 172,823 Disability-Adjusted-Life-Years (DALYs), based on systematic reviews of the literature and meta-analysis studies. With a 7.4 billion-person world population in 2014, these case counts translate to an estimated worldwide incidence rate of 0.31 per 100,000.

Italy

The frequency of certain dietary dangers in certain food categories. Depending on the problem, the relevant scientific insights, and the degree to which evaluation methodologies can be agreed upon by stakeholders, this assessment may be done in a variety of ways. In actuality, the evaluation might be anything from a simple qualitative risk estimate to a quantitative risk assessment.

The amount of "risk" to human health, i.e., either the present level of risk or a future level of risk, may be used to indicate the current or future public health status in relation to food safety. The latter situation occurs when governments or public health organisations establish objectives for improving public health and lowering the burden of chronic illness. Future

public health targets such as the Healthy People 2010 and 2020 target objectives established by policy makers in the USA are examples. The concepts of Food Safety Objective and Performance Objective are discussed as risk-based metrics that allow government risk managers to effectively communicate precise food safety goals to industry and trade partners. Under the auspices of the WTO, the term ALOP is an expression that has legal weight and should be understood as referring solely to the level of protection that is currently achieved in a particular country. Using the term PO to express such levels at earlier points in the food supply chain, the original concept of FSO proposed by the Commission in 2002 has been aligned with that of Codex Alimentarius in this update of ICMSF Book 7. FSO will continue to be used to describe the acceple level of a hazard at the point of consumption.

Codex Alimentarius defines FSO and PO as the highest frequency, concentration, or combination of both of these that is deemed acceptable for consumer protection in a specific food product or group of foods. To put it another way, when they are completed, these risk-based metrics explicitly state to the industry what amount of hazard is too high to allow in food, resulting in a risk at consumption that is in accordance with the ALOP. As a result, industry may then establish a sufficient food safety management system at the point where they are accountable to successfully control the danger in question to a level that is bearable. Several research linking ALOP with FSO and PO have been published recently in peer-reviewed literature. Despite the fact that FSO and PO are recently developed outcome objectives for food safety management, it is still possible to evaluate whether these targets have been fulfilled using the current food safety metrics, such as microbiological criteria. The Commission made a contribution to a working group report that was published in conjunction with the updated Codex recommendations on the use of MC. The article includes many hypothetical examples that show how MC may be used to operationalize a PO or FSO.

The principles of FSOPO as a risk-management tool and the best method to get values for these indicators are what ICMSF would recommend. In theory, values for FSOPO may be based on a reasonable assessment of the danger, but they may also, when there is a lack of time or a concentration of a hazard, be based on a hazard that is anticipated to maintain control of the situation. The Commission believes that even without creating a more comprehensive quantitative risk assessment, the achievement of relevant FSO or PO values for the specific hazardsituation at hand might be a useful foundation to tie the FSO to the ALOP.

On the basis of a quantitative risk assessment created for a specific pathogen in a specific food for the ALOP, when articulated, or to ensure that other benchmarks for food safety are being met in accordance with the food product and its intended use, a PO can be derived from an FSO derived by a competent authority from a stated ALOP, or directly from the ALOP without explicitly articulating an FSO. The new risk-based metrics FSOprocess control or for determining lot acceptance, which instead use on measures like MCs, vary in a number of important ways. FSOsPO are presented as instruments to articulate and explain the required degree of consumer protection in concrete ways. Information on some of the various techniques that have been utilised to describe the public health situation is also provided in the sections that follow.

Keeping Risk at a Tolerable Level

The food supply chain is complex, spanning primary production, harvesting, processing, marketing, distribution, and food preparation. From the food's original source to its final preparation, hazards may enter at any point along the chain. There are several infections that may be found on raw agricultural products and seafood, in particular, for which there are no efficient control techniques. At best, it is conceivable to lessen their presence in these foods

while still offering them to customers in their raw, unprocessed form. The fact that removing one danger may raise the possibility of additional hazards or other unfavorable effects makes eliminating foodborne risks much more difficult. Hence, food control programmes are designed to ensure that foods are as free from dangers as is reasonably practicable via effective hazard management.

In many cases when the successful implementation of GMP coli, Clostridium perfringens, Escherichia coli O157:H7, Listeria monocytogenes, Salmonella enterica, Staphylococcus aureus) and one para-site, the TLR is essentially the absence of illness. Between $5.6 and $9.4 billion was estimated to have been spent in 1995 on medical expenses and productivity losses related to these 7 diseases. The public health burden of 14 pathogens was estimated to be over $14 billion by a more recent study using the most recent surveillance data from the USA, with 90% of the burden falling on just five pathogens: Salmonella enterica, Campylobacter spp., Listeria monocytogenes, Toxoplasma gondii, and norovirus. The expenditures connected with Listeria, Campylobacter, and Toxoplasma were shown to be significantly influenced by long-term consequences resulting from acute infections.

It must be realised that expenses go beyond just financial considerations. Another example is to imagine that altering a customary, religiously based food handling behaviour might lessen the likelihood of contracting a foodborne disease. While changing these habits could marginally reduce the risk, the cost of losing the ability to practice one's religion may be too great, and the people in question may choose to accept the slightly increased risk instead.

Parallel to this, it is important to acknowledge how food control regulations affect customers' ability to choose the foods they eat. However, there are a number of situations in which the risks that some consumers are willing to take as individuals will not be tolerated by society as a whole because the potential public health effects of particular foods or food handling practises may be so severe that consumer choices may need to be restricted. For instance, a number of nations prohibit the commercial sale of non-pasteurized milk for consumers owing to worries about the potential existence of a variety of various dangers that might endanger consumers. Additional examples are restrictions on the import of fish from the order Tetraodontiae because of the danger of tetrodotoxin poisoning or prohibitions on the sale of eviscerated salted fish that are in place in a number of nations.

With the exception of prohibition, the items in these cases are intrinsically riskier than the governments that are impacted can accept. Risk managers in certain countries came to the conclusion that the risks were intolerable in the context of their society because of the lengthy history of public health issues associated with the aforementioned foods. But, it's also feasible that a different civilization might place a high value on these meals and be prepared to accept the negative effects on the general public's health.

When risk management methods implemented to minimize the risk of one hazard are likely to raise the risk of another hazard or will have other unfavorable impacts, such as on nutritional characteristics, this is another cost that must be taken into account. The use of chlorine to disinfect water for drinking and food preparation is a relevant microbiological example. Although chlorination lowers the dangers of water-borne microbial infections, the creation of organo-chlorine compounds poses a concentration-dependent risk. Despite this, these compounds are a highly important source of calcium and other minerals that are vital for good health. These circumstances need risk management choices that strike a balance between two opposing interests so that the advantages exceed the risks.

The majority of cultures see consumer protection as a moral obligation worthy of high importance. Media coverage of foodborne disease has risen, and as a result, people are more

aware of its prevalence and effects on the public health. The government and business are under more pressure to implement measures that will further minimise risk as a result of the heightened knowledge. Future levels of risk that will be accepted for different foodborne microbiological dangers may reflect this.

For each of the aforementioned categories, choosing not to act has consequences as well. Choosing which actions need to be performed is the end result of weighing the different risks and advantages. An aim must be established in order to carry out the operation. This aim may be stated, for example, as a public health target about risk in the population or as a tolerated level of hazard in a meal. As previously said, stating population level objectives in terms of risk does not provide the industry or inspection and control agencies in the government the precise direction they need. Perhaps expressing a goal in terms of the degree of danger will help to guarantee that the activities done will lead to the achievement of that objective. This is a major justification for the development of the new risk-based measures FSO and PO and Codex Alimentarius' endorsement of them.

The Value of Epidemiologic Information

For competent national authorities to use in developing risk-based public policy, including setting ALOP or TLR and choosing appropriate management actions to reduce the overall public health impact, including FSO and fingerprint of hazards by animal or plant of origin, geographic origin, production practises and conditions, and season, accurate knowledge of disease incidence and severity is essential harvesting, preparing, and distributing Programs for microbial surveillance and monitoring at the national level should be able to rank particular foods according to how frequently they are contaminated, identify subtypes of hazards by food, practise, process, behaviour, geographic origin, and season, and describe consumer-induced risk factors for foodborne illness.

Public health monitoring

Systems for monitoring public health at the national level should be able to estimate the frequency of dangers that are often foodborne and monitor the incidence of illnesses that may be spread from diseased people to consumers via tainted food. The proportional fraction of significant risks attributable to various foods should be possible to be determined, at least within broad food categories, through routine epidemiologic investigations of sporadic foodborne illness.

Investigations and monitoring of outbreaks at the national level should be able to pinpoint the food sources of epidemic diseases and assign the proportional amount of important risks to certain foods, procedures, practises, behaviours, and hosts for epidemic illness. For proper evaluation of the public health efficacy of management initiatives, improved attribution of foodborne disease to particular foods, procedures, and behaviours is crucial. monitoring information produced from food sampling for regulatory and non-regulatory purposes, such as microbiological food testing and environmental health information on the activities and procedures of food workers.

It is crucial to gather, evaluate, and comprehend data from a variety of sources in order to determine the impact of sickness on a community and the effectiveness of measures taken to prevent foodborne illness. Nowadays, a variety of methods are used to track and report the prevalence of foodborne illnesses:

1. systems for passive notification
2. systems for active surveillance
3. Case-control research
4. investigations into outbreaks

Sentinel Analyses

None of these techniques provide the whole set of information required for a quantitative risk assessment, and several often overlook food as a potential source. Passive notification systems observe trends in illness and might be beneficial for assessing the effect of changes in technology, preventative measures and regulatory regulations.. Each state's local sources provided reports that were used to generate the statistics, which each state subsequently sent to the Centers for Disease Control and Prevention. Also, some "notifiable" disorders have to be reported by doctors. This necessary requirement may increase the accuracy of the data yet many occurrences remain unreported. Similarly, records from labs may uncover patterns for instance in non-typhoidal Salmonella infections and flag for public health authorities shifting dangers to the community. in more precisely the incidence of salmonellosis and illnesses caused by E. coli O157 in Europe. Another objective of EnterNet is to eslish a system to detect epidemics throughout Europe from a common food supply. First eslished in the USA in 1996, and later duplicated and implemented in several regions around the world, the Foodborne Diseases Active Surveillance Network or, FoodNet, is an active, sentinel site pro- gramme that collects weekly updates from clinicians in certain regions of the country for specific foodborne illnesses, including Campylobacter, Cryptosporidium, Cyclospora, Listeria, Salmonella, Shiga toxin- producing Escherichia coli O157 and non-O157, Shigella, Vibrio and Yersinia. Isolates of chosen pathogens are analysed for commonality to discover outbreaks related to a common dietary supply. FoodNet estimates the number of foodborne diseases in the USA, analyses changes in frequency of spe- cific foodborne illnesses over time, links illnesses to particular foods and locations, and disseminates

Data on foodborne disease is also collected through case control studies by interviewing patients to learn their food consumption history and to identify food sources. In parallel, a number of individu- als are picked to act as controls. This technique has been used to uncover not just the foods that may be related, but also risk factors that the patients may share and that may explain higher vulnerability to the illness. Case-control studies are important for discovering pathogen-food combinations when it has been difficult to extract the pathogenic organism from the food source or the function of foods in illnesses with extensive incubation durations before start of symptoms.

The identification of the food source and circumstances contributing to foodborne disease may also be discovered via epidemiologic examinations of outbreaks. Unfortunately, not all outbreaks are adequately investigated or described fully in the scientific literature, particularly those that do not provide new information. Thus, such literature is typically of little assistance in respect to eslishing the real frequency of their occurrence and consequently the risk associated with the disease agent. Case control studies may also be used to assist identify the source of sporadic occurrences of foodborne disease and the variables that contribute to their frequency. Diverse sources may be more essential in occasional occurrences than in epidemics. In the USA, epidemics of Campylobacter jejuni infections in the spring and autumn are mainly caused by drinking raw unpasteurized milk or untreated water, although occasional cases appearing in the summer seem associated to handling or ingesting undercooked chicken.

A sentinal research monitors chosen health events in a sample of individuals typical of the general population. Laboratory testing may be restricted, e.g., to individuals experiencing diarrhoea, or may involve screening of all faecal samples for a spectrum of pathogens. This technique, for instance, has been used to estimate the incidence of campylobacteriosis and salmonellosis in The Netherlands and England.

However, a large portion of the data gathered by various systems regarding foodborne illnesses cannot be used to directly inform policy because: not all cases are reported to health authorities, creating significant uncertainty about the true burden of illness; frequently, only a small portion of illnesses caused by food-related pathogens are actually foodborne because transmission can also occur through the environment, direct contact with animals, or from person to person; and, finally, because not all cases are reported to health authorities, resulting in considerable uncertainty about the actual burden of illness;

In a number of nations, including the U.S., Canada, and the U.K., the occurrence of a disease is on the rise. Mead et al. calculated underreporting factors for a variety of disorders, including 2 for listeriosis and botulism, 20, 20, and 38 for campylobacterioses and salmonellosis. While there are some discrepancies, generally speaking, the estimations produced by such research represent a similar degree of magnitude across nations with comparable economic growth, demography, and healthcare infrastructure. European studies that were comparable to the American study indicated that the underreporting factors for campylobacteriosis were 7.6 and 10.3, while the underreporting factors for salmonellosis were 3.2 and 3.9, respectively.

Assessment of Risk

The government risk manager is responsible for conducting a risk assessment to assess the problem's severity and determine if or what steps should be done when a food safety issue or the need for improvement in the food safety status is found. Depending on the information and resources at hand, a risk evaluation and the documentation associated with it might take many various forms. For instance, one or more experts, a panel of experts, or other qualified resources may carry out the risk appraisal. The risk assessment may be completed as a risk profile, a descriptive analysis, or both. Although there is not yet a consensus on when a quantitative risk assessment is required or which statisticalscientists, NGOs, and consumers should be involved, WHO and Codex Alimentarius have issued detailed guidance for risk managers on this in the context of the Risk Analysis framework that underpins the work of Codex Alimentarius committees and the work of the Joint Expert Meeting on Microbiological Risk Assessment. Background data is often compiled by a government risk manager utilising easily accessible data and information to adequately define the issue, maybe with participation from subject matter experts from public or private organisations. It has been suggested that the identification of a food safety issue in the Codex environment be stated via the construction of a risk profile. Global risk profiles for pathogen-food pairings have been established by a few areas and nations.

Governmental risk managers must determine whether to instruct risk assessors to conduct additional research and put together a more elaborate qualitative or quantitative evaluation in order to obtain the information required for risk management decisions, such as an understanding of the level of risk to populations of consumers, factors contributing to risk, and potential hazards. A risk manager has to be able to make judgements after doing a risk assessment. The kind of risk assessment that should be sufficient for the risk management to base their choice on will rely on the issue with food safety, as well as aspects like the datainformation with timely access to resources. When a risk assessment is requested by a risk management, it is critical that both the risk managers and the risk assessors have a shared knowledge of the issue. This understanding should then include the risk assessment format that will be used, the resources that are available, the time constraints, and the desired form of the output, for example, a risk estimate and WHO 2004 or a "terms of reference for scientific advice" risk assessment are constrained, or where there are few management options. These panels may also be consulted where, in particular, epidemiological research shows that a risk

is not under control and that enhanced consumer protection is necessary. A shift in eating patterns, food processing technology, or food packaging or distribution networks may also generate problems. Such worries must be assessed, and if logical and backed by data from the scientific community, risk management measures must be adopted.

By defining the frequency or confinement of a microbiological danger that should not be surpassed at the time of consumption, risk managers may establish the stringency under which food control systems must work. In practice, they will consult with experts on the specific pathogen. Hence, it serves as the foundation upon which control authorities may establish standards or guidelines and determine if a facility is in compliance and producing safe foods, i.e., the foods will satisfy the established FSO under typical commercialization and usage settings.

Notwithstanding the fact that FSOs and POs may be established for any food hazard, this book solely takes into account risks that are microbiological in nature. Thus, it will be assumed that the FSOsPOs as ideas have grown over the last ten years and that the variety of viewpoints is well worth evaluating. The ICMSF website has information on these ideas in both layman's terms and with graphics. Examples of FSO sor concentrations of microbiological hazards in food that are deemed acceptable for consumer protection at the time the item is consumed. Similar to this, a PO denotes the acceptable amount for a certain early stage of the food chain, such as production or retail. It is crucial to underline once again that the main goal of an FSOPO, associated risk management metrics, and established standards for their usage in risk management are still relatively new ideas that have not yet been included into food regulation. Nonetheless, other countries do include the phrases in their laws, however they do not specifically identify pathogen-food pairings or 100 g.

Aflatoxin levels in peanuts intended for further processing cannot be higher than 15 gg. Salmonellae levels in fruit juice must be less than 1 cfu10 L in order for it to be distributed. *L. monocytogenes* levels in cold-smoked salmon with a two-week shelf life at 4 °C should be less than 25 cfug at the time the product is put on the market .With an emphasis on either concentration or prevalence as an acceptable limit for a danger, maximum hazard frequencies and POs are given as "lines in the sand" for governments to adhere to. The authority establishing the metric needs to specify the expected level of control beyond a simple "line in the sand" limit, for example by defining what proportion of the distribution of possible concentrations must satisfy the test l. While Codex does define FSOsor concentrations of pathogens that are considered tolerable, various authors have argued that it is important to account for both frequency and concentration of a hazard when articulating an FSOPOs useful in practise. In other words, the "tolerance" for testing compliance—the percentage of the lot that may be beyond the ostensibly acceptable level—must be indicated.

This requires knowledge of the distribution of potential contamination concentrations that is normal for the product in question. Considering that the anticipated level of control is defined, it is obvious that some food units will exceed the values designated as the limit in the FSOPO. Hence, it may be concluded that the food safety management system controls the hazard to the expected level.

As an illustrative example, let's say that a competent authority established the value of a PO for the absence of E. coli O157 in apple juice at the conclusion of manufacturing as: 99% of 100 ml units of apple juice.

The theoretically "acceple" level is then set by the authority at 1%, leading to an understanding of the PO as the 99th percentile of a cumulative frequency distribution of log concentrations. The industry's general recommendation is that the food safety risk

management system is functioning as intended if less than 1% of product units with 99% confidence in the test exceed the PO. Setting the value for PO and its tolerance are risk management choices since they unmistakably affect whether a danger is present that is deemed acceptable.

Making judgements about the frequencyPO value and the desired degree of control is also essential for determining if the food safety management system is functioning properly at the point when the FSOlots are created. When the proper scale of required FSOsPOs has been set down by the government. Also, industry should routinely check that their food safety management system is working as intended while in continuous operation. Control authorities may depend on inspection processes to assess whether industry-adopted food safety management systems are adequate for operating FSOPOs from both a government and industry standpoint.FSOs and POs vary from microbiological criteria in a number of areas, while first seeming to be comparable. The FSOs provide an outcome-oriented aim for the whole food supply chain, but the HACCP and GMP systems and applications of performance criteria, processor acceptance criteria, do not.

FSOs and TLR are Articulated in this Way

Drive the necessary improvement in the pathogen-product combination's food safety status in light of a long-term public health objective or the requirement to mitigate a food safety status that is deemed inadequate, such as by aiming for more stringent food safety industry regulation or a shift in consumer behaviour.

In both situations, industry is obliged to establish one or more suitable POs, PCs, and other control measures based on coordinated interactions different food business operators in the relevant food supply chain, in order to achieve a level of food safety in conformity with the FSO.The government may establish POs to direct a specific industry in implementing appropriate control measures at particular points in the food supply chain, for example, when the government believes that this industry typically lacks the resources to enact such measures on its own or when such measures are crucial to the effectiveness of the overall food supply chain.

While considering a PO as a viable risk reduction measure, competent authorities may make reference to FSOsinformation on the dynamics of a hazard between the site of consumption and the upstream point in the food supply chain. Use quantitative risk assessment techniques created for the relevant pathogen in a specific diet, ideally for POs to ALOPs.To coordinate overall management and guarantee that, when an FSO has been established by government, the food safety status of a food anticipated at the point of consumption is properly attained, industry may find it advantageous to establish one or more POs throughout the food supply chain. Any of the techniques used by competent authorities for generating POs from FSOs may be used by individual food business operators operating in and along the food supply chain. Operators of food businesses may establish a PO based on an FSO established by a competent body or on an assessment of the destiny of the hazard in the particular food supply chain, which eventually leads to an estimate of the risk.

So, both industry and regulators can easily understand and use the principles of FSO and PO. They also have a great deal of practical utility. FSOPO has been met, so. Additionally, operations that have been created and verified to fulfil the relevant FSOsGHP and HACCP standards may be trusted to produce food with a high degree of accuracy.

There is no need to create an FSO for every meal or every identified hazard-food combination. An FSO may not be required in certain circumstances because the possible microbiological risks associated with a product pose such a low danger when consumed. In

other instances, it is impossible to pinpoint the items for which FSOs should be established since the origins of a disease are so unpredictable. Shigellosis is an example of the latter, which may spread via a variety of channels, most of which are more significant than food, and it is unpredictable which particular food may be involved next.

The FSO is also helpful for assessing the safety of new items. When novel foods or new goods are introduced to the market, their safety must be at least as good as that of similar items already on the market. New infections and novel pathogen-food combinations are always being found as part of the investigation into foodborne illness. An example of a newly identified food-borne pathogen is the development of listeriosis as a foodborne illness in the 1980s as a consequence of outbreaks linked to coleslaw and Mexican-style cheese. The discovery that raw vegetables and unpasteurized juices may act as carriers for E. coli O157:H7 is an example of a novel pathogen-food combination. To stop more instances or outbreaks, a prompt action may be required in such circumstances. The publication of an interim FSO might be the first step in informing the food sector or exporting nations of the highest level of danger at consumption that is accepted. An interim FSO may be modified when new information regarding the risk, the food, and the illnesses that they cause becomes available and efficient control strategies can be identified.

As previously said, risk managers in government might utilise FSOsPO to inform affected stakeholders of the amount of control anticipated and compel the necessary adjustment.If imported peanut goods are tainted to the same level or less, they cannot be rejected under WTOkg. FSOs provide a way to put into practise the idea of equivalency found in WTO Agreement on Sanitary and Phytosanitary Measures. Members must recognise the SPS measures of other Members as comparable if the exporting Member can prove, via objective evidence, that its measures meet the required level of sanitary or phytosanitary protection for the importing Member.

Completion of an FSOPO

When risk managers are unable to commission comprehensive qualitative or quantitative risk assessments due to a lack of time or resources, they should focus on the hazards that are deemed bearable. Before an FSO can be completed, some fundamental information is required. In order to guarantee that these fundamental requirements are addressed, panel members should be chosen based on their expertise, experience, and access to information. The panel must, at the at least, be knowledgeable about the microbiological risk, its potential source, the circumstances that may exist throughout the food chain and result in foodborne disease, as well as the variety of host susceptibilities. A mix of passive and active epidemiological programmes, case-control studies, and other related public health research, such as those mentioned above, may be used to shed light on the association between the microbiological hazard, the food, and the illness. studies on foodborne illnesses.

CHAPTER 5

EVALUATION OF RISK BY DETAILED QUALITATIVE

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For instance, before a relevant FSOPO on the concentration of S. aureus or on the concentration of staphylococcal enterotoxin in the meal, it would be necessary for the pathogen to grow on cooked ham during storage at room temperature to the high cell concentration necessary to cause disease. As another example, the incidence of listeriosis has ranged from 0.1 to 1.3 per 100,000 per year in a variety of nations. When the cars were found, there were several organ- isms in the food. Since *L. monocytogenes* is widespread and low concentrations are regularly consumed by consumers without having a negative impact, the ICMSF, speaking as an expert panel, suggested that such low concentrations are unlikely to pose a risk to healthy consumers and, as a result, proposed an FSO of 100 cfu. Qualitative Risk Evaluations

The goal of a thorough qualitative or quantitative risk assessment is the same as that of the expert panel risk evaluation discussed above: to offer risk managers scientific guidance so they can choose the risk management strategy to implement in order to reach the desired level of consumer protection. In-depth qualitative or quantitative risk assessments include specialists from a variety of relevant fields, including those with computer or mathematical competence in the case of qualitative assessments. These experts are comparable to those on the expert panels listed above. Because of the inherent complexity of the food safety problem, the need to gather and review pertinent data and information, to conduct some level of research into significant data gaps, or to develop and validate new data handling models, such assessments typically have longer timelines for eslishing risk evaluations.

Exposure Evaluation

The prevalence and degrees of microbiological contamination of the food product at the time of consumption, as well as the quantity of the product ingested at each meal by various consumer groups, are estimated through exposure assessment. Programs for measuring food intake and consumption patterns are often offered nationwide and may be used to calculate exposure. Measuring the amount of pathogens at the moment of ingestion may be the extent of the exposure evaluation. Nonetheless, models are created to predict how elements such the prevalence of pathogens in raw components, the possibility for pathogen development in the food, and the influence of handling and preparation techniques affect the frequency and levels of pathogens eaten. Probable exposure estimates for pathogenic bacteria have been developed using data from baseline surveys of pathogens in foods and predictive microbial modelling methods. Food inspection data from many nations have amassed a significant quantity of information on microbial levels, which might be used as another source of data on the microbiological state of foods just before consumption.

To ensure that the findings from several research are similar, the sensitivity, specificity, and validity of sampling and testing procedures should be taken into account. Nonetheless, there may be genuine variance owing to ecological circumstances, different food safety control systems, and different animal health management programmes. Some apparent discrepancies in pathogen prevalence in the food chain may be attributable to under-reporting or

methodologies applied. For instance, temperature management in food delivery networks varies from nation to nation. The variations in societal cultural, social, economic, or demographic systems that may affect consuming habits and behaviours should also be taken into account in exposure analyses.

Risk Assessment Via Detailed A certain group of customers will have qualitative sickness after exposure to a specific quantity of a pathogenic microorganism's and toxin. A microbial pathogen or microbial toxin exposure may have a variety of outcomes, from no noticeable effects to infection without signs of sickness, acute illness, long-term effects or sequelae, and even death. The susceptibility of the host, the characteristics of the food in which the pathogen is carried, and the characteristics of the microorganism itself all play a role in the likelihood that exposure to a specific dose of a particular pathogen will result in any one of these outcomes. These characteristics of the microorganism itself, of pathogenesis, virulence factors, and ability to resist the host's defences) vary among strains and may be altered by prior conditions.

The ability to determine the relationship between the population's frequency of exposure to different numbers of the pathogen in the food at the moment of consumption and the number of illnesses per year has to be achieved in order to derive estimates of the numbers of pathogen that may cause illness and the severity of illness relative to dose from experimental studies with humans, from animal models, and epidemiological data.

Characterization of Risk

To provide a comprehensive picture of risk, risk characterization brings together the data produced in hazard identification, exposure assessment, and hazard characterization. The outcome is a risk estimate, which, for example, shows the amount of illness that will occur in a population over time as a consequence of the given exposure. The risk to the public health posed by consuming untreated raw milk is examined in 2.4, which includes an example of a risk categorization. This study was conducted in New Zealand and took into account a number of risk scenarios related to various milk production and handling procedures, such as the temperature of milk storage at the farm level.

In order to evaluate the reliability of the risk assessment's models, data, and assumptions, the resultant risk estimate should, if feasible, be compared with epidemiological data or other sources of reference information. The risk estimate should provide a distribution of risk that accounts for elements that might alter the pathogen's growth or inactivation, the variety of people's reactions to the microbial pathogen, and the estimate's level of uncertainty.

Insights into the nature of the risk that cannot be captured by a straightforward qualitative or semi-quantitative statement of risk should also be provided by risk characterizations. Examples include identifying the most significant factors that contribute to the average risk, the uncertainty and variability of the risk estimate, and gaps in data and knowledge. Any default assumptions made available to the risk assessment team should have their effects recorded. The risk manager may explore risk management choices by using the risk assessor's ability to compare the efficacy of various risk reduction strategies. If necessary, the calculated risk estimate may be compared to the acceptable level of risk set by governmental risk managers. If the calculated risk estimate is greater than the acceptable level, steps should clearly be made to lower the risk.

The phrase "deterministic" or "point estimate" risk assessments was used to describe these risk estimations. The variability of various and dynamic biological processes is overlooked in these methods, and it is also not taken into account how much ambiguity there may be in the data and how it can affect the risk estimate. Similar to the "safety assessment" paradigm used

for controlling the hazards associated with chemical pollutants, deterministic risk assessment may be combined with the use of "uncertainty factors". For any parameter that is represented as a distribution of potential values, probabilistic evaluations reflect all the information that is currently accessible. Analytically, it is exceedingly challenging to create a mathematical description of the production and consumption of a meal using probability distributions. A compound model of food production incorporating pathogen development, destruction, and infection is too difficult to analyse without computer tools, even though some analysis is practicable on extremely tiny and basic models. Using commercial tools, probabilistic risk evaluations for food safety are possible. An analytical technique for models incorporating probability distributions is the Monte Carlo simulation.

Creation of an FSO on the basis of a Quantitative Risk Assessment

Risk analyses may be used to show how the frequency and are relatively erratic and unpredictable. As a result, it is seldom simple to capture the dosage response connection in a risk assessment research. To describe the effect of a danger on consumers by a hazard characterization curve so that risks may be calculated, however, educated decisions will need to be taken. A hazard characterization curve's slope depends on the risk, the food, the illness, and the consumers for whom it was created. An ALOP or TLR value may be placed on the y-axis and the matching degree of danger, which indicates the FSO value, can be found on the x-axis if such curves for the incidence of illness for a particular pathogen-food combination are available. Risk managers may need to adopt a more circumspect stance and choose a more rigorous FSO value that is established at a lower level of the hazard due to the uncertainties and variability that underpin the establishment of hazard dose-consumer response connections.

The public health objective is often to get close to "zero cases" for a particular pathogen-food combo. While this is not always possible, it could encourage the adoption of FSO values that are more conservative. The price of achieving this aim, nevertheless, can be more than what a community is willing to bear. It must be kept in mind that the FSO will depict how the costs of minimizing risk and the costs of tolerating risk are balanced.

It is crucial to remember that when evaluating hazard characterisation curves like those, infectious agents' connection between consumer risk and hazard level may seldom, if ever, approach zero until the hazard has been eliminated. Instead, the population size and the period need to be changed when the expected incidence decreases below unity. Even though the anticipated value of the risk to a population is less than one case per year, this does not mean that there is no danger.

Implementing Control Measures to Meet FSO and PO

Before launching a new food product on the market, FBOs must complete two crucial stages. For each food product, they first create a verified product and process design that will satisfy the relevant safety target. Finally, this design is practically executed by responsible authorities using the proper risk management procedures at the point in the food supply chain where the FBO is operating. Operators of food businesses may define their own goals if authorities haven't already done so, either in the form of POs or other formal forms. Objectives are in any case met in actual operations by implementing one or more controls intended to avoid, eliminate, or lessen microbiological dangers, such as those covered in this.

Please take note of the definitions of FSO and PO that were reached at the level of Codex Alimentarius. The greatest frequency and/or concentration of a risk in a product at a certain point in the food chain prior to consumption that gives or contributes to an FSO or ALOP, as applicable, is the food safety objective.

Control Techniques

While microbiological criteria have been crucial in determining which food microbiological specifications have been accepted, they cannot be taken alone as actually beneficial for the management of microbial dangers. It was acknowledged in ICMSF Book 4 that the implementation of specific, focused control methods should get greater attention than food microbiological tests. The importance of testing to confirm the efficacy of a food safety management system as well as the use of data to evaluate process control and product acceptance. It did this by providing real-world examples of useful methods that could be used for a variety of product categories, as well as for different types of foods and processing environments. The most effective control methods are recommended for each product category based on the ideas presented in ICMSF, an updated version of which this book is. ICMSF advises food makers to regulate their control methods, processes, and operating environments to ensure that their products would fulfil the necessary microbiological standards if sampled rather than relying only on microbiological testing as an effective control measure. To assure the safety of final goods, numerous food producers and other food industry operators throughout the globe have used food safety assurance systems in the present. These methods depend on the selection of safe product designs and manufacturing procedures for the foods as well as the supervision of food operations via the use of HACCP and precursor programmes. In doing so, programmes based on the concepts of Hazard Analysis Critical Control Point are used, such as clear safety expectations, FSOs, or Good Hygiene Practices.

The first program's excellent practices are a component of a larger collection of systems collectively known as pre-requisite programmes. The fundamental sanitary conditions and procedures that must be maintained in order to produce safe foods are referred to as good hygiene practises. It also involves other auxiliary tasks like choosing raw materials, labelling and tagging products, or recall processes. GHP application that is effective serves as the building block for the development and implementation of the second programme, HACCP. A methodical approach to the identification, assessment, and management of all categories of food safety risks in a food operation is required for the establishment of an effective HACCP system. On the other side, failing to build and maintain precondition programmes might render a HACCP system ineffective and lead to the production of contaminated food. Consider the risks that are most likely to exist in each individual food operation. Pay close attention to the components of the precursor programmes, particularly GAP and GHP, as well as HACCP, that will have the most impact on risk management.

Suitable Hygiene Procedures

The General Principles of Food Hygiene emphasize the essential programmes connected to cleanliness and list the following as the main GHP elements: design and facilities.

1. Operation management.
2. Routine maintenance and cleaning.
3. Personal grooming.
4. The act of travelling.

Consumer education and product knowledge are expected to have a big influence on reducing the risk. For instance, generic incoming material requirements may be utilised to manage the risk of certain chemical and biological dangers being employed in a variety of finished items all created without a heating phase to decrease risks). If a food product can be depended upon to be properly heated as a stage in food preparation, then incoming material requirements related to biological risks may be of less relevance for a food product that will be thoroughly

boiled to eradicate any possible enteric pathogens. Thus, not all food businesses give the different parts of a prerequisite programme, like GHP, same weight. In order to effectively limit these risks, GHPs must be applied after taking into account the general hazards that are most likely to exist across all of the foods produced in a certain operation. This does not imply that the other GHP elements are not important. Some, for instance, can be crucial in ensuring that a dish satisfies established quality standards.

Some GHP components may have special relevance in specific circumstances and should be included in the HACCP strategy. For example, for big continuous ovens used to cook meat products, equipment calibration and maintenance are crucial. In this case, a verification method for the HACCP plan may include the process and frequency for doing checks on heat distribution during cooking. The accuracy of the thermometers used to monitor oven temperatures during cooking must often also be confirmed.

a broad variety of epidemics connected to environmental contamination brought on by subpar equipment design, subpar raw and cooked food separation, subpar equipment maintenance or cleaning, or subpar transportation control management. A high proportion of outbreaks from food service establishments may be related to inadequate personal hygiene in addition to environmental contamination. ICMSF volume 4 discusses information on sanitary design of facilities and equipment, cleaning and disinfection, staff health and hygiene, and education and training.

Critical Control Point Analysis and Risk Assessment

The following seven guiding concepts are included in the Codex publication on the Hazard Analysis and Critical Control Points System and Guidelines for its Application:

1. Do a risk analysis
2. Identify the crucial safety valves
3. Critical limitations for Eslish
4. Eslish surveillance techniques
5. Correctional measures and verification methods in Esl
6. Eslish practices for preserving records and documenting things

A systematic approach to the identification, assessment, and management of food safety risks in a food operation is required for the establishment of an effective HACCP system. The steps to be done in a food operation to manage food safety concerns are specified in HACCP plans. Moreover, HACCP plans outline the records that must be created throughout the process in order to verify that critical limits have been fulfilled at the operation's Critical Control Points. This could require gathering and examining samples from the whole ambiguous food supply. The methods for food sampling outlined in this article may be used to evaluate a questionable lot's safety and determine how to properly dispose of the food.

"A step at which control may be applied and that is important to avoid or eliminate a food safety hazard or decrease it to an acceptable level," according to Codex Alimentarius, is what is meant by a crucial control point. The HACCP team and the other competent authorities have the final say on what constitutes an achievement level. The concepts of FSO and PO may be utilised by competent authorities to explain to food company operators the degree of control required for a danger to be decreased to "an adequate level," as was covered in the previous two sections. Operators of food businesses may also utilise the PO idea to coordinate amongst FBOs throughout a food supply chain.

Food company owners must carefully utilise precondition programmes like GHP and the concepts of HACCP to build and execute a whole food safety management system that will control the major dangers in the foodPO in order to produce safe food or food components.

To that aim, they may provide the industry default or "safe haven" recommendations on control mechanisms that, when properly implemented by Industry, would be able to assure that the specified goal is attained consistently. As previously mentioned, such measures may consist of a single control measure or a mix of control measures. Advice may also be given on possible handling and process-related concerns that the industry has to be aware of in order to establish the necessary efficient food safety management system.

To meet the PO established by a government or the operator of a food business, food manufacturers design processes at a specific step in the food supply chain, making sure they can deliver the Performance Criteria across the step that is needed to convert the hazard level entering the step to the PO. The influence of the PC on frequency and PO is technically possible. Governments that are thinking about establishing an FSO and PO should choose one or more control measures that are operationally managed via GHP and HACCP. With the impacted food industry owners, using the FSOPO was agreed upon. When choosing a control measure for their food safety management system, individual food business operators, for example, will choose one that is validated to meet the overall Performance Criteria necessary to meet the POPO. If this option is found to be technically impossible with the available tools and resources, other options could be pursued.

The FSOPO Is Technically Achievable time may be modified by the government in stages. If no technically feasible fixes or acceptable adjustments can be identified, it could be required to outright prohibit the product or change the POs for certain food items. The FSO, as previously mentioned, is the highest amount of a hazard that a regulatory body would permit in a product at the time of consumption and that, in theory, in accordance with Codex, allows for or aids in ALOP. A risk-based restriction that should be operationally attained throughout the food chain is articulated by competent authorities by establishing an FSO, which also allows for flexibility for various production, manufacturing, distribution, marketing, and preparation methods. It's a good idea to have a backup plan, especially if you're going to be away from home for an extended period of time. For all, at this stage in the food supply chain, it would be exceedingly impractical to verify that the FSO has been satisfied within the context of regulatory enforcement.

An operational risk-based limit for the highest hazard level permitted in a product at a particular point in the food chain is thus provided by the establishment of POs by a risk manager, which should not be surpassed if one is to have confidence that the FSO or ALOP will be maintained. A PO's value should take into account the effects of the stages in the food chain both before and after it since it is conceptually connected to the FSO and ALOP.

As part of the adopted food safety management system, control measures must typically be put in place at one or more various phases in the food supply chain in order to accomplish the stated FSO or PO. The consequences in terms of the degree of risks may either be actively regulated at this point, for example, by implementing control measures that restrict or lower the prevalence andFSO.

Control measures may be adopted so that the change in hazard level throughout a specific step is actively managed when achieving a safe product and process design and implementing this in the food safety management system of a step. Codex refers to the Performance Criteria as the expected result of the control measures used in the food safety management system throughout a specific phase in the value chain. The PC is described by Codex as the impact in frequency and MC, Process CriterionPdC, and the anticipated degree of public health protection, first specified at the population level and then translated to supply chain level expectations. An operation that has a particular food safety management system in place,

such as primary production, slaughter, storage, distribution, manufacturing, retail, or food service, would be considered a phase.

Typically, a number of different control methods are used in concert to achieve a certain PC for PCs spanning a stage in the value chain, such as cold storage of ingredients, heat processing, quick cooling, physical separation of raw and processed materials, and packing. The justification for this is because several control measures provide important contributions to the PC that cannot be satisfied by a single measure, such as preventing recontamination after a significant reduction step, lowering hazards to target levels, or regulating the increase of hazards. The degree of danger in the end product may not be under control and the POvegele juice if a very significant decrease in hazard levels by pasteurisation or sterilisation were not followed by methods that regulate recontamination up to the time of packing.

Operators may choose particular equipment and process criteria that are best for their operation and product in order to achieve the savings required by these performance requirements.In order to reach the desired hazard level in the material or product exiting the step, additional control measures are often required to support such hazard reduction processes in concert.In this context, the term "PC" might be used to refer to the necessary overall control of a hazard in a step, which is operationalized by achieving the requisite individual control measures to, for example, restrict growth, minimise a hazard, andFSO is fulfilled.

While establishing PC values, it is important to take into account adjustments that may be necessary to a hazard's starting level, which is referred to as "H0" in the text below, in order to provide the PO or the FSO.Events or opportunities throughout a phase may influence the degree of a hazard in a food or the proportion of component components that end up in the final product. Single events may sometimes cause increases or decreases, but several occurrences at various periods in time throughout an operation might also have these impacts. All of these could have an effect on how a hazard actually behaves as food materials move through a step and must be understood and managed to ensure the frequency] of safe food materials, which presupposes a specific concentration of a hazard across all portions of a food material steps in the value chain.

Advice on the equation's broad applicability as well as for particular circumstances where a more detailed understanding of the hazard dynamics inside a step necessitates specialised techniques will be provided. However, even at the high level that the equation may be used across a step, in many cases it may support decisions related to the design of a suitable food safety management system at that step and assist in evaluating potential control measures that may be able to achieve a desired outcome target, such as a specified PO100 g or 2 log10 cfug. Due to no rise in the degree of danger throughout the stage, both expansion from the original population and contamination from outside sources may be fully avoided. The PC was necessary to convey the PO PO across the step. This is comparable, for example, to a performance standard that achieves a 5D decrease in the pathogen level together with control measures that entirely prevent re- or cross-contamination from occurring after the 5D reduction treatment.

It is crucial that the operator of the preceding step's food safety management system, which provides the incoming material to the present step, ensures that the outgoing danger level is less than 1000 cfuPO. When a supplier is delivering the appropriate entering raw material or component, it may be necessary to establish a strict microbiological criteria to ensure that the incoming danger level is no more than 10 cfuPO. For instance, a H0 = 0.1 log10 cfug beginning population after storage for 14 days at 8°C would produce a R 0.8 log10 cfug: H0 R + I = 2 0.1 0.8 + 2.7 = 2.This strategy might be regarded as accomplishing the FSO

precisely. The data from Szabo et al. are used in the example that follows for illustration purposes. However, in order to fully understand the impact of process variation it is necessary to move from point estimates to distributions that describe the variability of control measures in the risk management framework.

Suppose that I has a standard deviation of 0.59 and that the distribution of L. monocytogenes' log growth is regularly distributed. H0 and R levels do not take variation into account for the sake of computation and explanation. In order to consistently fulfil the FSO, the producer must aim for a lower average level of L. monocyto-genes in the final product due to the dispersion of I. If the same average level was increased by the threat's expansion

C stands for the cumulative increase in risk via cross-g or at 1 cellg or 1000 cells. Recontamination and mixing both function as "additives" on a linear scale rather than a logarithmic one.It should be noted that using characteristic numbers as outlined by Zwietering may be a straightforward method of estimating the log values for the different factors in the conceptual equation, including those for increase via growth and contamination.

Transfer of danger cells onto a bulk of food stuff that is being handled constitutes a contamination event during a step. There are thus 7 log10 cells in the whole 1000 kilogram me batch. Suppose that one gramme of material contaminated has 1000 cells. A level of 10,001,000 cells in 1000.001 kg will be reached as a consequence of this occurrence, which will add 1000 cells to the batch. 10.001 cells make up the new danger level concentration. 0.00101 cfug is the concentration in a batch of 1000.001 kg. Depending on how serious the threat is, going from 5 log10g equals a 2 log increase in total concentration and may have a substantial effect on public health.

Interestingly, there is a tipping point in the impact of the size of post-process contamination compared to the magnitude of hazard reduction for the batch and contamination event presented in the final case above. This is shown in 3.10 for a batch of food weighing 1000 kg and calibrated at 1 log10g. This demonstrates that for inactivation levels below 4D, the amount of danger left over from H0 significantly influences the final level of hazard, but for inactivation levels above 4D, the amount of post-process contamination significantly determines the final level of hazard.

Criteria for Process and Product

Implementing process requirements, such as the duration and temperature of a heat treatment, together with other elements like a lowered pH, water activity, or shelf-life are advised, might satisfy a performance criterion. The Codex Alimentarius-issued Principles and Guidelines for the Conduct of Microbiological Risk Management give more examples of how to relate product and process requirements to performance objectives.

Usage of Performance Criteria and Microbiological Sampling

More and more people are realizing that end-product testing is not as successful as food safety management systems centre on reducing risks via GHP and HACCP. There are two recognised applications of microbiological criteria in the ICMSF framework for controlling microbiological risks:

1. To confirm that the performance requirements for the controls are met.
2. To assess the suitability of a product when there are no other viable options for ensuring its safety, i.e. when it is unknown if GHP and HACCP have been correctly implemented

In order to produce safe foods that fulfil the FSO or PO, as necessary, a variety of control procedures or choices may be used. Yet, it still has to be shown that these metrics are

equivalent to the established performance criteria. This may be described in terms of the frequency or concentration of a microbiological hazard in the food for a variety of procedures and products.

The "defect rate" has traditionally been used to describe how well sampling strategies and microbiological criteria operate. By linking the effectiveness of attribute strategies to the concentration of a danger, the fraction of faulty samples may be calculated. This is done by utilising the distribution of bacteria. It is necessary to assume homogeneous distribution or random sampling.

Understanding the likelihood that a certain sample strategy would identify a specific amount of product contamination and then reject a non-conforming batch is helpful. It has been shown that contamination is often not evenly spread over a lot. In other words, a population is not characterised by a single distribution but rather by a combination of many distributions. The mean concentration is often not constant at the size of a lot or between lots, but instead fluctuates according to a lognormal distribution. The number of colony forming units in a sample changes arbitrarily in accordance with the Poisson distribution, yet at the local scale of a sample, the mean concentration may be thought of as constant.

In many cases, just a small number of samples from a tainted batch will test positively. Nonetheless, these few could be able to spread disease. As a result, while choosing or creating an attributes sampling plan, it is important to make sure that the average concentration in the batch is sufficiently low to guarantee that, under a certain level of confidence that takes variation into account, no samples from the batch have non-acceple levels.

The lack of any positive results is sometimes used as proof that there are no contaminants in the whole batch when an attributes sampling strategy is based on the detection of a microbe in a certain amount of food. It is more accurate to say that presence25 g or 0.04 cellsg. In these instances of low to extremely low pathogen concentration, this overly simplified technique implies that cells are evenly dispersed across the lot, and even under these conditions, the likelihood of finding a positive in the sample is not 100% but rather just 63%. It is important to take into account the variation in cell concentration within a lot and the randomness of collecting microscopic particles within larger samples. Because it cannot ensure detection, obtaining more random samples increases the confidence that the findings are typical of the whole batch.

As compared to absence in the sample, extremely low pathogen concentration levels are normally deemed present. This is because when fewer objects are detected in the sample, the sampling error is bigger. Absence approaches rely on the observation of only one or a few cells in Poisson processes, where the standard deviation is equal to the square root of the mean number of target cells. Hence, although the standard deviation associated with a count of 100 cells is 10%, the standard deviation for a test requiring observation of a single cell approaches 100%.

It has been shown that foods often have a log-normal distribution of microbe content. Hence, provided the overall geometric mean and standard deviation are known or can be deduced, the normal distribution of log counts may be used to predict the fraction of faulty samples in a lot. The standard deviation can never really be known, however. That has to be calculated. Estimates of these values, however, may be used to ascertain the relative likelihood of accepting a faulty food lot for a certain sampling strategy.

A sampling strategy can never accurately determine the lot's mean concentration. Only at a certain degree of confidence can it estimate the concentration. Knowing the quantity and size

of the samples examined as well as assuming the variability in cell concentration within the lot are necessary to evaluate the effectiveness of a sampling strategy. The detection threshold of a given characteristics sampling plan may be interpreted while taking the Poisson effect in sampling into consideration. There includes information on sampling strategies as well as a spreadsheet tool that makes it possible to do the required calculations and take the Poisson effect into account.

Using a variety of standard deviations, ICMSF utilised the tool to determine the geometric mean that yields a 5% chance of lot acceptance under the various sample strategies advised in this Book 8. Because the actual standard deviation of the distribution of contaminant concentration in a lot is uncertain, the examples in the ICMSF's Appendix A take a variety of cell concentration distributions into consideration. The standard deviations utilised only account for variance related to analytical techniques and are applicable to the distribution of cell concentrations.

It could be useful to establish default criteria for certain control measures in the absence of an FSO. These fail-safe standards, created by advisory committees of experts or control authorities, are necessary less flexible since they are designed to prevent dangers under "worst-case" scenarios. They could first anticipate a larger than usual degree of a risk and its products;

Additional Expertise

Each approach has advantages and disadvantages, and in certain situations, it is desirable to employ many methods for validation. Data gathered via laboratory challenge tests may include information on food, culture medium, or other suitable materials. Nevertheless, this entails the employment of surrogate test microorganisms. Challenge studies in a food processing setting may give a better degree of confidence about the capacity to achieve performance parameters. For the purpose of process validation, pathogenic microorganisms should never be introduced into the environment used for food production or processing. It could be conceivable in certain circumstances to monitor changes in the population of naturally occurring pathogens while a procedure progresses. For instance, such research might be carried out when meals are being prepared and processed from raw agricultural products. In a perfect world, validation would include laboratory challenge testing using pathogens, followed by revalidation once the control mechanisms have been put in place. This, however, could not be feasible when a disease is uncommon and a high number of samples are required to provide useful data.

CHAPTER 6

LABORATORY CHALLENGE TESTS

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Pathogen intrinsic resistance: Studies to assess a pathogen's resistance to various factors that may be included in a control measure should be performed using a variety of strains, including isolates from the food in issue that are involved with an outbreak. While establishing effective control settings, strain resistance is a crucial consideration. The inocula should be produced under circumstances that result in pathogen resistance suitable for the technique. For instance, pathogenic E. coli and salmonellae vegetative cells that exhibit the greatest resilience to heat and acidic conditions while in the stationary phase after having been cultured at high temperatures should be employed. It is important to utilise enough pathogens to completely eradicate any biological variability effects.

When they are not linked to public health issues for the specific food or circumstance at hand, isolates with unreasonably severe resistances or growth characteristics should not be included in the strains to be evaluated. Since heat-resistant Salmonella spp. have been linked to outbreaks involving chocolate, roasting is the single most crucial pathogen inactivation step in this scenario. For instance, Salmonella Senftenberg 775W is appropriate to evaluate survival of Salmonella spp. during the bean roasting step of chocolate production. As a result, the test organism serves as a highly heat-resistant contaminant to use in determining if the thermal process design is adequate. Salmonella Senftenberg 775W is not the ideal non-pathogen to use for design validation since Salmonella spp. linked to outbreaks involving liquid egg production are not very heat-resistant. If non-pathogenic microorganisms have been proved to have the same growth pattern or resistance as the pathogen of concern, then using them to validate control methods in a food operation may be done. For instance, in thermal process validations for almonds, dairy products, meat, and juice.

The food's composition. Inactivation, survival, and kg may all be impacted by dietary composition, and assumptions regarding spore loads can be employed for numerical analysis and risk evaluations. Moreover, there could be chances to get microbiological information from samples taken during food processing. This in-plant data might be utilised to validate a process or to validate laboratory findings. An appropriate instance for in-plant validation is monitoring changes in the population of a pathogen in raw materials while the food is being prepared. Therefore, it could be required to track changes in the population of a non-pathogen with resistance to the pathogen that is comparable to or larger. For instance, when the numbers or frequency of the disease are too low to provide useful data, this may be essential. Season, operating area, raw material supply and kind, as well as processing circumstances, may all have an impact on a pathogen population's unpredictability. While gathering information for use in process validation, these and other aspects should be taken into account.

Variability in Process

While establishing the critical limits connected with control measures, the variability that takes place in a food operation must be taken into account. Performance and dependability of the equipment, the integrity of container seals, processing durations and temperatures, pH,

humidity, flow rates, and turbulence are a few examples of characteristics that might affect a process' variability. Setting critical limits must take into consideration the diversity of process factors and product composition. In general, the conditions required to manage a hazard as stated above may be closer to the critical limits at a CCP for a process CCP running under a high degree of control. The essential limits must be more cautious and restrictive for a process that is less well regulated. In other words, critical limits must be predicated on the process's capacity to meet a certain requirement under typical operating circumstances while taking variability into consideration. In order to identify when the process is running outside of this typical variability, monitoring and verification methods outlined in a HACCP plan should be created.

Correct implementation of three distinct processors has been done. For instance, pH measurements, process standards, and microbiological standards. The first is the UK Advisory Committee on the Microbiological Safety of Foods' recommendation to industry of four control strategies to reduce the risk of psychotrophic C. botulinum in refrigerated prepared meals with a shelf life of more than 10 days. An aw of 0.97 or less throughout the meal and across all components of complex foods, a heat treatment of 90 °C for 10 min., or equal lethality, and a pH of 5 or less were all advised. Option one aims to eliminate vegetative cells and spores of psychrotrophic strains of Clostridium botulinum that may be present in the raw materials used in the production of the food. It does this by using a combination of heat and preservative factors, which have been repeatedly shown to prevent growth and toxin production by non-proteolytic C. botulinum. The goals of options two and three are to stop the organism from expanding and, as a result, stop the generation of toxins. Option four can entail heat destruction or inhibitory substances to stop the outgrowth of C. botulinum spores that have survived.

Unstated performance criteria underlie each control choice. As a 10 minute heat treatment at 90 °C is what is expected to happen, the performance requirement for option one might be described as a 6D reduction in psychrotrophic strains of C. botulinum spores. Less than a 1 log growth of C. botulinum within the use-by date when kept at the advised storage temperature might be the performance requirement for choices two and three. The ACMSF report gives extensive background information on the likelihood that the danger may occur as well as potential controllable variables.

The danger of E. coli O157:H7 and related enteric foodborne pathogens in fermented sausages is the subject of the second illustration. On the West coast of the United States, a foodborne disease epidemic in December 1994 was brought on by E. coli O157:H7 in a fermented sausage product. In response, the U.S. Department of Agriculture established a rule requiring all manufacturers to use procedures that lower the risk of E. coli O157:H7-related disease. In this instance, the government suggested a performance standard and left it up to business to choose how to meet the standard while still producing goods of acceptable quality. The agency's suggestion for a 5D kill was based on little information indicating that up to 1000g might be used. One such sampling procedure would include analysing 15 samples that were taken when the meat mixture was stuffed into the casings.

Before distributing the final product, implement a hold and test procedure. 15 samples per lot of products that are meant to be cooked before serving would be taken. Items that are often eaten cold would be tested at a rate of 30 samples per lot. Each sample evaluated would have an analytical unit of 25 g.This option offers the adoption of alternative procedures that provide the equivalent of a 5D decrease in order to accommodate new technologies or concepts.The goal of each choice is to guarantee that the raw sausage mix contains no more than 1 cellg of E. coli O157:H7. The procedure requirement for option one is based on

research data showing a 5D kill of salmonellae and E. coli O157:H7 in beef and is drawn from an existing rule for roast beef. Since that the roast beef standard did not provide for a hold period at 62.8 °C, the 4 minute wait time was an additional need. The higher rate of death that would occur with a decreased pH of a fermented product would not be used by processors opting for this option. The second performance requirement calls for a 5D kill of E. coli O157:H7. This option requires the processing facility to have research data validating that the being employed method will result in a 5D death on file and accessible for evaluation. The validation study has to be created using a USDA-approved technique.

The third option includes a microbiological-based reduction stage as well as an elimination step. Microbiological tests and a 2D kill performance criteria are used to confirm that each manufacturing lot's E. coli O157:H7 level does not exceed 1g in the raw sausage mix. There is no appreciable decrease in sensitivity of detection when fifteen 25 g samples of raw sausage mix are composited for analysis, according to later studies funded by industry. It would be 95% likely that the amount of E. coli O157:H7 in the mix is no more than 1 cell125 g if, for instance, a processor chooses to gather 15 samples from throughout a lot during stuffing and have each analyzed using a 25 g analytical unit. For thirty 25 g samples, a negative result offers a 95% likelihood of no more than 1 cell4).

Using and Choosing Acceptance Criteria

Statistics on the food trade show how widespread the global food commerce is. For instance, the total value of international commerce in agricultural products for the year 2008 was between 1060 billion and 1105 billion US dollars. Around 61 million metric tonnes of food were imported into the USA in 2014, falling into 13 different categories. In addition to these flows of food products from one nation to another, it is impossible to assess the size of the intra-national commerce in food that occurs between food enterprises in each nation. All of these food items have to be secure and of high quality. Yet, customers and food companies must depend on their supplier to produce raw materials of acceptable safety and quality or else rely on the efficacy of governmental food regulations since the safety of a product cannot be verified by sight or smell.

In many cases, a government or food company lacks the dependable tools necessary to confirm the safety of all incoming goods. Commercial partners often engage into a "business contract" when product liability is a significant problem in acknowledgement of this constraint. As a result, commercial purchasers are able to set requirements for the food they are buying and have a wide range of tools for checking supplier compliance. For similar reasons, export and import regulations that might result in various types of verification are often adopted by mutual agreement between trading states and applied to international food commerce. Controls for governments, on the other hand, should be based on international standards, such as Codex Alimentarius, or else be founded on science and implemented solely to the extent required to safeguard human, animal, or plant life or health.

The use of criteria to assess the accuracy of specific lots or consignments of food as well as the accuracy of the food production processes will be covered in this article. Many criteria that are based on sensory, chemical, physical, or microbiological factors may be used to evaluate the acceptability of a large quantity of food. Further information, such as the quantity of samples to be collected, how and where the samples are collected and stored until analysis, the analytical unit, the technique of analysis, and what is regarded as adequate, should be included in the lot acceptance criteria. Through inspections or audits, commercial purchasers and control agencies may assess the accuracy of a food business. A choice on whether or not to sample a food may also be made based on the findings of an inspection or audit.

The food's country of origin has a significant role in determining how well it is accepted when it is welcomed at a port of entry. Foods cannot be inspected and approved by the control authorities of an importing nation while they are being manufactured in another nation or area. As a result, the sanitary precautions connected to the food inspection and certification system in the exporting nation or area must be relied upon. Thus, it is required to design a framework for acceptance based on mutual agreement between the authorities in the participating nations in order to ease the movement of foods across international boundaries. The SPS Agreement established the idea of equivalence. It acknowledges that having comparable inspection and certification processes in exporting and importing nations is not always feasible, but it is nevertheless important to determine if they provide an equal degree of consumer protection. According to Codex, equivalence is "the situation where sanitary measures employed in an exporting nation, albeit distinct from those applied in an importing country, accomplish, as shown by the exporting country, the importing country's adequate degree of sanitary protection. The exporting nation is responsible for making the case for equivalentity. But, the importing nation's sanitary measures must also be compliant with its own ALOP, and they must be able to prove it. It may not be required to apply additional acceptance criteria at the port of entry if equivalence can be agreed upon. Both importing and exporting nations are subject to responsibilities under the Codex Alimentarius process for determining equivalence. The reader is advised to the relevant Codex standard for further information.

The obligation to show that a sanitary measure or group of measures fulfils the ALOP of the importing country is one of the main obstacles in the achievement of equivalence of various sanitary systems. The SPS agreement permits ALOPs to be expressed either qualitatively or numerically, although few nations have done so, and even when they do, they are almost never quantitative. In Australia, for instance, the ALOP is described as "offering a high degree of sanitary and phytosanitary protection, aiming at lowering risk to a very low level, but not to zero." When the statement "extremely low level, but not to zero" is not accompanied by a quantitative definition of what is intended, it is difficult for an exporting nation to prove equivalency against such a qualitative ALOP. These choices always include qualitative evaluation and discussion between the exporting and importing nations, which may be problematic.

Codex Alimentarius has eslished the notion of 'objective basis of comparison' to further facili- tate equivalency talks. This enables the exporting nation to show that their control measure produces the same results as those anticipated by the control measure employed in the importing nation. This is predicated on the presumption that, whether or not it is mentioned, an importing nation achieves its control measures to fulfil its ALOP. ALOP and FSO are interrelated. They talk about how the FSO is quantitative and how it provides for or influences the ALOP. Similar to the PO, the FSO is intended to be met or contributed to by a PO, which is a quantitative statement of the total quantities of microorganisms in a meal. Thus, this value may be utilised as the "objective basis of comparison" if an importing nation has articulated an FSO or, in certain circumstances, a PO. Given their inherent connection to the importing nation's ALOP, it is theoretically possible for the exporting country to show that a sanitary measure or set of measures for a food are equivalent based on the attainment of the importing country's FSO or PO. While the applicable Codex standard on microbiological risk management does not address the function of FSO and PO in the achievement of equivalence, it is a logical step that follows from the adoption of that standard and is in line with the stated purposes of FSOs outlined in Annex 2 of that document.

Approval criteria typically fall into three categories and may entail a wide range of parameters:

Standard: a condition that must be met in accordance with a law or legislation

A guideline is an advisory standard created by a regulatory body, an industry group, or a food processor to outline what may be anticipated when best practises are used.

Specification: A clause in a contract between a customer and a food supplier that specifies requirements that may be obligatory or optional depending on their intended usage. The establishment of food rules and policies that guarantee the safety of the foods for which they have regulatory authority is the obligation of the government. As a result, food control authorities act as risk managers and may create FSOs and, where necessary, POs for food risks. A FSO or a PO, details the results anticipated from food processes that are subject to efficient control techniques. A microbiological hazard's frequency or maximum concentration in the food may be used to convey the result. In addition, the FSOPO is a target that food operators may use to guide the development and implementation of their food safety management system, serving as a foundation for the establishment of acceptance criteria.

Standards

Standards may be established for a broad range of factors, but they are most effective when the danger to consumers is severe enough and adherence to the standard is necessary for ensuring consumer protection. Governments establish standards that specify the requirements that products or processes must satisfy in order to be in conformity with legal or regulatory requirements. Performance targets, performance criteria, and microbiological criteria are all examples of standards. Equivalence determination is sometimes combined with process criteria and product criteria. The Codex guidelines and those further specified should be followed when developing standards that specify microbiological parameters important to the safety or quality of food. So, in a perfect world, microbiological standards would be created after a quantitative risk analysis and the completion of an FSO or suile PO. European Regulation 2073 and other rules provide some examples of these standards, which often provide more information on what is regarded as best practise for a food manufacturing process. Government and business may both utilise guidelines as the foundation for determining the reliability of a food operation or food lot.

There may be published guidelines for a procedure or a product. When a procedure is involved, the circumstances that need to be under control for the food to be produced safely is outlined. The product features and microbiological standards that the operator should pursue in order to generate an acceptable product are often provided in a guideline. When best practises are used, such criteria outline a food's features. Guidelines could be the best way to let food businesses know that a certain area of the industry has to improve. In other cases, when inadequate information prevents the establishment of a standard, recommendations may be used to explain the circumstances thought to resolve a newly identified microbiological issue. Guidelines may also act as a stopgap solution, offering direction to food businesses until enough data and technology are available to establish standards. A guideline by itself could be sufficient to bring about the improvements required to increase food safety and quality, removing the need for standards. Examples of acceptance criteria in a guidance paper on the prevention of Listeria monocytogenes contamination of ready-to-eat foods and the standards for determining their safety. A regulatory government agency created the first example, while a non-regulatory government agency created the final.

Specifications

Food production must satisfy regulatory criteria as well as consumer demands. Food firms commonly specify purchasing criteria for food ingredients and other supplies to guarantee compliance with these standards as well as the company's own needs for quality and safety.

The need for the buyer to choose whether to accept or reject ingredients, food, and other commodities is implicit in the transfer process between buyer and supplier. The experience the customer has with the product being bought is the most crucial aspect in this selection. The value of having pre-established acceptance criteria that are agreed upon with their supplier is recognised by purchasers as they grow more sophisticated and aware of the danger connected with certain meals. Such buying requirements are intended to lessen the possibility of accepting an unaccepted product or component.

When an ingredient is added, the finished product must fulfil all standards for safety, quality, wholesomeness, and other factors that are important to the customer. Buying specifications specify the desired qualities of an ingredient. Nowadays, buyers in the food supply chain often establish their own requirements for the commodities they acquire, especially where the safety of the raw material or ingredient is a CCP in the buyer's HACCP plan. In some situations, a food company may also outline the operational requirements in the form of operating instructions or best practises recommendations. Examples of supplier standards are not often made public, however the World Food Programme has issued criteria of a similar kind.

Request for Approval Criteria

Control agencies are in charge of examining whether standards are being followed. Inspections of food establishments and, if judged required, food sampling and testing may be part of this. A kind of licence that is necessary for continuing operation is often issued to certain food manufacturing activities that are compliant with standards and other legislative requirements, depending on the jurisdiction. Compliance may lead to further benefits, such as a decreased inspection frequency, in other food enterprises where licences are not necessary. Sanctions such as the revocation or temporary suspension of a licence, a formal directive to change one or more procedures, or the recall of a food lot may be imposed for failing to adhere to regulations and standards. Some jurisdictions permit control authorities to shut down a food company in the interim awaiting compliance in severe cases when food safety has been seriously damaged to the point where public health is at jeopardy. Governments should arrange the findings of their evaluations to ascertain if patterns call for modifying rules or taking enforcement action. The control authority's decision to sample and test certain food categories for conformity with established criteria may be influenced by these data.

Control authorities may also decide to look into rule compliance. Governments often establish these regulations. Guidelines are not required to be followed, thus not doing so may just lead to suggestions for improvement. Nevertheless, control authorities often compile the results of testing against standards to analyse the whole food chain's compliance and determine if a standard should be developed after a risk assessment and the creation of any relevant risk management metrics, such as FSO or PO. Verifying conformity with recognised best practise may be done by internally or externally evaluating food businesses against standards. Compliance could also be required if the regulation is a component of a programme for industry certification that results in a certification of conformity in some way. Businesses in the food industry might create their requirements for suppliers using acceptance criteria in guidelines.

A method of evaluating and approving a supplier's overall system for the management of food safety and quality, like that detailed in is the best way to ensure that suppliers control the dangers. If a food company sets requirements, it might opt to audit suppliers directly or indirectly via a third-party certification agency. Depending on the supplier's history of compliance, this occurs less often after the first evaluation of a new provider. Vendors who are deemed to be insufficient are taken from the list of authorised suppliers. In order to ensure

compliance, suppliers of raw materials that must be tested for microbiological safety or spoilage often must test their goods themselves against the client's specifications and provide the buyer a certificate of analysis for the necessary parameters with each shipment. After receiving certain raw materials, the buyer may additionally evaluate them. Tests that are routinely performed may examine a number of variables, like the pH, AW, moisture content, etc. of the product. Microbiological examination for pathogens or indicator organisms may be used in other testing. The variables affecting the analytical findings must be taken into account regardless of the criteria. These significant aspects control the usefulness and dependability of the criteria used to distinguish between acceple and unacceple lots. More focus should be put on the circumstances under which foods are produced, i.e., the use of GHP and HACCP, given the limits of microbiological testing to assure the safety of individual food lots. Establishing Supplier Acceptance via Approval in Business-to-Business Partnerships

FSO and PO's role in approving a supplier

The first stage is to ascertain if an FSO or PO that has been established for a commodity is being fulfilled. If there is agreement on the accomplishment, additional specific information may be supplied on the con- trol methods that have been implemented to fulfil the FSOPO consistently. Throughout the auditing process, the FSOor microbiological tests may be employed for this reason. The number of lots that should be examined should represent dangers and risks and the penalties of non-compliance with the criteria. When it has been established that the supplier can dependably satisfy purchase standards, then product testing may be ceased or significantly limited. In contrast to the FSOPO via process control has better value than relying upon microbiological test- ing of incoming batches.

Approval Procedures

At each transfer point in the food chain there is generally a buyer and a supplier. Eventually, the ultimate purchase will be a customer. Buyers and suppliers might be in government as well as in business. Suppliers may be domestic or in another nation. The recommended way to controlling the safety of food is to pick suppliers that can be relied upon to regularly produce components or meals that fulfil food safety regulations. Food safety methods focused on prevention are significantly more successful than trying to discern safe from dangerous batches by microbiological testing. Although there may be a place for testing some products or meals, microbiological testing should be done judiciously and utilised as a complement to other information, particularly the circumstances under which the item is produced. A checklist of criteria that may be used to approve vendors appears. The total outcome of these operations is to build a base of suppliers that can be reliably relied upon to produce raw materials that will be safe when utilised as intended. This strategy demands that all parties be informed on the potential dangers that may be related with the meals offered. The FSOC onsignor Port of loading Vesselor providing nation for compliance with all eslished standards. Another is the anticipated consequence if an improper choice is made to accept a faulty batch. The extent of an adverse impact will depend on the likely presence and severity of a hazard in the food and whether its intended use can result in a decrease, no change, or an increase in the hazard prior to consumption.

Extensive knowledge about a food and the control system under which it was produced, leads to a high level of confidence that the ingredient or food will meet the FSOIEC 17021:2011. The principles are as follows:

1. Integrity of the auditors
2. Fair presentation

3. Due professional care
4. Confidentiality
5. Independence
6. Evidence-based approach

The audit programme itself, consisting of one or more audits, is broken down into a cyclical activity involving four main components called Plan-Do-Check-Act. The planning stage involves eslishing objectives for the audit programme and developing the details of the audit programme which in turn includes the personnel and audit procedures. The implementation stage details the activities involved in actual audit undertaken as part of the audit programme. The monitoring stage is where an overview of progress is maintained including issues such as performance of the audit team members and stakeholder feedback. Finally, these stages are followed by a review of the audit programme and any adjustments necessary to improve the process prior to starting a further programme cycle.Food companies that use standardized audit procedures as a basis for supplier audits are more likely to ensure consistency, transparency and fairness in their audit activities which provides a sound basis for good supplier relations and development of the supplier base.

CHAPTER 7

ENLISTMENT OF MICROBIOLOGICAL CRITERIA

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The Codex Alimentarius Commission establishes standards for microbiological criteria that are accepted globally. These guidelines were first established by the World Health Organization, or concentration of a microbiological hazard in a food at the time of consumption that offers or contributes to the appropriate level of protection. A PO is a related idea that applies at an earlier stage in the food supply chain to help meet the FSO or ALOP. FSO10.1007Performance Goals if any. Using this methodology, this will also disallow the use of MC for environmental monitoring.

Uses and Implementation of Microbiological Food Criteria

Creating significant MC for a dish is a difficult task that takes a lot of time and work. As a result, MC should only be implemented when necessary and when it can be shown that doing so would effectively safeguard the public's health and make HACCP systems practicable to execute.

Examining the microbiological state of foods in light of the acceptance standards agreed upon by operators of food businesses.Confirming that the chosen control methods satisfy the pathogengenicity or other traits-related POs and zeolites at a particular point in the food chain.

Various Microbiological Standards

Different general acceptability standards for food lots were recognised. These are specifications, rules, and standards. They may be clarified in the context of MC as follows:

Microbiological Standard

An obligatory requirement that is put into a legislation or ordinance

An advisory criteria known as a microbiological guideline is used to notify food operators and others of the level of microorganisms that may be anticipated in a product when best procedures are followed.Microbiological specifications are a requirement or recommendation depending on the usage of a food purchase agreement between a customer and a supplier.

Biochemical Standards

The microbiological profile that foods must fulfil to be compliant with a legislation or policy is typically defined by regulatory agencies via the use of microbiological criteria. Foods that don't comply with a standard may be taken off the market or need remedial measures to bring them into conformity. Standards may be established for a broad range of reasons, but they work best when the danger is significant enough and compliance is necessary to preserve the public health. These foods should be subject to microbiological standards, which might also promote fair trade. The criteria should ideally be based on a reasonable level of risk for the relevant hazard that has been expressed through an FSO or PO. Government and business may both utilise microbiological criteria. Foods from both local and foreign sources should be subjected to the same microbiological requirements established for lot approval.

Biological Principles

Many players may achieve microbiological standards. They provide information on the anticipated microbiological profile of a product prepared using a food safety control system or one of its component parts, such as GHP. Microbiological guidelines are used by food operators to build their control systems. Guidelines are only suggestions and could not result in a dish being rejected. Particularly in cases when inadequate data precludes the establishment of a microbiological standard, microbiological recommendations may be the preferable method of informing and directing food companies within a particular sector of the industry to improve its operations. A microbiologically based recommendation by itself, together with advice on what best practices are, could be sufficient to bring about the adjustments required to enhance food quality and safety. This could make it unnecessary to establish a standard.

Microbiological Requirements

To lessen the possibility of accepting an ingredient or a finished item that may not meet their standards for safety or quality, buyers often specify their buying requirements. For example, microbiological specifications frequently establish the microbiological limitations for a component, so that when it is utilised, the finished product will fulfil all standards for safety and quality. In the food supply chain, it is standard procedure for purchasers to establish microbiological requirements for the goods they acquire. Unless otherwise specified in a contract, the requirements are often advisory. The materials are often only sampled when necessary. In other situations, each incoming batch could be examined by the receiving firm or via a "Certificate of Analysis" by the supplier.

Using Microbiological Standards

Regulatory Authorities Applying

For further information, please visit the website. The recommended acceptance criterion used to force the food industry to take required action is based on microbiological standards. Others are used at intermediate processing stages and are used to show that a certain procedure is being run in line with acceptable sanitary practice. Some are applied to lot acceptance at a specific point in the food supply chain, such as the port of entry or items in commerce. Rejecting lots, recalling food, or reprocessing are a few instances of obligatory action that might occur in the first scenario as a consequence of noncompliance. while in the latter scenario of standards applied to intermediate processing stages in the food supply chain, the required action is more often a mandate on the food company to enhance their hygiene processes rather than the removal of the item from the market.

By independently evaluating foods against the standards and/or growth during subsequent handling, storage, and use intended use of the food, the consumers concerned, including relevant sub-populations, and consumption habits, regulatory authorities apply microbiological standards in the context of their official food controls. Therefore, Salmonella might be present in a raw agricultural product that is meant to be completely cooked before consumption, but not harmful, with proper processing. Normally, a strict sampling strategy for Salmonella in such a product would be useless. Effectiveness might be improved by using proper labelling and clear directions for preparation and usage.

Consideration of the intended use should also include who is going to make the product and the group of customers for whom the meal is designed. When producing or preparing food particularly for these customers, extra care must be taken since they are more fragile than healthy adults, such as babies, the elderly, immunosuppressed people, etc. The strictness of MC and any supporting sample strategies should reflect this.

Taking into account cost or other restrictions deemed suitable for food. Depending on its objective, a sampling plan should specify the number of samples to be obtained, the size of the analytical unit, the acceptance number, an indicator of the statistical performance of the sampling plan's analytical procedures, and the actions to be taken when the MC is not reached.

In order to make the best use of money and resources, it is crucial when using an MC for product assessment that only appropriate tests are applied to foods and at points in the food supply chain that offer the greatest benefit in providing consumers with foods that are safe and wholesome for consumption. The MC should be implementable for food firms on a budget. Sampling also has to be taken into account, namely the kind of sample, the sampling plan, and the sampling frequency.

Implementation of Microbiological Standards

This comprises the indicators of microbiological pathogenicity or other features where process technology, either as pathogens, as indicator organisms, or as spoiling microorganisms. MCs shouldn't include microorganisms whose relevance to the stated meal is in question. For instance, the mere discovery of certain microorganisms during a presence-absence test that are known to cause foodborne disease does not always indicate a hazard to the general public's health. Before being regarded as a substantial concern, these bacteria often need to proliferate in food. Consideration should be given to testing for pathogens rather than testing directly or indirectly for indicator organisms when pathogens may be readily and consistently identified. As will be covered below, testing for an indicator microbe may be helpful.

Using Indicia Organisms

Foods or substances are regularly tested using indicator organisms. Verifying process control and finding possibilities for process changes are essential uses of indictors. Nevertheless, indicators are not just utilised for pathogenic issues; the majority of the factors used to establish MC also apply to indicators. When utilised as indicators, microorganisms, their cellular components, or their meolic products may reveal:

The potential for a disease or toxin, as well as the suitability of a food or substance for a certain use.an estimate of the perishable foods' ability to maintain quality under projected handling and storage settings as well as the potential for changes in the food due to fungus activity that might make it less acidic and perhaps more dangerous.

The Efficiency of Sanitation and Cleaning

Indicator microorganisms and agents may be classified as post-processing contamination, faecal contamination, signs of possible human contamination, and survival of a pathogen or spoiling organism. Aerobic bacteria, coliforms, Enterobacteriaceae, E. coli, yeasts, moulds, proteolytic bacteria, and thermophilic bacteria are a few examples of microbes that may be examined numerically or qualitatively. ATP, ribonucleic acid, endotoxins, and different enzymes are a few examples of biological components that may be exploited. Hydrogen sulphide, carbon dioxide, lactic acid, ethanol, diacetyl, and ergosterol are a few examples of meolic products that are utilised as markers.The following are some qualities of a perfect indicator organism:

Existence suggests a chance of decay, a flawed procedure, or a flawed practice.Simple to find and of product. Similarly, it is unlikely that many kilos of pasteurized goods contain enteric germs. Limits for pathogens in processed foods that undergo a validated kill phase should not be set at random; rather, they should only be set if there is a requirement to identify

contaminated goods. It is ideal to get limits for use in buy requirements from data gathered during routine manufacturing while the operation is under control. It is usual for a business to establish stricter standards for internal usage in order to guarantee adherence to consumer and legal obligations.

Microbiological limits, m and M, are used in sampling by attributes processes to determine the existence of HACCP. M distinguishes between slightly accepeveryle and unaccepeble units in a three-class design.

The microbiological limits are swapped out with other limits in sampling processes that are not based on characteristics. For instance, for variable plans, the maximum percentage of the lot that may be accepted with concentrations over the limit, the accepted microbiological quality limit. Sampling Planning, Sampling Processes and Handling of Samples before Analysis.

Sampling plans should include the sampling process as well as the decision criteria that will be used to evaluate the results after a certain number of sample units and subsequent analytical units of a specified size have been examined using specified procedures. No sampling strategy can guarantee the absence of a specific microbe from a whole lot, but a well-designed sample plan can determine the chance of finding microorganisms in a lot. Sampling strategies should be economically and administratively viable. In particular, hazards to public health connected with the hazard, vulnerability of the target consumer group, heterogeneity of distribution of microorganisms where variables sampling methods are applied, and randomness of sampling should all be taken into consideration when choosing sample plans.

the population as a whole and a sample of it The idea of a population was presented in the part before, and it was said that the sample of the population should exhibit characteristics that are typical of the population. These notions would be expressed, for example, by all the counts that would be seen by looking at every unit in the lot and the counts actually seen based on the few units that were looked at, in terms of standard plate counts. An analyst or bacteriologist would refer to each one of these units as a "sample," but statisticians use the phrase "sample" for a set of units that are extracted to estimate the characteristics of the whole population. In order to avoid misunderstanding, the term "sample of the population" will be used to refer to both the whole group of units from whom the sample was selected and the sample units themselves. It is also assumed that a sample unit is a recognizable unit that can be identified repeatedly. The analytical unit's size is then determined and it may then be a part of the sample unit. Yet in this, the sample unit and the analytical unit are referred to as being identical.

The Functional Operating Characteristic

If we pick 10 of the 10 g blocks we consider sample units out of the lot, and we determine that these 10 units make up a representative sample, then we should choose these units such that each sample unit in the lot has an equal probability of being selected as one of the sample units chosen. Using stratified random sampling, where the lot is seen as a collection of sub-lots that have a similar trait, is another strategy. Thereafter, samples and tests are chosen at random from these sub-lots. Random sampling is often difficult to achieve in practise, and this might be especially important for goods with incomplete mixing or unknown origins. But, we should at the very least make an effort to collect test data from all areas of the batch, regardless of time spent in a process or location inside a load or vessel. If the sampling plan is to provide impartial data and unbiased conclusions based on the sampling plan's decision-making criteria, proper sample selection is crucial. We are able to lower the possibility of

making biassed choices by sampling randomly. Unless otherwise noted, all statistical calculations in this book assume that the sample was chosen at random.

Sampling Strategy

To determine whether the whole lot should be approved or refused, the findings of tests conducted on sample units will be compared with certain criteria. The sample plan refers to the specific selection of the sampling technique and the decision criteria. This is a simple hypothetical sample strategy example. Analyze the presence or absence of the target microorganism in each of the ten analytical units of 25 g of food taken from the lot. In this case, the whole batch of food is accepted if just two out of the 10 sample units exhibit the organ-ism. However the whole batch must be discarded if three or more sample units provide a positive result. A sampling plan is defined by two parameters, namely: n = number of sample units to be tested and c = the maximum number of sample units that may yield a positive result for the lot still to be considered complete, in addition to the characteristic being evaluated and the tolerated level.

For presence samples, as well Pa is the anticipated percentage of times that testing conducted in accordance with the sampling strategy will show that the lot is adequate. In other words, Pa is a measure of our, or the sample plan's, dependability.

Binary Distribution

The binomial distribution in probability theory and statistics indicates the likelihood of discovering a certain number of positives when taking samples from a lot and using a test that only has two potential outcomes, such as a positive or a negative result. This occurs often in microbiological testing for pathogens in particular since it is anticipated that the contaminant will only be present at very low levels, if at all, and will either be present in a sample unit or not. A test that determines if a microbiological count is above or below a certain numerical limit m, however, also returns a result that is either positive or negative. Pa may be calculated using the binomial distribution as well. Think of 1000 chocolate bars, 100 of which are tainted with salmonella, as an example. A 1-in-10 likelihood that a sample unit includes a Salmonella cell would be predicted if a sample unit were picked at random. On the other hand, the sample unit has a 90% probability of being free of the contamination. Each sample unit has a 1 log if two are chosen at random. By replacing in the threshold of accepility and the needed confidence of acceptance of a non-compliant batch, we discover:

Fundamentals of Sampling and Probability Concepts

It has been argued whether to accept or reject a lot based on a sampling strategy connected to a certain microbiological test or characteristic. Only the objective for which the test was conducted will determine whether the judgement of accuracy or not is applicable. A meal unsuile for one purpose may nevertheless be suile for another; for example, although rejected for humans, it can still be suile for animals. Instead, if a meal is rejected, it may be reprocessed, bringing the actual fault rate down to a level where it passes the test and is acceptable for the intended use. So, a rejected lot will often only be kept until the appropriate food safety manager determines what to do with it. Depending on the situation, this might mean returning it to the supplier, ordering reprocessing, prohibiting its use for human consumption, or ordering its destruction. The phrases "accept" and "reject" are used in this text only in this specific meaning, i.e., accepted or rejected for the intended application of the sample plan.

A lot is, ideally, a significant amount of food or food units produced and managed in a consistent manner. By taking into account microbiological levels and distributions, the assumption is that there is homogeneity within a lot, which seldom happens in reality. The

distribution of microorganisms within batches of food is often diverse, but it has been shown that under specific conditions, the logarithms of the counts from a batch of food are likely to be regularly distributed. The difficulty of interpreting sample data is exacerbated by this variability in situations when a lot is ill-defined, for as when it is a small portion of a greater amount of the product.

So, it is advantageous if vendors provide recognisable code numbers to food batches produced over short time intervals, for certain operations. According to the kind of process and level of batch homogeneity, the choice of coding system will change from one process to the next. For instance, a batch process, like batch retorting, may need the coding of just a small number of product units as one lot, but a continuous process may yield a pretty homogenous product, in which case a lot might encompass units generated continually over a relatively long period. If a batch of production is mixed together in a consignment and regarded as a single lot, the rejection of one batch might have a negative impact on the whole batch, even if just a few of the manufacturing batches within the batch may be of low quality. At the price of additional analyses, treating the various manufacturing batches as lots and applying the proper labelling allows for more accurate detection of subpar food and may lead to the rejection of fewer units from the whole shipment. This is an adaptation of the more fully stated stratified sampling plan idea. Food generated with the least amount of variance feasible for a certain technique or product should make up the majority of the diet. The term "lot" is often used in this book in its statistical meaning, as a collection of units of a product, whose acceptability is assessed by studying a sample taken from it, because to the uncertainty involved in commercially designating a lot.

An Exemplary Example

As closely as is practical, a representative sample represents the makeup of the batch from which it is taken. In such case, how should a representative sample be selected? In order to make a judgement on a lot with confidence, bias must be avoided and a sufficient number of sample units must be drawn. Bias may be avoided using the widely accepted method of sampling at random. For testing, random numbers are used to choose the liquid or solid volume units. Of course, there is no assurance that a random sample will reflect the characteristics of the lot exactly, but the randomness of sampling serves as the foundation for calculating the likelihood that a sample will produce a particular result and increases the likelihood of finding every variation in the lot.

Using a stratified random sampling strategy, which involves selecting a predetermined number of sample units at random from each stratum, is also an option and could even be desired. The proportion of sample units from each stratum should match to the percentage of lot units in that stratum. This implies that the number of sample units per stratum should be the same if each stratum has the same amount of product as every other stratum; otherwise, they should vary depending on the percentage of the lot included in each stratum that is being evaluated. When one knows in advance that the consignment may not be of uniform quality, one may utilise stratification as a way for addressing known causes of variance. If parts of a consignment are transported in various vehicles or holds of a ship, or if it is known that the consignment is actually made up of several lots, perhaps representing various production days from the same plant, different plants owned by the same company, or different suppliers, the consignment may not be of uniform quality. If the data for several strata seem to be homogenous, they should first be evaluated independently before being aggregated. According to research by Jongen burger et al., stratified or systematic random sampling is sometimes more efficient when there are contamination "hot spots" since these methods function as well when there is homogeneous contamination. Given that systematic sampling

may not perform as well with systematic contamination and that random sampling may perform worse with clustered contamination, stratified random sampling may be the best option.

Random sampling may not be possible due to practical or physical restrictions, therefore the probability computed may not be accurate. The degree to which reality resembles the ideal circumstance of random sampling determines how reliable they are. This should be kept in mind while analysing the findings that follow.

Confidence in Findings Interpretation

As was previously said, test findings based on sample strategies cannot provide complete guarantee that the choice would be the right one. There are a number of variables that may impact how confident we are in the sample findings. Several of these are connected to the methods we use. In this, it is expected that the analytical method will accurately identify any contaminants it encounters and will not provide any false-positive results. In reality, these presumptions are never true, and data about the performance of the analytical technique, such as sensitivity and specificity or reliability and repeatability, may not be accessible even for conventional microbiological methods. When such information is known, the OC function may sometimes be calculated differently or have different critical limits.

Our ability to precisely identify the kind or amount of contaminants present might also be impacted by how the sample is handled and processed. Depending on the material being examined, the preservation system being employed, and the analytical approach, a different handling technique will be used. Such handling measures must be chosen with competent microbiological judgement. As previously mentioned, statistical concerns are connected to our sample plan assumptions. The following is a list of some of the topics covered in the course. The design of sampling plans for quantitative analytical outcomes depends on understanding of underlying distributions and the normal variability between sample units. Either the computation of acceptance probability or the selection of critical limits reflects this. Chap. 7 goes into additional depth on these topics. Also, it's crucial to think about whether the whole sample process and any underlying presumptions are appropriate for the choice that will be made about the lot.

Moreover, it's crucial to keep in mind that selecting a sample technique might be influenced by a variety of non-statistical factors. For instance, the financial expense of sampling and the possibility of cross-contamination as a result of excessive sampling. When working with perishable or commercially sensitive goods, it may be necessary to employ less-than-ideal statistically or with less-discriminatory sample strategies. Several of the sample strategies used in food microbiology, including $n = 5$ or even $n = 1$, are primarily driven by these problems. These small figures reflect arbitrary, non-statistical limits that restrict the degree of confidence that conclusions based on such sample strategies are sound. A sample strategy should be created taking into account these constraints and the accuracy of any presumptions made in order to be suitable for the intended purpose. The choice of the sampling plan, including critical limits, and the quantity of sample units to be examined, should be decided according to the intended purpose. If a product promotes the development of a potentially harmful disease, or if the test is used as a gauge of hygiene or general microbiological quality, for example, the choice of n should reflect the stringency suitable to the conclusion being made about the lot. The suggested method would be to first determine the appropriate acceptance and rejection probabilities for lots of specified acceptable and unacceptable quality, and then to calculate the number of sample units needed to accurately differentiate such lots.

Sampler Programs

This paper focuses mostly on strategies that may be used for large quantities of food offered for admission at ports or other points of entry. The receiving agency often has little to no knowledge about the methodology used to prepare the food or the track record of prior success of the same processor. Plans with attribute-based compensation are acceptable in this situation. Only if this distribution is understood are variable sampling strategies, which rely on the nature of the frequency distribution of microbes inside several foods, viable. Furthermore, variable sampling plans are not appropriate for making a determination of a lot's presence or absence. For example, in a sampling plan with n = 10 and c = 2, ten sample units are taken and tested; if two or fewer show the presence of the organism, the lot is accepted; however, if three or more do, the lot is rejected, though it need not be destroyed. Data from the application of a two-class plan based on presence10.1007g can only provide an indication on the expected concentration of that microbe inside the lot when the lot is tightly mixed. Only when the analyst is certain that the threatening microorganisms are distributed uniformly can such procedures be used. n and c determine how well the sampling scheme performs. The difference between acceple and unacceple lots is easier to distinguish the bigger the value of n at a given value of c. As a result, the plan n = 15 c = 2 is stricter than the plan n = 10 c = 2, whereas the plan n = 5 c = 2 is more liberal. On the other hand, if c is reduced for a given sample size n, the meal must be better to have the same probability of getting passed. In contrast, as the likelihood of Pa acceptance rises, if c is raised, the plan becomes more tolerant and will more often pass food lots with unacceptable quality. acceptance probabilities for a group of plans are provided. To demonstrate the characteristics of several two-class attributes plans, the operational characteristic curves for a couple of these plans. When we use an alternative strategy to evaluate lot quality, the significance of the sample size, n, is also emphasized. Imagine that a sampling strategy was used to determine whether to accept or reject the lot as well as to provide an estimate of the percentage of defectives in the lot. Such estimations should be validated by the actual sample findings and provided as confidence intervals with a range of values. N has a significant impact on the correctness of the estimations and the breadth of these ranges. For illustrative reasons, lower and upper bounds of 95% confidence intervals for various combinations of sample sizes, n, and actual numbers of positive sample units, k, for a homogeneous contamination with a certain faulty rate per sample are provided.

The impact of the lot's size is another factor that may be taken into account. In a two-class scheme with c = 0, a sample size of, say, n = 30 sample units might be randomly selected from a lot of any size. One gets an OC curve similar to that illustrated in 7.2c if the lot has a very high number of sample units. By employing the plan n = 30, c = 0, a lot with one faulty unit out of 40 will be accepted around half the time, according the OC curve. This probability was calculated using the binomial distribution model.

Proposals for Three-Class Characteristics

Plans for three-class attributes were developed for circumstances when the concentration of microorganisms in the sample units allowed for the division of the product's quality into three attribute classes. In a three-class scheme, counts over a concentration m that distinguishes between excellent and somewhat accurate units are desired, however some may be permitted. A lot is rejected if any count for the n sample units exceeds M, which is the second concentration over which no count for any sample unit may be accepted. Based on the notion that analytical findings for sample units taken from a lot are of a quantitative character, this concept is put forward. In this instance, the amounts of microorganisms present in sample

units may be defined in terms of frequency distributions that can be characterized by certain parameters of location and spread.

How different frequency distributions of microbiological content within a lot are impacted by the values of m and M for three-class designs. Curve 1 shows a perfectly acceptable batch, with few bacteria overall, a low average count with minimal variance, and no counts higher than m. Curve 2 shows a lot with a comparable average count but with a considerably greater range, therefore it should, in theory, be computed using a different distribution model for different values of p. This impact only becomes significant when one-fifth to two-thirds of the lot is used as a sample, which in bacteriological investigation of food lots never really happens.

Comparing Sampling Strategies

A suile sampling plan's choice or design will rely on the specific goal, i.e., the sampling material, the kind of microbiological result being evaluated, and any previous knowledge about manufacturing processes and frequency distributions of sample results in lots that may be available. In the paragraphs that follow, certain statistical considerations for selecting a sampling strategy will be compared between two-class and three-class attribute plans, as well as three-class attribute plans with variables strategies. You may choose between several sampling strategies only when the outcome of a microbiological examination is provided as a count or in another quantitative way. Only two-class designs are suitable for outcomes that are purely qualitative. Concerns about the frequency distributions of sample results and if there is any prior knowledge on their shape, location, and spread emerge when dealing with quantitative analytical data for sample units in a lot. g represents a typical distribution. The selected value of 0.8 is based on documented observations of mesophilic Clostridium spores in raw beef, pig, and poultry as well as related findings in other food sources. Each and every component of the distribution above m in each place is flawed. To demonstrate how the percentage defective rises with mean log count, the proportion defective in the distribution at each position is shown against the mean log count. Lastly, given a plan with five samples, the operating characteristic curve for the specified plan is utilised to calculate the probability of acceptance from the fraction faulty at each mean log count. The mean concentration is displayed versus this acceptance probability.

Proposals for Two-Class and Three-Class Qualities are Compared

Compares, using similar sample sizes n, acceptance numbers c, and lot quality, the operational features of the two kinds of attributes schemes suggested in this article. Lot quality is calculated as the percentage of the lot that is below level m to make comparisons easier. Thus, the same value of c is chosen for the two-class plan as the c of marginally adequate units for the three-class plan. Similar to three-class plans, the two-class plans do not discriminate between values between m and M and those above M. Regardless of how much each individual result exceeds m, the lot is accepted if not more than c sample units provide findings that are higher than m. Nonetheless, the related three-class design does distinguish between the two by adding a further subdivision of lot quality since the limit M distinguishes between marginally adequate and faulty units.

The Log Count

It is clear from comparing OC surfaces for three-class plans with constant sample sizes n but variable values for c that the surface heights fluctuate mostly in the pm-direction, or for different percentages of marginally accurate units in the lot. The explanation is that M can never surpass the 0 limit on the number of sample units. A three-class plan might really be seen as a combination of two two-class plans, one of which refers to the limit M and the other

of which refers to the limit m. One of these two-class strategies may, in extreme cases, predominate the decision-making process. Nevertheless, the actual effectiveness of a three-class strategy often relies on the range of pm and pd combinations that are likely to occur in practice.

Hildebrandt et al. studied the performance aspects of two-class and three-class sampling plans in case log-normal lot distributions can be assumed by comparing the two-class plan n = 5, c = 1; m = 5 × 104 CFUml. To investigate the effect of the lot standard deviation on the probabilities of acceptance for two- and three-class attribute sampling plans when the lot under consideration contains 5%, 20%, or 50% of sample units > m, four different types of lots with lot standard deviations of = 0.1, 0.2, 0.4, and 0.8 were taken into consideration.

CHAPTER 8

CONSTRUCTION OF THREE-CLASS PLANS

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Without challenging the rationale behind the selection of the provided microbiological limits, m and M, the prior section's discussions began. Nevertheless, these limits are often stated as the maximum level of the target organism M that would be acceptable under normal production settings and the limit of the target organism M that, if exceeded, would be deemed unacceptable, or faulty.

The design of sampling plans could be based on knowledge of production technologies leading to values for m and M that take into account the maximum mean concentration of contaminants as well as the maximum extent of heterogeneity, under conditions of good manufacturing practise, according to the reference to GMP-conditions and empirical studies describing frequency distributions for quantitative analytical results that are achievable under these conditions. It is debatable whether to reject a lot satisfying GMP criteria only because of a single sample result above M, particularly in the case of non-pathogenic microorganisms like total bacterial count or signs of cleanliness that pose no health risk to the user. In this situation, the difference should be calculated such that lots with low microbe concentrations and high or even inevitable heterogeneity run only a small, known risk of rejection as a result of a single sample unit that is over M.

An "indifferent lot" for the two-class sample plan n = 5, c = 2 is initially taken into consideration on the presumption that sampling outcomes follow a normal distribution. The phrase "indifferent lot" denotes that there is a 0.5 likelihood that the lot will either be accepted or rejected. An indifferent lot is said to have a mean concentration of microorganisms that is just at the limit m: = m, or just at the maximum achievable mean concentration under circumstances of excellent manufacturing practise, according to the definition of this two-class sampling plan. The likelihood of rejecting a lot becomes higher than the probability of accepting it as soon as lot mean concentrations go beyond this threshold. As a result, the hypothesis evaluated by this two-class design relates to the lot under investigation's mean contaminant concentration. Reduced acceptance probability may result from applying a three-class plan to the same lot with n = 5, c = 2, i.e., adding a second restriction M and the condition cM = 0. Nevertheless, the distance between m and M in regard to lot heterogeneity determines whether or not the difference between employing the two-class and the three-class designs is significant. Regarding these relationships, it is suggested that as a first step, the required decrease in acceptance probability for a lot with marginal mean concentration of contaminants = m and marginal spread be defined. This will help to clarify the additional risk of rejecting an indifferent lot with a given, accepted, heterogeneity. A number for the upper limit M that satisfies this criteria should be selected as a second step.

These factors show how to choose M once m has been established. This process is mostly focused on creating sample programmes for non-pathogenic microorganisms like hygienic indicators. Yet, pathogens might also benefit from the link between M and m. One would simply begin by first establishing M in terms of safety and selecting m in accordance with

that value. A three-class sample plan may be created using this process to satisfy specified criteria about its stringency in contrast to the corresponding two-class plan. The three-class plan's assertion of two hypotheses, one regarding the marginal mean concentration of contaminants in an accepted lot as m and the other about the marginal spread that is accepted by establishing the distance between m and M, is one of its distinguishing features.

It's a good idea to have a backup plan, just in case. It is based on assigning one of three large concentration bands a concentration measurement. It forgoes discriminating in order to attain this simplicity. The three-class characteristics approach, for instance, would give the same degree of concern to pollutants 1001 and 9999 in a sample unit if m were 1000 and M were 10,000. Additionally, it gives pollutants with numbers 999 and 1001 a whole other degree of worry. The benefit of the variables plan mentioned above is that it provides excellent discriminating between different concentration readings. The variable plan, on the other hand, requires more mathematical knowledge to use and comprehend, and the accuracy of the assumptions made about the frequency distribution determines how well it performs.

When the real standard deviation exceeds the expected value, the decision rule of the variables plan is unable to react, while the decision rule of the three-class plan stays the same and reacts to higher measured concentrations. This comparison demonstrates that both plans are quite stringent in their ability to distinguish between homogeneous lots of excellent and poor quality. Nevertheless, the variables plan discriminates at mean concentrations closer to V = M, since this is the beginning point to establish the marginal mean contamination V k that is significant for this kind of plan, in contrast to the three-class plan, which focuses on the marginal lot mean concentration m.

The slopes of both OC curves grow less steep as lot standard deviation rises; the three-class plan is more affected by this trend. Although acceptance probability for lots of accepted quality continue to be relatively high under the variables plan, they decline more quickly than under the three-class plan as soon as the lot quality shifts from accepted to unaccepted. If past information is employed in the construction of the three-class plan, particularly when determining the proper distance between m and M, closer operation comparisons of these kinds of sample plans might be obtained. It's crucial to understand that these two strategies are fundamentally different from one another. The characteristics schemes will function effectively under such conditions against their predetermined parameters since they were created for situations when it was impossible to make assumptions about the underlying distributions. Other methodologies may allow for alternate sets of assumptions and more cost-effective sample plans connected to these assumptions when it is feasible to have high confidence in the underlying frequency distributions. As a result, the sampling strategy used should be determined by the lot's characteristics and planned purpose.

Pathogen-Related Risk Factors: Many Variables

The severity of the illness should be reflected in the microbiological criteria and sample schedules, which should also be suitable for the food. It has been discovered that several well-known food-pathogen combos exist. It is vital to have some knowledge of the factors that influence whether a meal is likely to contain infections or their poisonous meo-lites. These correlations often reflect significant geographical and cultural influences.

Factors for Epidemiology

Typhoid, cholera, and hepatitis A infection epidemics have been linked to water and various kinds of seafood. Salmonellosis outbreaks are often linked to meat and poultry as the source of the illness. Pastries with ham and cream filling are regularly linked to staphylococcal foodborne disease outbreaks. Vibrio parahaemolyticus outbreaks of gastroenteritis are often

linked to seafood. The typical carriers of C. perfringens-caused enteritis outbreaks include cooked meat and poultry, as well as stews and gravies that have been overcooked in terms of time and temperature. *L. monocytogenes* is often linked to cooked meat, smoked fish, and chicken meat, whereas campylobacteriosis is linked to raw meat. Botulism is an uncommon illness that is often brought on by eating improperly prepared, home-preserved foods, especially cured pig products, fermented fish, fish eggs, or marine animals, as well as low-acid foods like vegetables. Histamine poisoning, a seldom fatal condition, is often connected to scombroid fish species. Salmonellosis, brucellosis, campylobacteriosis, and more recently enterohaemorrhagic E. coli infection have all been linked to raw milk in the past. Moreover, listeriosis, brucellosis, staphylococcal poisoning, bloody diarrhea, and hemolytic uremic syndrome brought on by enterohaemorrhagic E. coli have all been linked to cheese prepared from raw milk.

The link between cooked rice that has been overheated and the bacillus cereus gastroenteritis is well established. Enterohaemorrhagic E. coli O157:H7 infections and other EHECs may be spread via undercooked ground beef, while recent outbreaks have also been linked to unpasteurized dairy products, fermented pork products, fresh fruit, and polluted water. Cyclosporiasis outbreaks have been linked to produce like raspberries and basil, although polluted water has been the main source of cryptosporidiosis. Foodborne enteric viruses like noroviruses and hepatitis A are also tightly linked to fresh vegetables, seafood, and RTE meals that need a lot of manual handling. Low-moisture foods, such as cereals, chia powder, tree nuts, peanut butter, and chocolate, have often been linked to salmonellosis epidemics.

Environmental Features

Animal, human, and environmental reservoirs are among the main sources of foodborne microbial infections. After food infection, the pathogens' activity is regulated by the food's composition, the presence of other bacteria, and its environmental circumstances.

The agricultural environment is home to several pathogens that are harmful to humans, including Salmonella spp., Campylobacter spp., L. monocytogenes, Yersinia enterocolitica, pathogenic E. coli, C. perfringens, and S. aureus. Despite the fact that animal products have traditionally been associated with foodborne illness, numerous significant outbreaks in recent years have been linked to produce, including lettuce, sprouts, cantaloupes, and raspberries. Man is also a reservoir for several foodborne pathogens, including S. Typhi, Shigella spp., hepatitis A, and small-round structured viruses like noroviruses, some of which may survive for weeks or months in the carrier form.

Due to potential contamination during production and harvest, their inherent qualities that affect microbial growth and survival, traditional preparation and handling practises specific to that food, and, frequently, the absence of a CCP that will eliminate the hazard, some food products present a greater risk than others. Consuming raw food poses a significant danger to consumers who are sensitive since goods like oysters may have been harvested with norovirus or Vibrio vulnificus contamination. Unless there is a chemical that prevents development or a competing anti-listerial microflora in the product, ready-to-eat meals may get re-contaminated with L. monocytogenes, which may multiply during subsequent refrigeration.

The scope and diversity of foodborne diseases are significantly influenced by local traditions and community hygiene norms, particularly those pertaining to food, water supply, and sanitation. It is important to think about how well the current regulations protect sources of water, milk, and shellfish collecting regions. The appropriate use of refrigeration in processing plants, food-service establishments, and homes, as well as the detection, recall, or

condemnation of contaminated foods, vermin control, public health supervision of food-service establishments, all contribute to lowering the incidence of foodborne illness and have an impact on the choice of sampling plans for specific commodities from specific sources.

Foodborne dangers are also influenced by regional dietary traditions. For instance, the practise of eating uncooked chicken in Japan has contributed to the disease's very high occurrence there. Similar to Alaska and Canada, type E botulism is more common there due to the use of numerous fermented traditional marine foods by aboriginal tribes.Raw fish, raw beef, and other meals all include members of the Aeromonas hydrophila group. While individuals with different forms of diarrhoea may have high A. hydrophila levels, its significance as a cause of foodborne diarrhoea is yet unknown. Water and unprocessed aquatic products may be used to isolate Plesiomonas shigelloides. While P. shigelloides has sometimes been found in high concentrations in individuals with diarrhoea, its contribution to foodborne or waterborne sickness is still debatable.

Clinical Traits

There is an intrinsic link between certain foodborne bacteria and serious diseases in humans. For example, C. botuli-num types A, B, E, and F may generate toxins that, even in very little doses, can result in neurological disease in healthy individuals. The case fatality rate may surpass 50% if not appropriately treated with antitoxins and given respiratory support, albeit this is uncommon nowadays with rates normally being less than 5%. Some strains of S. Typhimurium, S. dysenteriae I, V. cholerae, enterohaemorrhagic E. coli, and C. perfringens type C have the virulence necessary to cause serious illness and even death. In malnourished instances, 50–70% of dehydrated cholera patients pass away unless they get the proper treatment, which includes oral or intravenous fluid and electrolyte replenishment. *L. monocytogenes* primarily affects vulnerable populations, usually pregnant women, newborns, the elderly, and those with impaired immune systems; nonetheless, death rates among these patients may reach 25%. Individuals who have underlying chronic diseases, particularly men with a history of heavy drinking, are more likely to have V. vulnificus infections, which are linked to iron overload in the patient.

The "classical" enteropathogenic E. coli strains were once thought to be pathogenic E. coli strains of certain O serogroups that mostly caused diarrhoea in newborns. Nonetheless, a number of other strains of E. coli have been identified and are worries for the modern food sector. In underdeveloped nations, enterotoxigenic E. coli is a significant contributor to infantile diarrhoea and is a common cause of traveler's diarrhoea. Shigella and enteroinvasive E. coli are similar in virulence and antigenicity. Shiga-like toxins are produced by enterohaemorrhagic E. coli, including E. coli O157:H7, which was originally recognised as a pathogen in 1982. Food-related outbreaks linked to E. coli O157:H7 have been well-documented and are reason for serious worry since infection is likely to occur at low doses, the disease may be severe, and kidney failure and death can sometimes result, particularly in small children and the elderly. Also, it is important to keep in mind that a variety of non-O157 strains, including O26, O121, O45, O145, and O104, have been linked to foodborne outbreaks.

Shigellosis may be brought on by S. dysenteriae at low dosages. Other Shigella spp., V. cholerae, and other salmonellae may also be more contagious in extremely vulnerable people such newborns, undernourished people, and those with impaired immune systems. Moreover, compared to healthy young people, the severity of enteritis caused by salmonellae, Shigella spp., and pathogenic E. coli is higher in the very young, the elderly, immunocompromised, and those with concurrent disorders. Even the often mild gastroenteritis brought on by V. parahaemolyticus, staphylococcal enterotoxin, B. cereus, or C. perfringens may sometimes

develop severe in these populations. Beta-hemolytic streptococci are relatively uncommon foodborne pathogens that may cause tonsillitis, severe glomerulonephritis, arthritis, cardiovascular disabilities, and other serious complications. Several other foodborne infections might take a long to recover from.

The initial symptoms of enteric infections may often be followed by persistent secondary sequelae in many foodborne diseases. Examples include hemolytic uremic syndrome, which is linked to E. coli O157:H7 infection, hemolytic uremic syndrome associated with antecedent C. jejuni infections, reactive arthritis following enteric infections caused by Salmonella, Shigella spp., Y. enterocolitica, and thermophilic campylobacters, depression from chronic diarrhoea brought on by Toxoplasma, and septic arthritis following Salmonella infections.

Clinical Considerations

The expertise of doctors and laboratory techniques are key factors in determining the diagnosis of foodborne disease. For instance, few doctors are likely to have seen botulism before, therefore misdiagnoses may happen even when the symptoms are common or even even when they are extremely mild or seem similar to other conditions. Since the clinical syndromes of many of these illnesses may be identical, e.g., bloody diarrhoea may represent symptoms of bacillary or amoebic dysenteries and Shiga-like toxin-producing E. coli, many enteric foodborne disorders may only be recognised via laboratory isolation of particular organisms.

When a laboratory reports a previously unknown foodborne illness, awareness often rises and further occurrences are made public. This argument is shown by the discovery of enterohaemorrhagic E. coli O157:H7 infection and foodborne campylobacteriosis, followed by additional non-O157 serotypes. For the routine isolation or detection of several foodborne pathogens from foods, such as Shigella spp., Y. enterocolitica, enterohaemorrhagic non-O157 E. coli, Cyclospora, Cryptosporidium, and foodborne viruses, in public health and food laboratories, completely satisfactory methods are not yet available. Hence, the sensitivity, accuracy, and precision with which their presence may be determined are constrained by laboratory methods.

Risk-Based Microbial Hazards Classification

The word "hazard" in this book only refers to microbiological issues that are connected to foodborne disease. They include viruses, parasites, toxigenic fungi, and bacteria-associated toxins or toxic meo-lites. Microbial dangers may cause a wide range of risks, from very minor symptoms that last just a short time to extremely serious, life-threatening infections. Health risks often fall into one of three categories depending on the amount of concern:

Moderate Risks

Moderate dangers seldom provide a life-threatening threat, have no lasting effects, typically last a short time, and produce symptoms that are often self-limiting but may inflict excruciating suffering. Certain microorganisms may pose substantial risks to some groups while posing only minor risks to the overall population. L. monocytogenes, for instance, may short-term diarrhoea and/or abortion in the general population.

Risk-Based Microbial Hazards Classification

Risks that are Substantial, Disability-Inducing, but Not Life-Threatening

These risks produce sickness that lasts a modest amount of time and often have no long-term effects. The lower, moderate category of risk is where certain infections, such Campylobacter

jejuni and other thermophilic campylobacters, are most often found. Nevertheless, some strains of C. jejuni may cause serious sickness, like Guillain-Barré Syndrome in susceptible individuals. According to estimates, one incidence of GBS develops per 2,000 C. jejuni infections, usually 2-3 weeks after infection. Most strains only produce mild diarrhoea that lasts for a medium amount of time.

Extreme Risks, Life Endangering

Some microbiological risks may cause significant chronic sequelae or have long-lasting consequences. They may impact both the general population and groups that are at high risk. Specific host susceptibility to infection, such as listeriosis in pregnant women, cultural practises, such as the consumption of potentially hazardous foods specific to certain subpopulations, or geographic influences, such as fumonisin intoxication linked to regions where mouldy maize is consumed, are all factors influencing the development of illness in high-risk populations. Hemolytic uremic syndrome (HUS) develops in around 5–10% of cases of acute E. coli O157:H7 infections, and patients who develop HUS have a 3-5% death risk.

The main microbiological infections and poisons connected to food are mentioned in 8.2 along with their effects on public health, frequency of participation in illness, kinds of foods that have been used as vehicles, and prominent causes of disease. The frequency with which these viruses and toxins generate outbreaks or cases of foodborne disease has not been attempted to be arranged in this list since it varies by locality, nor is it meant to be all-inclusive. If food microbiological testing or other preventative measures have been useful in reducing the risk and guaranteeing food safety.

Arcobacter butzleri and A. cryaerophila bacteria, Low; the precise modes of transmission are unclear and the incidence of Arcobacter infection may be overestimated.The two most significant modes of transmission are probably eating infected animal products and drinking polluted water.Patients may not have any symptoms. Acute watery diarrhoea symptoms that may last longer than two weeks or even two months, depending on the individual. Antimicrobial treatments may be successful and provide relief in a few of days.

Emetic Toxin-producing Bacillus Cereus

1. Rice that has been fried or cooked, reconstituted cereals, puddings, and custards
2. Typically faeces, manure, animal waste, septic tank waste, and septage.
3. tainted food, unpasteurized milk, untreated water, and unpasteurized apple juice.
4. While cryptosporidiosis is primarily an acute, short-term illness, it may become worse in kids and those with impaired immune systems.

Cayetanensis Cyclospora

Medium; eating tainted food or water may result in parasite infection. Individuals who live or travel in nations where Cyclosporiasis is common may be more susceptible to becoming sick. Only infections that spread through the fecal-oral route may infect humans. Berry-related foods, including raspberries, lettuce, basil, snow peas, cilantro, and water are also included. gastroenteritis accompanied by prolonged watery diarrhoea that lasts for days or weeks until it self-limits. Unless addressed, sickness may return with higher severity for immunocompromised persons.Giadia lamblia Medium; prevalent in underdeveloped nations, but rare in wealthy nations. The most prevalent link between giardiasis and water contamination is water drinking. Food contamination by diseased or contaminated food handlers, tainted water, and vegetables. Asymptomatic infection is a possibility. diarrhoea within a week after ingesting a cyst, followed by sickness lasting 1-2 weeks and, in some chronic instances, months or years.

Preservation Circumstances

It is important to take the specific microorganism in question's development needs into account while determining the food's preservation settings. Salmonellae cannot grow in foods with a brine content of less than 10%, while staphylococci may. In dried foods, however, Salmonellae may persist for a long time. As a result, these goods might be categorized under cases 6 for staphylococci and 11 for Salmonella spp.

Several pathogens may thrive on fresh meat, but not on dried meat with a brine concentration of less than 16% in the water phase. So, the danger would grow in accordance with cases 6, 9, 12, or 15 if fresh meat is kept at temperatures that permit multiplication, however for dried beef, there would be no change in risk and cases 5, 8, 11, or 14 would apply. Keeping temperature is very significant. Microbial populations and the hazards they pose typically rise between 10 and 20 °C, and they do so considerably more quickly at higher temperatures. Contrarily, chilling below 10 °C will mitigate the majority of risks since many germs do not reproduce or do so more slowly at low temperatures. For instance, case 8 rather than case 9 would apply to ham stored below 6 °C. When the storage temperature approaches zero degrees Celsius, the development rate of psychrotrophic pathogens, such as L. monocytogenes, Y. enterocolitica, and non-proteolytic C. botulinum, will decline. Little or no growth may be anticipated during storage at typical refrigeration temperatures for a relatively short length of time.

aggressive flora Pathogen growth may sometimes be halted by competition from other microbes. Although staphylococci development is often limited by the accompanying rotting microbiota, salmonellae can typically grow in most foods at the right pH, aw, and temperature. Since they also include several competing bacteria that inhibit the development of S. aureus, fresh raw meats and bacon are not often linked to staphylococcal food illness. When food has been prepared to minimise the microbial population and subsequently becomes contaminated with staphylococci, there is a risk of enterotoxin production.

eating habits Hazard and the selection of "case" are similarly impacted by custom. For instance, unless fish is refrigerated, V. parahaemolyticus grows quickly on raw fish. While globally dispersed, V. parahaemolyticus is a considerably less frequent cause of sickness in other nations because fish is cooked before ingestion, yet it is an important source of foodborne illness in Japan where raw fish is often ingested. Hence, for Japan, cases 8 or 9 would be acceptable for this virus, but case 7 would be suitable for a nation with different dietary patterns.

Reconstituted dried goods in relief zones, foods that have been pasteurized before delivery are sometimes consumed raw. The risk will be higher if a product is designed for those whose sensitivity to foodborne diseases is exceptionally high.Kind of risk if the aforementioned temperature and food composition parameters are suitable, several microbial dangers may rise. Several dangers, including poisons and poisonous meolites, have a tendency to be very resistant to environmental factors, including typical cooking. Viruses and parasites are examples of other dangers that cannot multiply but may decrease in concentration depending on the environment to which they are exposed.

Snacks for Hospitals, Long-term Care Institutions

Due to stress from various impairments, immunosuppressive medication, and being in intensive care, patients may be more vulnerable to infection and catastrophic consequences following intestinal illness. Interference with recovery from another illness. Due to their ability to transmit illness inside the hospital, staff and patients need to be safeguarded. Patients with AIDS, transplants, and cancer.

Those with impaired immune systems are particularly vulnerable to gastrointestinal infections. Especially dehydrated high-protein meals, comfort food Because of starvation and other stressful circumstances, those who need comfort foods are often very vulnerable and prone to significant problems. The confinement of the populace in congested regions, which often have unsanitary conditions, increases the chance of illness spreading from person to person. Reconstitution with polluted water, unsanitary handling, and poor storage conditions that promote fast bacterial growth are particular risks.

Availability to Risk for the Target Customer

The danger will rise if a product is designed for those whose sensitivity to foodborne illness is exceptionally high. Examples of specialised meals designed for consumer groups at high risk.

Storing and Getting Ready to Serve

Between the time the lot is tested and the time the food is eaten, only the typical circumstances to which the food is anticipated to be exposed should be taken into account. For instance, until it is cooked or warmed for serving, a frozen item is often kept frozen. The sampling plan could not provide the degree of protection anticipated if a meal is unintentionally mistreated after being tested and authorised. Technique for preparing meals the manner of food preparation is a crucial factor.

The examples below show how knowledge about microbial ecology, as well as how to store and utilise food, is combined when selecting a case:

Salmonellae are dangerous pathogens that often contaminate raw protein meals but are rendered inactive by pasteurisation. Salmonellae may, however, recontaminate pasteurised items; consequent drying or freezing cannot be depended upon to eradicate these germs. There is no difference in risk if such a dried item is taken in its dry condition; however, if usage after reconstitution is delayed and heating is not done before consumption, the case would be 12. After reconstitution, cooking as soon as possible would lessen the risk, therefore case 10 would be ideal. Testing for S. aureus in a raw meal that will be cooked is not necessary. However, if the food has been prepared and subsequently handled, contamination with S. aureus is a worry. Case 9 would be suitable if temperature abuse might occur, and case 8 if storage temperature is well controlled. The lowered aw inhibits the competing bacteria in certain salty meals where salt-tolerant S. aureus may proliferate. Hence, staphylococci instances would resemble those of pasteurised food.

Similar to *S. aureus, B. cereus* and *C. perfringens* produce spores that can withstand mild heating, making them moderate threats as well. Cases 8 or 9 are often acceptable since there aren't many methods that may lessen the risk that these germs provide. The usage that the food will get after that must be taken into account. Testing would not be acceptable for a food for which a delay between when the food is rehydrated and when it is consumed, for instance, if it is ingested immediately after reconstitution of a dehydrated meal.

The aforementioned instances highlight the fact that before an examiner can choose an acceptable case or even conduct a test for a specific reason, they must have some understanding of the microbial ecology and history of a meal. The various potential applications for the consignment of food must be taken into account when selecting instances based on the danger as indicated above. The choice of case should take this potential into consideration since certain usage may be more dangerous than others. Microbial dangers include the quantity of harmful bacteria present or the presence and concentration of poisonous meolite in food. The relevant instance is chosen after determining the danger category and how the handling and preparation of the food would affect the hazard. If the

organism in issue is to be assessed by presence or absence, then increasing the sample size is the only method to provide sufficient protection. But, as previously said, it could need four times as many sample units to twice the dependability. A pragmatic choice should be taken after weighing the expense of the extra testing against the possible increase in discriminating power. Yet, it must also be weighed against the effect a poor choice might have on the real amount of risk. It may not be possible to randomly sample the whole shipment. Just a small percentage of the consignment, from which the sample units may be taken, may be available for random sampling. If this is the case, the findings only relate to the sampled section of the consignment rather than the full consignment, thus it is important to think about whether they can be applied to the complete consignment. For instance, accessible containers may be ones that are close to a vehicle's entrance, a ship's hatch, or the outermost stack in a warehouse. The location of the sample of the whole lot is less significant than when a non-random distribution is suspected when the contamination in a lot is really random. The sample units selected may sometimes indicate the area of a lot or consignment that has been subjected to a higher risk due to contamination or environmental factors that encouraged microbial development. Despite how convenient they may be, by allowing consumers to choose from the area of the lot that is most likely to be dangerous, they may also increase consumer protection. This wouldn't be the case if the perishable food was packed too warmly and allowed to cool slowly in the pallet's middle compartments. The frequency distributions are another another issue that can only be resolved by drawing on particular information about the lot. A standard deviation of 0.8 is used in Sect. 8.11's estimate of sampling plan performance. Depending on whether the standard deviation of a frequency distribution is above or below 0.8, different sampling schemes will function differently.

As judgements to accept or reject lots are determined on samples obtained from the lots, situations occur when the sample findings do not represent the real state of the lot. The possibility that a "acceple" lot, if provided, may be wrongfully rejected is known as the "producer's risk". The “consumer’s risk” reflects the possibility that a “bad” lot, when given, would be wrongly accepted. For the purposes of this text, "consumer's risk" is defined as the possibility of accepting a lot even if the calculated values imply acceptable quality but the real microbiological content is below the standards established in the plan. When acceptance sampling is selected to assess if an FSO PO. Such an erroneous selection may raise consumer danger. In concept the lowest needed probability to reject a lot not satisfying the FSO PO, the issue may be posed if the plan can give any useful protection, even with a considerable increase in the number of sample units. This illustrates that microbiological testing will have very little benefit in process validation when the process is intended to yield a low number of damaged units. In reality, a likelihood of acceptance on three instances in four implies that one batch in every four will be rejected, a loss substantial enough to induce a producer to tighten microbiological control to a level much below the limit stated in the test utilised. Even the rejection of one lot in 20 may be sufficient to have this impact. Nevertheless, when utilising fewer sample units, such as, the protection provided to the consumer for a given lot is significantly reduced; as a result, it is advised to utilise high values for n when a direct danger is identified. In these cases, following testing and usage of microbiological criteria will result in more accurate guarantee of safety.

The sample plan stringency must be examined in perspective of the linked criteria as well. Take the example of a mild health threat, such as the presence of S. aureus in cooked peeled shrimp. If the microbiological limit m were put at a numerical level much below that likely to pose danger, one might routinely accept lots containing a large percentage of marginally acceple units, which would need just a lenient sampling strategy. If, however, m were placed nearer the hazardous level, one would accept such lots infrequently, requiring the use of a

relatively stringent sampling plan. If m were at a hazardous level, one would not accept lots containing any units exceeding that level, and 2-class plans of high stringency would be required. Adjustment is possible by choosing limits known by experience to be associated with safety. When this is done, even though a high pro- portion of lots with substandard units will be accepted, the probability of consuming food that would cause illness is kept low.

The choice of n and c varies with the desired stringency and hence with the cases in the grid. For stringent cases n is high and c is low; for lenient cases n is low and c is high. As n decreases with the attributes plans proposed in this book, the chance of acceptance of unaccepted lots increases. This fact must be taken into account if the number of sample units exceeds the analytical capability of a laboratory and n is reduced. The procedure for selecting the number of sample units, n, should be first to fix desired acceptance and rejection probabilities for lots of defined acceple and unacceple qualities. Then the number of sample units required for this purpose is derived. However, the choice of n is usually a compromise between what is an ideal probability of assurance of consumer safety and the workload the microbiology laboratory can handle. Consider first the nature of the hazard, then decide the appropriate probabilities of acceptance and rejection for the hazard in question.

The ICMSF Tool

The ICMSF has developed in 1998 a freely downloadable Excel spreadsheet, in which two-class and three-class sampling plans can be evaluated. OC curves are presented both for the proportion defective samples and for the mean of the concentration distribution. The Log-Normal distribution is used to describe the microbial distribution. Furthermore the Poisson-log- Normal distribution is included for two-class sampling plans where the microbial method has an enrichments step. Both geometric means and arithmetic means are reported. All quantitative data are easily available since it is a spreadsheet and the programme is flexible in the type of scenario that needs to be evaluated.

CHAPTER 9

SAMPLE HANDLING, SAMPLE ANALYSIS AND LABORATORY QUALITY ASSURANCE

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Samples of many different kinds are gathered and sent to the lab for examination. These are sample units from consignments or lots of ingredients or foods used to determine the lot acceptability. Others could be for investigative reasons to evaluate environmental control, look into the cause of an issue, or to verify a procedure. Some might have legal repercussions in the event of a lawsuit or for observing regulations. This article will go through some of the key aspects that must be taken into account while gathering sample units, sending them to a lab, preparing them for analysis, and carrying out analytical processes, including laboratory quality assurance.

A sample unit is a tiny section of the lot whose distribution of microorganisms, both in terms of quantity and variety, ought to be indicative of the whole lot. Only when the food and processing circumstances lead to a homogenous dispersion of microorganisms is this criteria fully met. Only thoroughly agitated fluid goods experience this. In general, it is assumed that microorganism dispersion is random. The distribution resembles a Poisson distribution when cells are uniformly distributed throughout the solid or liquid meal, i.e., with a variance equal to the mean. Nonetheless, cells tend to cluster or aggregate more often, and their spatial distribution is erratic, with the squared variance being greater than the mean, for example, indicating a negative binomial distribution.

The quantity and type of the initial microflora of raw materials, their structure, their quantities and ratios in recipes, and the processing conditions all have a role in the presence and dispersion of pathogens, indicator organisms, or spoilage microbes in final goods. The preparation and processing of the food may also provide certain germs the chance to grow in specific areas of the meal while preventing or even killing off others. Post-process contamination, especially when intermittent, may result in the haphazard presence of pathogens at low concentrations. When liquids like milk are sampled in bulk, representative samples may be taken if the product has been fully mixed before sampling. Although sufficient mixing may be accomplished in small amounts, mixing effectiveness declines as bulk volume grows. When it comes to dry foods, the difficulty of acquiring representative samples rises as the size of the particles decreases, for example, from small powders like milk powder or flour to larger bits like nuts or raisins. Similar issues arise with meat, including entire carcasses, huge chops, trimmed bits, ground meat, and meals that include several components, such prepared dishes.

The same restrictions apply to samples taken from the production line. The likelihood of finding deviations rises when samples are taken from locations where residues tend to collect. The issue is much more complicated for environmental samples, which should be crucial as an early warning of the entry and colonization by viruses. The distribution of pathogens like salmonellae and Listeria monocytogenes is significantly influenced by elements like the complexity of the environment, the existence of niches where multiplication may take place, interactions with rival microorganisms, and changes in environmental conditions like relative

humidity, temperature, or exposure to disinfectants. The limited number of samples that are often accessible is another crucial element. This underlines the need of having a well-thought-out sampling strategy, suitable sampling equipment, and properly trained employees to collect the samples. When an analysis unit is removed from the sample unit, the dispersion of microorganisms becomes even more crucial. The distribution is normalised by homogenization, which reduces the heterogeneous distribution of microorganisms in the analytical sample. The degree of homogenization, from whole pieces to minced and finely chopped meat, lowers the variation of counts from 0.334 to 0.075 and thus enhances the chance of identifying salmonellae, as shown using beef by Kilsby and Pugh. To get accurate findings, you must use the proper standard analytical techniques or verified alternative techniques. The successful detection or enumeration of the microorganisms depends on a number of crucial practical considerations, such as the requirement to adapt methods to specific food matrices to account for the potential presence of injured microorganisms, the application of Good Laboratory Practices, etc. The purpose of this is to evaluate the many procedures from sample collection through analytical unit analysis, including laboratory quality assurance, and to identify any possible issues that could have an impact on the outcome.

A Group of Sample Units

Based on the characteristics of the sample, a judgement is often made concerning the lot or it is confirmed that the manufacturing procedures are working as anticipated. The sample must accurately reflect the microbiological characteristics of the whole area under consideration in such decisions. Samples are taken at various points throughout the whole food chain, depending on the kind of information needed. Several parties may have quite varied demands, including regulatory agencies, food producers, merchants, and academics. It is important to think about the objectives of sampling at every stage of the food supply chain, including farms, processing plants, warehouses, retail outlets, and even homes of consumers. This makes it easier to choose samples, interpret data correctly, and form reliable conclusions.

Sampling may be done for research to better understand the presence of microorganisms in food items or to gauge how the bacteria behave during processing and storage. Regulatory agencies conduct sampling at certain stages in the food supply chain to assess if imported or commercially accessible goods adhere to the law. The same authorities would do extensive sampling to pinpoint the source of an epidemic. In business, sampling is done to ensure that suppliers and consumers are adhering to criteria. The food processor may confirm adherence to GHP and the effectiveness of preventative measures by taking samples at intermediate stages of the processing line, from surfaces of equipment in touch with the product, or from the processing environment. It is common practise to sample the finished product in order to assess the HACCP plan's effectiveness.

"Initial sampling" refers to the process of taking physical samples at designated sampling stations in accordance with a previously established sampling plan. Often, the sample unit is bigger than the quantity that is being examined. The term "sample handling" refers to each of the steps that come after, such as the description, recording, and labelling of the samples, their interim storage prior to dispatch, their transportation to the analytical laboratory, their reception, registration, and storage, and the preparation of the analytical unit before analysis. To guarantee the quality of the sample units and enable trustworthy traceability, including "chain of custody" for regulatory samples, all procedures leading up to the analysis must be under control. The recommendations made in this are considered to be best practises and ought to enhance outcomes.

Scientific Sampling

Investigation samples are often taken in reaction to observed deviations or "out of control" circumstances, to support regulatory measures, or to record findings. It is a to start In this case, random sampling is not appropriate; instead, the investigator should combine his or her knowledge of microbiology with that of the process, the equipment design, and other pertinent data to identify the sites and foods that need to be sampled, i.e., the implicated foods and locations where the microorganism is most likely to be found. Samples that might be used as evidence in court need to be carefully sealed, ideally with tamper-proof seals, to preserve their integrity and chain of custody. Before samples leave the facility, dated photographs of the samples is a helpful technique to record their integrity. All paperwork andl for at least 30 s before to use. No restrictions should be placed on the kind of tools used for environmental sampling, particularly at locations that are more difficult to reach. To test for food contamination on floors, walls, or other surfaces, sponges or swabs are best. To gather leftovers in fissures or beneath machinery, use brushes, scrapers, scoops, or spatulas. To collect water from drains and to sample the air, plastic pipettes and syringes may be used. Individually packed and pre-sterilized containers and utensils may eliminate the requirement for disinfection between samples. While collecting certain samples, disposable plastic gloves should be used, one fresh glove being used for each sample. Torch sterilisation of kitchenware is not recommended and could even be harmful.

Sampling Techniques

The sample technique should be modified to suit the goal and account for the environment, such as dry vs moist surroundings. The goal is to gather sample units that are typical of the lot in order to make decisions on lot acceptance. A regular procedure with sample locations and a technique of sampling should be created to evaluate control of the processing environment. The sampling locations, foods, substances, times, frequencies, etc. are not pre-determined and are left to the sampler's judgement when determining the cause of a problem. Although it would be ideal to make every attempt to acquire samples that serve the intended purpose, the safety of the sample collector and any potential impacted parties comes first.In order to prevent osmotic shock-related die-off, dry foods must be rehydrated gradually. This was validated by van Schothorst et al., who demonstrated how salmonellae recovery may be significantly impacted by the rehydration conditions of meals. Shaking had worse recovery rates than slow rehydration techniques like soaking. In foods, both the proportion of healthy, dead, and damaged cells as well as the severity of cell damage varies. This topic has been the focus of several investigations, and there are reviews available.

Regeneration of Damaged Cells

Certain foods that have undergone freezing, moderate heating, chemical preservation, low water activity, or low pH may have bacteria that are sub lethally harmed. Analytical outcomes may be significantly impacted by damage to microbial cells. For these bacteria to be successfully discovered by growth on or on selective medium, resuscitation conditions must be present. The wounded or damaged cells will have enough time to repair the damage and regain all of their normal phenotypic and physiological characteristics under the correct resuscitation circumstances. Before growth begins, something is required. This "time to repair" impacts the incubation times of both solid and liquid media by increasing the lag phase. The time frames suggested in conventional approaches are often the best for recovering the greatest number of microorganisms. Lowering this incubation time might result in significant counts being reduced or, in the case of qualitative approaches, false negatives. For Salmonella, for instance, this was shown when pre-enrichment for 6 h resulted in poorer recoveries than pre-enrichment for 24 h. Pre-enrichment that was extended to 48

hours did not result in a rise in salmonellae, and in some cases, the levels were even lower than after 24 hours. As shown by the discovery of Salmonella in various dietary matrices, food components may significantly alter both healthy and wounded cells' ability to recover. Rehydration rates may be lowered when dehydrated items, such milk powder or feeds, are rehydrated quickly. Higher dilution rates or the inclusion of chemicals are needed to counteract the inhibitory effects of salt or food components containing naturally occurring antibacterial components in dehydrated culinary goods like soups or concentrated bouillons. For instance, negative results may have bacteriostatic and/or public health effects. Even when a considerably larger percentage of the product is tested in the retest, retesting often does not corroborate the first good result. There may be a wish to think the initial finding to be in mistake, maybe owing to contamination during collection or analysis. Other causes, including non-random, heterogeneous distribution, low incidence of contamination, and pathogen die-off between the initial and repeat tests, should also be taken into account, even if laboratory or sampling error are likely ones.

Understanding how a microorganism may be spread inside a food product and the distinction between prevalence and concentration of contamination are necessary to comprehend the disparity between the original and retest findings. The frequency at which several samples from a certain product test positively is referred to as prevalence. The quantity of cells in a particular amount of a product is referred to as concentration. Think on the illustrations in 9.1. The prevalence is the same for both lots A and B, although there are varying concentrations. Lots A and B would likely provide 6 positives for every 100 samples tested if each lot was separated into 100 one-kg samples and each sample was tested separately using a technology that could detect one cell kg 1. Due to the presence of clumps of around 100 cells in each positive sample, lot B really has a 100-fold greater concentration. In real life, microbial contamination often occurs as clumps, as seen in lot B. The nature of the product, the source of contamination, and the sil- icity of the microbial contaminant in the product will all affect the number of cells per clump. The dispersion of the microorganisms inside the product must also be taken into account. If distribution is homogenous, each individual sample unit would have an identical chance of being contaminated at any point in the procedure.

Low Contamination Rate over Many Batches

The findings of tests might sometimes show that contamination happens regularly but at a very low level. In this illustration, a homogenous product is produced in 20 lots each month using a 500-L kettle, and each lot contains one Salmonella cell per 3750 g. Just two lots will test positive using a sampling strategy utilizing n = 15 25-g analytical units to create a 375-g composite. It may be argued that there was a one in 100 probability that two positive lots would have been discovered if sufficient retesting is done to establish that the prevalence of contamination in each of the two positive batches is one cell in 3750 g. Given the low likelihood, it is possible that some of the negative lots may also have been contaminated, but less often. If the contamination is widespread, it would be determined by a detailed study of numerous negative samples. In any case, it is important to evaluate and document product histories for rare occurrences of positive test findings that point to widespread, low-level contamination.

Change in Contamination Concentration

The quantity of live cells in a contaminated ingredient, food, or food processing environment may rise, decrease, or stay constant over time. The concentration of contamination will rise and become more obvious if a disease is proliferating. The likelihood of discovery will, however, decrease if death is taking place. A survival curve for Salmonella enterica that was

liquid-applied to spinach leaves or absorbed onto dust from chicken dung is one example. With the liquid application, the number of live cells quickly declines after 1 day without UV treatment, and Salmonella surpasses the plate count detection limit. It takes around 5 days for a standard Salmonella test to provide a positive result. During this period, the concentration of live cells has fallen to below the detection limit if one continues to utilise the plate count approach. There is a higher chance that the second test won't confirm the first positive result if a retest is requested using the plate count technique without switching to a method with a lower level of detection, such the Most Probable Number method. Depending on the product, pathogen, and storage situation, different rates for the first quick decline and the following gradual reduction apply. A survival curve similar to that shown in 9.3 for liquid application is produced when pathogens like Salmonella are transferred from a high moisture growth condition into dry product, according to extensive experience with preparing inoculated samples for laboratory performance testing, evaluation of new methods, and challenge testing. Salmonella that has proliferated in a plant environment and is subsequently added to a dry food, for instance in many cases when hence tests are conducted, would be anticipated to follow a very similar curve. The accuracy of the procedure, or the capacity to produce outcomes that are equal to or nearly so, defines the quality of the results. When the same sample is analyzed by the same analyst under the same analytical circumstances, two unique outcomes are achieved, and this difference is represented by a method's reproducibility. On the other hand, reproducibility illustrates the distinction between two laboratories. Many ISO papers provide examples of r and R-values for various analytes. The quality of errors impacting the data acquired by analytical labs has been thoroughly addressed. The size of the analytical unit sampled, the homogeneous or heterogeneous distribution of microorganisms in the primary sample, the accuracy of weighing the analytical unit, and differences in handling procedures either in the laboratory or during the sampling process are a few examples of laboratory sampling errors.

Inaccuracies in diluent volume resulting from different diluent volumes used during serial dilutions and in the quantities of diluent used during the initial homogenization of samples, which will influence the accuracy of the colony counts obtained. Fluctuations in liquid dispensing quantities due to pipette volume mistakes, which may also be caused by bacterial adhesion to pipettes or pipette tip walls and correct pipette calibration. The homogenization and maceration of the food sample, which should be carried out using the proper apparatus that enables sufficient homogenization of the sample and doesn't raise temperature, preventing sub lethal harm to microbial cells. The amount of time that must pass between dilution preparation and plating, which should always be constant and as brief as feasible. Pour plate and associated procedure problems, which include things like not thoroughly cooling molten agar before it is poured, inadequately mixing the agar with the inoculum, spreaders hiding other colonies, and variations in oxygen exposure that will impact colony counts. Errors in the surface plating approach include insufficient inoculum spreading, insufficient agar surface drying, and partial microbial unit adhesion to the spreading device. False positive and false negative findings for molecular approaches may happen for a variety of reasons. They consist of:

Falsely Positive Outcomes

Contamination coming from a positive source, such as another food that has been inadvertently contaminated with the target organism or a positive laboratory control. Lack of appropriate test stringency, such as non-specific food matrix material binding to PCR probes or target genes for PCR probes that are not unique to the intended organism. False negative outcomes because there weren't enough target sequences in the volume examined; inhibitory chemicals being present in the dietary matrix.

PCR's Lack of Specificity

ANSI 17025, which lays down general standards for the proficiency of testing and calibration labs, is accessible, and laboratories may apply for certification by suitable accrediting bodies. These organisations provide independent assessments of the laboratory's adherence to the chosen standard and enable the laboratory to prove the competence and dependability of its practises. A number of organisations have put up guidance documents for the application of laboratory quality standards to help labs in this endeavor.

Guarantee of Laboratory Quality

Verification of Procedures

Since trustworthy findings from microbiological techniques for testing foods are essential for a range of applications, it's crucial to identify a method's performance characteristics via validation. A method's performance against a set of technical requirements is shown via validation, which is a crucial step in producing findings that can be relied upon every time. The end user may have trust that a technique has been shown to be able to identify or count the microbe or group of microorganisms indicated via validation.

International, national, and trade organisations, as well as national governments, have created standardised methodologies. AOAC International and ISO are a few of them. Both qualitative and quantitative approaches are often validated in food microbiology by comparison to a standard- ized, traditional method; these methods are frequently regarded as reference methods. Many alternative, quicker techniques for detection and IEC 17043:2010 have been developed during the last 20 years. It has been created to provide all interested parties a uniform foundation for judging the skill of companies that offer proficiency testing. In addition to providing recommendations on the use of proper statistical techniques, it also provides information on the main forms of proficiency testing schemes that are used by labs, accrediting organisations, regulatory authorities, and other interested parties.

A chance for development is provided by labs' participation in proficiency exams run and provided by national, professional, or commercial organisations. Such proficiency exams facili- tate benchmarking performance of a laboratory by providing labs with a way of objectively measuring and showing the dependability of the data they provide. They may be utilised as a risk management and performance-improving tool since they help in identifying flaws. There is a concern that labs that operate in isolation and do not often have access to other laboratories' data may have biases, inaccuracies, or major variations in their own data. In order to ensure consistency of analytical data from a laboratory, proficiency testing may also be used to evaluate and enhance the performance of analysts inside a laboratory.

It is crucial to remember that the kinds of samples used for proficiency testing have restrictions relating to their preparation and the viability of the microbial population. As a result, not all food matrices have samples accessible for proficiency testing. Competitive microbiota are not always present, and pathogen concentrations in proficiency test samples are typically rather high. As a result, these samples don't test the lab's capacity to find even a small number of damaged cells, which may appear in real food samples. In order to evaluate the effectiveness of a procedure in the lab or its dependability, reference materials containing just very small amounts of damaged cells may be more helpful. Different microorganisms have their own reference materials generated.

CHAPTER 10

IMPACT OF SAMPLING CONCEPTS ON THE EFFECTIVENESS OF MICROBIOLOGICAL METHODOLOGIES

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The phenomenon known as "damage" is one of the basic methodological issues connected to food microbiological testing. Physiological ageing of microbial cells as a consequence of exposure to diverse stimuli is involved here. This is a state that often happens when microorganisms are inactivated. Injury is often a transitory condition when the applied stress has started to harm the morphological, physiological, and me-olic systems inside the cell but not enough to render it non-viable. In the end, the stress causes enough accumulated damage that the cell can no longer recuperate and is rendered non-viable. Injury often follows first order kinetics, with just a fraction of the cells becoming harmed over time, similar to inactivation. It is crucial to stress that wounded cells may heal, proliferate, and cause illness if given the right circumstances.

The use of selective and differentiating microbiological medium is crucial for the isolation and identification of microorganisms by culture methods. For the microbe of interest, selective plating or enrichment medium may use a range of chemical agents or circumstances to which it is resistant, allowing it to flourish while inhibiting the development of other germs. For instance, to inhibit the development of Gram-positive bacteria, crystal violet is often added to medium that are used for the selective isolation of Enterobacteriaceae. The molecular mechanisms that enable the microbial cell to withstand various stressors, such as poisonous elements, antimicrobials, antibiotics, dyes, emulsifiers, unfavorable pHs, decreased water activity, and increased incubation temperatures, are often disrupted when cells are harmed. The underlying elements that are commonly employed include a microorganism's capacity to withstand one or more of the aforementioned substances or circumstances. Environment of 83.3 mM acetic acid at 37 °C for 7 hours. Agar types: selective and non-selective the most common technique for determining the degree of damage in a bacterial population is selective media. Both the selective and non-selective media provide estimates of the overall number of viable cells as well as the non-injured cells.

The aforementioned event has the immediate effect of invalidating the presumption that if the target microorganism is present in the analysis unit, it would be identified by microbiological procedures that directly employ selective media. As a consequence, there will be a noticeable decrease in the number of detectable cells, which will be seen in a drop in the number of positive reactions in presence.

Percentage of cells with damage

Salmonella at a mean concentration that is not harmed. The aerobic model for Listeria monocyte-genes from the USDA Pathogen Modeling Program was used to determine the growth of the target microorganism and forecast the length of the lag phase and generation periods. The likelihood that the lot from which the sample was obtained would be approved as lacking *L. monocytogenes* was then calculated using the levels anticipated in the enrichment broth.

The findings unequivocally show that the length of the incubation time and the pH of the enrichment broth affect the likelihood of finding a contaminated lot. A high level of confidence that the initial contamination levels would be found is given by an incubation time of 18 hours, particularly if several analytical units were checked. Nevertheless, if the enrichment broth's pH was pH 5.0, this would not be sufficient. Particularly in environments that encourage fast development, the change from low to great detection probability is rather dramatic. This is a result of the shorter lag periods and the exponential proliferation of microbes. This example shows quantitatively how enrichment procedures used in sample plans are dependent on the target microorganism growing enough.

The second scenario builds on the one we just spoke about by taking injury effects into account. Let's suppose for the sake of this example that a heavily mixed food product undergoes a light heat treatment that damages 99.99% of the cells but is inadequate to inactivate *L. monocytogenes.* For the sake of this example, we'll suppose that the lag phase of the wounded cells is prolonged by 4 h so that they may recuperate before growing in a selective enrichment medium. We will further assume that 100 CFUml = 1.0 for total cells and Log represents the concentration of *L. monocytogenes* in food.

This example had to take into account how wounded cells behaved while they were healing. In order to keep things simple, it is assumed that the recovery time is separate from the lag phase, that is, it is added onto the lag phase, and that it is unaffected by the pH or incubation temperature of the enrichment broth.

The growth kinetics results are identical to those for unharmed cells after the recovery time. The impact of injuries on the likelihood of lot acceptance is given based on this supposition. Similar to the previous case, if an enrichment step is necessary to establish a high possibility that the microbe is present in the final analytical unit utilised for detection, the possible detection of *L. monocytogenes* was significantly impacted by the length of the enrichment period. The capacity of the enrichment conditions to promote maximum growth also has an impact on this. The process is further impacted by injury and other sub-lethal stimuli because they lengthen the wounded cells' lag phase. As can be seen in this illustration, injuries prolonged the necessary enrichment procedure, highlighting the difficulties in attempting to shorten the currently advised enrichment incubation times. As was previously said, the main focus is on designing efficient enrichment media and methods, tightening the requirements for intra-method sampling, and avoiding what may be a significant source of type 2 mistakes.

The target microbe operates independently, which means that its development during enrichment is unaffected by the presence of other bacteria. This is often one of the implicit assumptions in the testing of foods for particular microorganisms. When dealing with low concentrations of the target bacterium and other microbiological species, this presumption often remains true. In respect to situations involving large concentrations of other microbes, such as the likelihood that food with a low level of *Listeria monocytogenesa* will be accepted is hypothetically affected by injury, the length of the enrichment incubation, and the pH of the enrichment broth.

These conditions could arise during an enrichment step with foods that have a significant microbial burden, either naturally occurring or purposefully added. This would be especially true if non-selective enrichment were being used. Two separate issues might possibly develop. The first occurs when the target microbe's development is impeded by the growth of another microorganism. The second occurs when the target microbe's identification is hampered by the development of a closely similar microorganism. We'll explore examples for each of the scenarios below.

The Jameson Effect's Possible Influence on Sampling Plan Effectiveness

One of the characteristics of microbiological systems with two or more competing microbes is that if one of them has a noticeably quicker growth rate, it will inhibit the highest level that the second germ may reach. The Jameson Effect is the name given to the phenomena. Usually, when the dominant microorganism reaches the early stages of stationary phase, the competing microorganism has 109 CFUml of population. Based on the outcomes of the enrichment, the likelihood that a lot would be accepted.

For presence-selective plating medium, probability of acceptance values were computed using the ICMSF model, and then representative colonies were chosen to verify the isolate's identification. Nevertheless, if a second microbe develops on the plating medium that cannot be clearly separated from the target species, this approach may produce severe type 2 mistakes. If the second microbe grows more quickly or is much more concentrated in the first enrichment broth than the target pathogen, this might be a specific issue. As an example of how the variation in growth rates might affect the efficacy of identifying the presence of the pathogen, it has long been known that the presence of Listeria inoculate in food can hinder the correct detection of *L. monocytogenes.* This will be used as a hypothetical example.

We'll suppose for the sake of this example that a homogeneously mixed meal includes *L. monocytogenes* at a concentration of 1 CFU/100 g, or 1 CFU/g. A suitable enrichment broth and a food sample are combined at a ratio of 1:9 such that the starting concentration of *L. monocytogenes* in the enrichment broth is Log = 3.0, 2.0, or 1.0. The enrichments are then incubated at 25 °C for 24 hours, and a 1.0 ml aliquot of the enrichment broth was properly diluted to yield between 100 and 300 colonies per plate when spread plated on PALCAM agar. Selected colonies were examined to see whether they belonged to *L. monocytogenes* or *L. innocua*. This lot would be acceptable if, with n equal to the number of colonies tested and c = 0, none of the colonies chosen were *L. monocytogenes.*

For the sake of this illustration, it is assumed that *L. monocytogenes'* lag phase and generation times were respectively 3.0 hours and 1.0 hours and 2.5 hours and 0.8 hours. 10.4 shows how *L. monocytogenes* and *L. innocua* are expected to develop. Using a hypergeometric distribution, the probability of acceptance is determined as follows: P =! The concentrations of *L. innocua* are listed in 10.9. In this hypothetical example, there is a strong possibility that at least one *L. monocytogenes* would be found if the final concentrations of the two microbes are almost the same at the conclusion of enrichment, especially if at least five colonies are checked. The relative abundance of *L. monocytogenes*, however, reduces sharply when the ratio of *L. innocua* to *L. monocytogenes* rises, and the likelihood of finding a colony of *L. monocytogenes* diminishes dramatically. This may be somewhat mitigated by increasing the number of colonies investigated, but the practicalities of evaluating such a high number of colonies would limit the usefulness of this. Instead, creating an enrichment medium that inhibits the development of *L. innocua* during enrichment or a plating media that enables direct distinction of the two bacteria might be a preferable option.

From the several examples given, it is clear that an enrichment process must be used to amplify the pathogen's levels in order to identify particular pathogenic microorganisms at low concentration levels with a high degree of certainty that at least one cell is present in the analytical unit. The starting concentration of the target microbe in the meal, the rate of development of the microorganism in the enrichment medium, the presence of competing microorganisms, the size of the analytical unit tested, the technique of analysis, etc., all affect the degree of confidence. Finding a way to concentrate the target microbe without increasing the concentration of rival microorganisms, changing the properties of the enrichment medium, or harming the target microorganism would be the perfect answer.

Powerful magnetic field that draws magnetic beads to its location and holds them there. The original sample volume is decanted, and the beads are then washed in buffer to remove any remaining food homogenate or enrichment broth. The magnetic field is subsequently withdrawn, the beads with attached target cells are re-suspended in a smaller volume, and the concentrated suspension of cells is transported to a detecting device. The predicted concentration would be 16 CFUfor food matrix) to the beads as a way of showing the influence of immunomagnetic separationml and that a 10 ml sample was treated to an immunomagnetic concentration which was 80% efficient and then re- suspended in 0.5 ml of buffer. Both will have an impact on the concentration step and restrict the advantages to the identification of batches with low defect rates. The suitable sample statistics that have to be employed with these kinds of technologies have only been examined in a few number of direct evaluations of immunomagnetic concentration technologies.

The last decade has witnessed a major shift to several quick approaches based on the identification of certain genes or proteins linked with the bacteria of interest. The underlying sampling and methodological statistics involved with the assessment of foods and water using immunologic and genomic-based technologies have, however, only been the subject of a small number of research. Nonetheless, a few ideas may be deduced from biological principles. As has been explained throughout, the fact that microorganisms are unique particles and cannot be indefinitely diluted substantially influences the sample statistics for culture approaches. So, the likelihood that an analytical unit truly includes a microbe will eventually affect the likelihood of detection. By collecting additional samples, taking larger samples, or enriching the sample, this may be avoided. One would anticipate that the use of genomic-based approaches, especially those that depend on the identification of a single chromosomal DNA region, will follow a similar path. As a result, if an analytical unit is collected before DNA extraction, the presence of a microbe will be the limiting factor. On the other hand, because there is only one DNA copy per original cell, there will be a question as to whether the analytical unit includes a copy of the target DNA sequence if the DNA is extracted first. Based on the efficiency of the DNA extraction processes and the existence of any PCR inhibitors that are co-extracted with the DNA, the method's real effectiveness would be further reduced. The effectiveness of the PCR reaction and the reaction volumes used in PCR reactions will both affect the performance of the detection process. This suggests that the effects of sample size and the need for enrichment will be comparable to those previously outlined for cultural techniques. The few research that have examined the sample statistics of cultural and genomic-based approaches provide credence to this hypothesis.

While taking sampling statistics into account is acknowledged as a critical component in the success of food microbiological testing, the collection of food samples has received the majority of attention. The aforementioned examples, however, show how sampling theories may also be used to explain other methodological performance traits. Developers of methods have a tendency to treat microorganisms as chemicals. The chance that a microbe or one of its components is genuinely present in the analytical unit under examination must be taken into account due to the particle nature of microorganisms. When assessing the efficacy of microbiological procedures, these parameters must be taken into account, especially when enrichment techniques are employed to increase the levels of the target micro-organism. As was seen above, this has a significant impact on a method's effective LOD and, therefore, the efficiency of a sampling strategy as a whole.

Use of Investigational Sampling and Tightened Sampling

Tightened and investigative sampling are typically used in circumstances where there is a higher level of concern or perceived risk, such as when a process deviation has occurred, a

performance criterion has not been met, a product has failed to meet microbiological criteria, the food comes from an operation with a history of inconsistent control, the food comes from a region where there has been a recent increase in illness involving the same or similar type of food, or When there is inadequate information on an ingredient that is especially sensitive or a food is meant for a sensitive population, there may be a greater need for tighter sampling since it is more important to determine the suitability of the food. For instance, shipments from a new supplier or from a nation where the production and sanitary standards are unknown may call for greater sampling. Both industry and regulatory agencies may encounter these situations, and both may use investigative sampling and tighter sampling. The objectives and methods, nevertheless, could vary. Consumer protection is the main concern of regulatory bodies. In maintaining that objective, business must also safeguard the company's financial interests. Both aim to distinguish between acceptable and unacceptable products, but it's also crucial for business to understand how mistakes might be made and why they could have happened. Control authorities may have less knowledge regarding the origin and production of a specific suspicious lot than the industry, and in many situations they would be need to rely on the industry to identify the impacted lots.

Aseptically packaged goods that are sterilised in-line and subsequently placed into sterile containers are another example of stricter sampling. Product sampling at high frequency is customary during the commissioning of new lines as well as in the wake of contamination-related issues. The sample frequency may be decreased once it is clear that the defect rate is below the acceptable threshold. For further information, please contact us. When dealing with goods that have a limited shelf life and are very perishable, this is a particular worry. Due to the possibility that the product has already been launched into the market, speed is often of the utmost significance when testing in such circumstances. Sensitivity, specificity, repeatability, and reliability are additional factors that must be taken into account when choosing the test technique to be used. Test procedures must be valid and appropriate for their intended use. The capacity to identify low concentrations of pathogens in the case of infectious agents is often crucial, and such tests frequently entail some kind of non-selective pre-enrichment, transfer to a selective enrichment, and then detection trends.

A poor trend in a hygiene indicator has been seen, and a component has lately been linked to sickness. Tighter sampling may be used as a result of these and other factors until accumulating data shows that it is no longer required. Also, when contamination proof is sought in legal disputes, both tighter and investigative sampling are often used.

Enhanced Sampling Strategies

Increasing the number of samples that are taken and examined is one way to make a sampling strategy more exacting. The number of analytical units must be raised when 2-class plans are involved and m is fixed in order to produce a more severe plan. Another alternative for 2-class sampling programmes is to increase the size of the analytical unit for testing rather than changing n. The likelihood that this choice will improve confidence in recognizing a faulty sample unit within the lot and, as a result, result in the rejection of the meal will rely on the geographic distribution and independence of the defectives across the lot. Making c smaller or n bigger may make acceptance criteria more demanding when 3-class plans with fixed m and M are involved.

Other information may already be available to show which lots may be impacted by an issue, and this will guide the development of more exact sample plans that may be used to detect date coding, CCP monitoring data, etc. With this data, stricter sample strategies may be used to examine specific lots to see whether they are impacted and to validate the failure rate. It is possible that the information currently available indicates a specific rate of failure in specific

lots, but it is also possible that other lots are also affected, possibly with a lower and steadily declining rate of failure that is not immediately apparent due to the time it takes for the physical defect to manifest. In order to properly manage risk and prevent incurring extra expenditures for recovering unaffected lots, it is crucial to identify all of the impacted lots. It is also likely that the apparent incidence of failure is overestimated based on preliminary findings since they are linked to a specific kind of contaminant that is absent from other potentially impacted packs but is capable of producing less obtrusive contamination symptoms. This is another important reason to carry out more stringent microbiological testing to identify all affected lots. This is crucial when informing the public about recalls since it's common for people to react badly to recalls that grow beyond their original announcement.

Use of a more rigorous sampling strategy is required to prove that the process is under control after a contamination occurrence. Even while steps may be taken to avoid recurrence, it is not always possible to determine that the issue has been resolved in contaminated scenarios. In these instances, more rigorous sampling might provide useful information and assurance. It is more confident that the defect rate is within the acceptable level thanks to the use of a stricter sampling methodology.

The purpose of the sample should be well understood prior to selecting a sampling strategy. Is the sample meant to distinguish between acceptable and unacceptable products or to look into and find the source of an issue? The sampling goal will have a significant impact on the strictness of the strategy and the usage of biassed and unbiased sampling.

When there is "zero tolerance" for the characteristic being tested, such as Salmonella and E. coli O157:H7 in an RTE product, adopting a sample strategy for investigative or acceptance sampling may be very challenging. No sampling strategy, short of a complete investigation of the food, can guarantee a flaw is completely absent. Hence, the investigator is in a position where a choice must be made about what level of stringency is appropriate yet still practicable to implement. When a Food Safety Goal, Performance Objective, or other eslished constraint is present, choosing a plan is significantly simpler. The degree of plan rigour required to identify the defect's levels may then be established. The established limit may sometimes be too low to allow for realistic sampling and testing for either research reasons or to distinguish between acceptable and questionable products. Even if it is not feasible, the investigator may at least identify the level of plan rigour that would be required.

Even if the stated regulatory stance is "zero tolerance," there may be commonly accepted required sampling programmes. In the United States, salmonella is an example of this. Although having established a "zero tolerance" for Salmonella in the majority of processed foods, the FDA still uses sampling strategies that are comparable to ICMSF cases 13–15, which are referred to as categories I, II, and III in the FDA Bacteriological Analytical Manual. A batch containing 2% defectives would test negative 30% of the time, even under the strictest conditions. In order to provide some assurances that the flaw may be identified with more sensitivity than if the usual attribute plan were used, it is typical in the U.S. to choose a plan with a higher stringency. Also, the population to be selected must be chosen while taking into consideration any possible issue sources. Think about a scenario where three slicers are used to cut three different types of meat into slices, which are then combined to create a package that has a piece of each. One slicer in particular, especially during the first hour or more of slicing, may be a source of contamination if it has not been cleaned correctly. The cause of the issue won't be found by a sampling strategy that doesn't choose samples from each slicer or that doesn't include enough samples to be reasonably guaranteed to include product from each slicer. A biassed sample that guarantees the malfunctioning slicer

is represented will obviously be more effective than a random sample with a large enough sample size to be reasonably assured that each slicer is represented if the samples can be linked to a particular slicer. This is particularly true if one wants samples from each slicer for each time segment since the contamination may be time-related.

Fewer Samples

Reduced sample frequency may result from factors related to the meal or its supplier. When an audit of a food operation. Also, a continuous sampling programme might, over time, provide a degree of assurance that will let testing to be lowered, a sign of excellence in the manufacturing process. Moreover, items with minimal risk could be tested less often. Reduced sampling often entails sampling less frequently, sampling the same amount of sample units more frequently, or, if the situation calls for it, not sampling at all. Nonetheless, it is advised to use the sampling strategies and when lots are sampled. In other words, the acceptance standard is equivalent to the standard for the matching plan for routine inspection. It is important to notice that decreased sample has lower discriminating ability than regular inspection. Unless data indicates that regular sampling should be resumed, the lower level of sampling may be employed. By completely excluding certain lots from the sample process, the pace of sampling may be slowed down, freeing up precious resources that can be used elsewhere. A tried-and-true method for cutting down on sampling is known as skip-lot sampling, which works by deciding at random, with a set probability, whether a lot offered for inspection would be accepted without examination.

Sampling in Skip-Lots

To adopt skip-lot sampling, a single sample strategy that details the risks must first be created. The "reference sampling plan" is what we're talking about here. Beginning with this reference plan, normal lot-by-lot testing is conducted. A transition is made to sample just a portion of the reference sampling plan after a certain number of consecutive lots are approved. The individuals that make up that proportion need to be chosen at random. When a lot is rejected for whatever reason, the original sampling plan is continued or a stricter plan is put in place until confidence is restored that the performance has recovered to an acceptable level. A technique called skip-lot acceptance sampling enables the exclusion of particular lots from testing. various dangers of ANSI producers. The "reference sampling plan" is the name given to this strategy.Begin by doing a routine sample check while utilizing the provided reference plan. Change to checking just a portion of the lots after a certain number, I of consecutive lots are approved. As with the reference plan, the members of that fraction are chosen at random. Return to regular inspection as outlined in the reference plan when a lot is rejected under the skip-lot plan.

Mistaken Methods for Strict Sampling Plans

In the context of incorrect uses of severe sampling programmes, the two instances provided below are relevant. If the plans n = 60, c = 0 and n = 95, c = 1 provide equivalent probabilities, for instance, it may be argued that an operator discovering one positive in 60 analytical units would then check another 35 in the hopes of clearing the lot if all of the latter were negative. Nevertheless, this method is really a two-stage strategy with $n1 = 60$ and $c1 = 1$, plus $n2 = 35$ and $c2 = 0$, which has a higher likelihood of accepting an unacceptable lot than n = 95 and c = 1. The actual probability Pa is 0.07 as opposed to the one-stage plan's 0.05 value. Even though there isn't much of a difference in this case, two-stage methods like this one might sometimes result in more serious errors. The chance of approval for two-stage plans will nearly always be higher than for one-stage plans, therefore employ them with care. The OC curves of two-stage sampling plans should be calculated, and the resultant

probabilities of acceptance should be assessed, where they are really being employed. Similar issues might occur when a strategy requires a significant number of expensive analytical units. Assume that the plan has n = 95 and c = 1, but that initially only 20 units are tested for economy's sake. If a unit from this first group were to malfunction, an analyst may investigate the other units with the thought that if a problem was not discovered in the second group, the first might be disregarded. In reality, however, the sample scheme that was used corresponds to n1 = 20, c1 = 1, and n2 = 75, c2 = 0. There is no basis for "preferring" the outcomes of the second series. The double sampling plan would be used instead of the original n = 60, c = 0 plan if an analyst was unsure about finding a defective unit in the first 20 samples and the lot was approved based on the remaining 40 samples being negative. This allows for the acceptance of more defective lots than the original n = 60, c = 0 plan would. This serves as an example of how sampling processes should be used correctly and results should be evaluated.

The US Department of Agriculture Food Safety Inspection Service's Pathogen Reduction absence testing has used the "moving windows" method. Each facility's performance is evaluated using a set of size n samples, which is a collection of samples. The number of positives in independent samples was expected to follow a binomial distribution when sampling with two alternative outcomes. A facility operating at the baseline prevalence for Salmonella in carcass or ground meat samples was determined to have an 80% chance of passing by USDA-FSIS. This was chosen to strike a compromise between the necessity to identify achievements likely to be functioning above the prevalence and the need to prevent an achievement from failing purely on chance. Next, depending on the baseline prevalence, a sampling plan or performance is created in a way similar to that previously stated in this to provide each achievement an 80% chance of passing.

CHAPTER 11

SAMPLING TO ASSESS CONTROL OF THE ENVIRONMENT

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This article discusses the significance of microbiological testing to determine how well control mechanisms put in place to stop product contamination from the environment are working. Emphasis is placed on preventing contamination of ready-to-eat meals. While the topic is restricted to the validation of pathogen control techniques, the ideas may be used to address microbial deterioration. Regular environmental sampling is used less regularly at other points throughout the food supply chain and more frequently at food processing facilities. As recontamination of food from equipment or other environmental sources is a substantial contributor to foodborne illness, this concentrates on the verification of processing environment controls in facilities making foods. There is also a role for environmental contamination at various stages of the food supply chain, including initial food manufacturing, distribution, retail and foodservice locations, and consumer environments. While possible uses are briefly explored, microbiological sampling under these conditions may be more difficult. Across the whole food supply chain, efficient control mechanisms must be put in place to ensure the microbiological safety of foods. To reduce the quantity and prevalence of pathogens during primary production and harvesting of fruits, vegetables, and animals, good agricultural practises are crucial. Foods produced industrially need the proper design and execution of Hazard Analysis Critical Control Point systems and Good Hygienic Practices. The essential criteria for pathogen control and the execution of a successful HACCP plan are included in GHP. Risks arising from increases in growth or recontamination may be reduced by using good practises during storage, distribution, and subsequent handling in the food chain. For many goods, understanding the microbial ecology of the environment is crucial. Many foodborne outbreaks linked to commercially produced foods demonstrate how poor GHP implementation may result in pathogen post-process contamination. outbreaks connected to fresh fruits and vegetables have been brought on by environmental contamination in the field. Cross-contamination has also been linked to outbreaks in the restaurant and retail industries. Prerequisite programmes like Good Agricultural Practices, Good Storage Practices, and Good Hygiene Practices play a crucial role in reducing the presence of pathogens throughout the food chain in food safety management systems. Critical Control Points are used in HACCP systems to prevent, remove, or limit microbiological risks to acceptable levels. Although minimally processed foods may require adjustment of one or more parameters, such as low temperature storage, decreased aw, lowered pH, etc., especially tailored to minimise pathogen development, many goods have CCPs that typically include a kill phase. The safety of the goods is helped by the attainment of the proper critical limits at particular processing phases, taking into account any changes throughout operations.

Experience shows that, until the product is in a sealed container, even the best implementation of precondition programmes or HACCP plans may not provide a guarantee against contamination from the production environment. Consequently, the chance of contamination when food is exposed to the processing environment may be decreased, but not entirely prevented or eliminated. Manufacturers may choose to establish methods to implement zoning that identifies high hygiene regions and GHPs to reduce recontamination

in these areas once this potential is realised. Moreover, the establishment of a regular monitoring programme to evaluate environmental management may be required in cases when environmental pollution might lead to dangerous foods.

The control measures that need to be in place before a HACCP programme is implemented are referred to as "prerequisite programmes". General Principles of Food Hygiene provides a description of the fundamental components of GHP for food processing. Other nations may publish their own standards, such as the Current Good Manufacturing Practices of the U.S. FDA. Codex Alimentarius has produced a number of rules of sanitary procedures for various goods. Arrangement of processing lines and supervision of people and mobile equipment mobility to limit cross contamination from raw materials to final product are some of the most significant components of preparatory programmes that may reduce contamination from the processing environment.

Design of the equipment and placement for cleaning,reduce the likelihood for disease transmission by using high pressure air or water improperly, and employ suitable cleaning and disinfection processes that are targeted at the pathogens of concern for the specific food and process.Proper trash disposal, planned preventative maintenance to reduce problems during operation and.

Regarding the Target Diseases, Staff Behaviour and Training

To evaluate GHP efficacy and adherence, visual and other sensory inspections are useful. Although such inspections may be carried out by professionals who have received the necessary training, all operators should be taught to remain watchful at all times and to report any deviations from the standard. Monitoring the microbiome for infections or the right indications may help determine if the environment is under control. While creating and implementing GHP programmes, the risk for contamination should be taken into account. If contamination is expected to happen, a method for gauging the program's success should be used. To reduce in-process contamination, each manufacturing facility must take into account control methods that are suitable for the operational circumstances, the food, the process, and the pathogens of concern. When issues arise, these facilities must also undertake root-cause analyses.

To enhance hygiene and food safety in the food supply chain, several rules and regulations have been developed. Guidelines for Good Farming Practices, Standards for the Growing, Harvesting, Packing, and Holding of Produce for Human Consumption, and documents specific to particular commodities, such as pumpkin, lettuce and leafy greens, mushrooms, or nuts with addenda for pecans and almonds, are a few examples of primary production.

Also, the FAO has a database of more than 800 papers on ethical agricultural practises. Guidelines on Good Hygiene Practices for Vending and Dispensing, for retail and food service companies, as well as guidelines for wholesale distributors, are also available.

Primary Production Contamination

As was already mentioned, there are rules and recommendations for both general and commodity-specific reduction of microbial contamination during primary manufacturing. It's a good idea to have a backup plan in case your computer crashes.

For products that are often eaten in the raw form, such as many fruits and vegetables, bivalve mollusks, certain fish, raw milk, etc., contamination control during primary manufacturing is important. There is more information available on possible sources of contamination during primary manufacture.

Prerequisite programme concerns for produce products that are consumed raw are used as an example to demonstrate broad ideas. They emphasise factors like: Water resources and crop-appropriate management, cleanliness of machinery, tools, and structures, controlling bugs and other animals that may damage the crop, Waste management in the field, worker training, and health, including cleanliness.

Investigations have been conducted to determine the sources of contamination in a number of outbreaks linked to raw materials. It has been shown in several instances that contamination occurs during primary production in the field. There are a number of ways to contaminate products like tomatoes, jalapenos, spinach and other leafy greens, and berries. They include applying organic wastes as fertiliser to agricultural fields, tainting irrigation water, or using pesticides to flavour the area where the crops are killed. After the recall of more than 23,000 tonnes of pet food, the producer halted operations in order to refurbish and sanitise the facility before restarting production. Further instances that were identified in May 2008 led to the plant's final closure and a fresh recall of 105 brands of pet food. It has been shown that *L. monocytogenes* contamination of food items during processing has contributed to various outbreaks involving chilled, ready-to-eat foods that encourage the development of this bacterium. Recurring problems are caused in large part by the pathogen's persistence in the processing environment for extended periods of time. Key components in the avoidance of contamination include the efficacy of hygiene control measures put in place at processing sites, such as the design of facilities and equipment, cleaning and sanitization processes, as well as employee awareness and training. We investigated factors that affect contamination, *L. monocytogenes* persistence in processing settings, and related economic and public health concerns.

After processing, contamination also happens at the food supply chain's distribution, retail, and consumer levels. Many case studies are available, including some that show that contamination is not a recent problem, such as those published by Salvat et al. and Carrasco et al. Pathogens may come through contaminated food handlers, uncooked foods, or other environmental sources in the area where food is handled and prepared. While contact with contaminated raw foods, aerosols, vomitus, and infected sores have all been associated to outbreaks, the fecal-oral pathway is the most common source of food contamination by handlers. Food service personnel were examined by Todd et al. in regard to 816 outbreaks.

Understanding the incidence, distribution, fate, and behaviour of the pathogens in food processing settings is necessary for developing effective GHP techniques targeted at the pathogens of concern. Whether contamination is brought on by temporary or chronic malnutrition, acidic low temperatures, high or sponge samples must be taken into account for each production plant. Food samples should be taken at intervals throughout the procedure when contamination could occur. It should be thought about taking samples at various manufacturing stages. As product residues are a form of composite sample from the process, they may also be collected.

Environmental samples may be taken from the general processing environment as well as from product contact surfaces. Various approaches and instruments are utilised to recover microorganisms from surfaces found in food processing settings and processing equipment. The choice of the most suitable sampling instruments and procedures should be suited to the kind and size of the surface to be sampled. Some samples offer historical information, monitoring accumulation over time. Examples include residues from hollow bodies, gaps and crevices; joints and seals between floor and equipment; water residues from syphons; drains; dust from vacuum cleaners; brooms, brushes and mops; air filters; and damp or old insulating material.

Sampling methods utilised in the food sector involve a broad range of instruments. Non-antibacterial sponges or big cotton pads are suile for this purpose. As the objective is to identify the presence of a pathogen, wide regions should be sampled without regard to dimension. Environmental samples should also be gathered at various times. Ismaïl et al. examined benefits and cons of regularly used approaches. Methods such as electrostatic wipes or roller samplers have been reported, and findings emphasise the necessity for careful selection of sampling techniques appropriate to the circumstances in the environment; e.g., dry or wet. For example, Rönnquist et al. tested the efficiency of several kinds of swabs to recover norovirus from surfaces in food processing facilities, and microfiber cloths proved to produce the greatest recoveries. In addition to evaluating for pathogens, samples may be analysed for indicator microorganisms relevant to the pathogen of interest. All the data should be structured in a way such that a baseline can be eslished for what is deemed normal when all GHP processes are under control.

Regular Environmental Sampling Program

Routine environmental sampling programmes are usually focused on one pathogen or indicator microorganism and involve a limited sampling regime. The goal of the regular sample programme is to identify increasing risk of product contamination before it actually happens. Data from the investigational phase are used to select sampling sites, times, frequencies, and types of samples that will most effectively fulfil that purpose. Some of the basic process control concepts may be applicable in the design and implementation of an environmental sampling programme. The use of trend analysis, in particular, is useful. Statistical analysis is seldom of value if the programme involves presence heated and protected by wrapping or packaging is of greatest concern. Samples from product contact surfaces of equipment that could be included in a routine sampling programme are identified. In addition, samples from floors and other sites in close proximity to the flow of the product are indicated.

The types of samples collected for a routine sampling programme should be determined from the investigational sampling data and experience as the programme is implemented. Environmental sponge samples are more commonly collected for analysis than product samples. Examples of sampling sites for four food operations are provided. 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 programme could include in-process product samples where this may provide additional benefit over sponge sampling. The type of material sampled depends on the type of product and processing line. ICMSF provides recommendations on potential in-process and environmental sampling that may be useful for different types of products.

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 their 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 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 start

of operation, which may provide misleading results. In another situation, the first product from the process may represent the highest potential for detection of the target organism because it may remove residue that collected in interior surfaces that are not readily accessible for swabbing. 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 a slashed, routine sampling plan with a defined minimum number of samples.

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 damage or contamination of the processing environment. To minimize the analytical workload and cost, samples may be composited before analysis. Compositing should only be done when the quality of the information obtained is known to be unaffected.

Many facilities are adding or strengthening their pathogen environmental monitoring programs to enhance their food safety risk reduction efforts. The two most common types of pathogen environ- mental monitoring programs are Listeria spp. monitoring as an indicator for *L. monocytogenes* and Salmonella monitoring. Monitoring programs for other pathogens, such as C. sakazakii in infant formula manufacturing facilities, along with hygiene indicators such as Enterobacteriaceae, share many similarities with the pathogen environmental monitoring programs dis- cussed here. Monitoring for more generic indicator groups, such as sampling for total aerobic bacteria to verify sanitation, differs from the pathogen environmental monitoring program discussed here.

For food manufacturing facilities where there is a science-based reason for a pathogen environ- mental monitoring program, common components that should be built into the program to make it as effective as possible. Guidelines on pathogen environmental programs have been published by different organizations, e.g., GMA for monitoring Salmonella in low moisture foods, GMAfor Salmonella in nut products, ABCfor almond products, Tompkin et al.for *L. monocytogenes* in meat processing plants.

Meaningful information is gained only if appropriate tools are used to collect samples. Sampling materials must be sterile to avoid contamination. Proper labeling and description of the samples is essential. Before analysis, samples should be stored under appropriate conditions so that the population of the target organism neither increases nor decreases. 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 and from surfaces, holes, crevices, etc. Paintbrushes can be used to collect dusty samples on surfaces of equipment or of the infrastructure. Cotton plugs, sponges or gauze pads are useful for liquid or moist residues as well as surfaces of with limited amounts of residues. Sponges must not contain anti- microbial substances. Other sampling tools such as pipettes for liquids or spatulas for vacuum collection material may be required depending on the situation. Flexibility is needed to adapt the means of taking samples to local situations. The sampling tools must not introduce other, non-microbiological, hazards into the processing system. In most manufacturing plants, for example, a strict policy exists that glass cannot be introduced into the processing environment. ABC provides illustrations on the types of tools that could be used in different locations in almond production.

Sample Analysis

Samples taken for microbiological surveillance are analyzed for specific microorganisms; i.e., pathogens of concern or indicators such as non-pathogenic 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 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. This type of sampling may identify e.g., biofilm formation, or a niche supporting microbial growth in the equipment. When pursuing a source such as a niche, it is usually necessary to completely dismantle 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 to avoid dissemination of the 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 included in routine sampling. Pre-conceived 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.

Statistical Process Control

Food operations must be controlled to produce foods of consistent quality and safety. A controlled process requires process managers being proactive and informed of the factors that influence variability. Process control thinking and technology can be applied to the manufacture of a single lot of food produced on 1 day, or multiple lots produced over days or years, and to both batch and continuous processes. This discusses sampling and testing to assess whether food process operations are under control.

HACCP is often referred to as a preventive system. However, from a statistical standpoint HACCP would be more appropriately described as a means for controlling the variability of a food system, as monitoring control points and critical control points typically will result in reduced variance of a particular process parameter. A statistical approach to safety can be effectively applied in HACCP systems. SPC methods provide an objective and statistically valid means to assess ongoing processes, and as such are particularly applicable to monitoring and verification of a food system.

Lot acceptance sampling can be used in verification and, to a very limited extent, in monitoring. SPC and lot acceptance sampling are two statistical techniques for controlling quality and safety are well developed and documented in many textbooks, manuals and periodicals. The statistics of lot acceptance plans have been dealt with in detail in. SPC methods with applications to food technology are treated. Or information on process control technologies, other texts are recommended. Further, for more information on process-oriented control systems in the food industry and the statistical tools used for that purpose, other texts are recommended. As we move forward with this discussion on SPC in the food industry, it is important to note that while SPC techniques can be used for many aspects in processing including process parameters as well as environmental parameters, the reader should consider the SPC discussions herein to be mainly focused on process parameters.

The application of SPC to food safety relies heavily on a detailed understanding of the hazards, the food and its ingredients, and the processes employed in its manufacture. There is no substitute for wide-ranging and complete knowledge of a process system. To the extent possible this process knowledge should be captured electronically. There are many techniques to assist process managers in this task: process flow diagrams and process mapping techniques are good resources to use to create one's process knowledge base.

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. Data acquired as part of SPC monitoring of process parameters can lead to better process understanding, identification of significant hazards and parameters contributing to overall process variability.

Knowledge of the Factors that are Necessary for Control

If significant microbial hazards have been identified, it is necessary to assess the conditions of processing and measure or step that must be controlled to prevent, eliminate or reduce their occurrence to acceple levels. Subsequently, control measures can be eslished that adequately and consistently ensure the required level of control. This will help designate the importance of certain steps in the process i.e., as CCPs, CPs or other prerequisite programs including GHP procedures for controlling the identified hazards. The use of SPC methods can aid in identifying the factors and process parameters to control and eliminate hazards.

Knowledge of the Extent of Variability and Factors that Influence Variability

Key to using SPC methods is understanding the factors that influence variability. Most food opera- tors understand the factors that influence the cost of producing a food and strive to control each factor according to their relative impacts on cost and profit. Likewise, this 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 unaccepted, 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 degree of variability that can occur. The information could, through the use of SPC, then be used to determine how this variability might be controlled within an acceple range. This should include both short term variability and variations that can occur over time such as seasonal changes in the levels of organisms associated with various ingredients.

Eslishing Criteria for the Actors that Must be Controlled

The information from item 3 above should be used to slash 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 slashed at critical steps in the operation, procedures must be slashed to monitor those limits to ensure they are met during operation. By using SPC charting along with information on what to do when a system is out of control, discussed later as an "out of control action plan" , factors to be controlled can be charted, followed and studied.

Eslishing Monitoring Procedures

A wide variety of measurements such as sensorial, physical, chemical and environmental are used for evaluation of food processes. The chosen method will typically 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 would be continuous with adjustments being made automatically. The measurements may include processing parameters, food collected at different stages in processing, finished product, and or quality manager assess the degree of control in the production facility and if needed, the corrective actions required to ensure safe and compliant food. Since one or more days may elapse between sampling and obtaining a result from a microbiological analysis, the data provides a history of past performance. While often not clearly articulated, the purpose of this sampling is to provide a "microbiological his- tory" of the food product and the processing conditions as well as verification of the effectiveness of hygiene control measures. By acquiring data over time on the microbial population, manufacturers eslishing a baseline for the level of control that is attainable when GHP procedures and the HACCP system are in control. Once swished, subsequent analyses that differ from the baseline indicate a deviation from the norm due to changes in operating conditions, including OPRPs. Additionally, these data can form the basis of manufacturing trends that might be useful for historical analysis and analysis of process deviations.

The extent of microbiological testing for verification is typically limited and not intended to provide assurance of the safety 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 for a single batch, except the data are collected over time and involve multiple batches. An underlying assumption is that when a process is statistically in control, the "between-batch" variability is small and the overall variability is mint.

CHAPTER 12

TYPES OF MICROBIOLOGICAL DATA FOR BASELINE

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Microbiological information may be gathered from at least five sources that vary by sampling site or time both above and below the mean. The normal distribution, which is derived from the "Law of Large Numbers" mathematical principle, is often used to regulate the desired properties or parameters of a meal or a process. In layman's words, this theorem implies that a collection of mean values from a large number of samples, all from the same initial distribution type, will exhibit the variance characterized by the Normal distribution, or the usual bell-shaped curve. As mean values are subject to the Normal distribution, a significant portion of SPC theory is based on the distributions of the mean values of various measurements. This analysis has proved to be both trustworthy and beneficial.

The means of even a tiny sample size from a distribution are often distributed almost regularly. Even when the number of samples being considered is very small, such as n = 5, the distribution of individual values for many targeted food attributes or control factors, such as pH and temperature, is basically normal. An individual sample may be seen as the result of several little samplings for various traits or features found in nature, such as the pH of a particular sample or the population density of bacteria in a growing medium. Hence, the distribution of individual findings will resemble a Normal distribution provided one can describe the parameter or feature in the proper units. When bacteria are cultivated in a setting that encourages non-steady state development, such that found in food, the population density often grows exponentially. Decreases under difficult circumstances are also approximately significant. Long-standing research has shown that when microbe "concentrations" are stated as logarithmic numbers, they have a Lognormal distribution, and as a result, a Normal distribution.

Unintended and unwanted traits, such as concentrations of spoilage bacteria or particular diseases, fall under the second pattern of variability. This is referred to as a "process capacity analysis," where the objective is to maintain the level as low as feasible based on technical, economic, and/or the original control chart data to establish warning and action control limits. Such investigations may be seen as a component of the process validation operations that establish a new process' or process step's ability to effectively manage a microbiological hazard. A process capacity study is only applicable to the process on which it was performed, much as the HACCP plan itself. A new process capability study would be necessary if the process were changed in a way that may affect product safety, and this research would also serve to verify the method. In a perfect world, validation via a process capacity study would go long enough to assess every potential process performance-influencing element. This is seldom the case, hence following verification activities are made to gather more information in order to improve the preliminary process capacity assessment. Two components make up a process capacity study:

obtaining measurements whose distribution reveals a process's planned behaviour; applying the data to sequential data patterns to generate one or more process limitations.

For this, attribute charts, which employ the same data but plot it in a cumulative fashion, provide a more understandable visual representation. Another method of displaying cumulative data is with MSUM charts. Knowing when a non-random impact entered the process might provide crucial hints for locating and then fixing the issue.

Waiting until a control chart reveals an out-of-control situation before acting is bad practice. There are several "rules" for evaluating control charts, and organisations are free to use some of them. It is essential that each process owner create and implement an assessment system. To warn of potential loss of control, some control charts include warning limits within the control limits. Cu sum charts are particularly helpful for spotting patterns early on. Several systems have a tendency to destabilize over time with plenty of notice. Mechanical parts may wear out, control devices may wander, cleaning and disinfection procedures may be less rigorously followed, or the composition of the contents may alter. Even a sudden change in one technique may sometimes lead to a gradual change in another.

The foundation of statistical process control is the formulation of upper and lower control limits. Yet, it may not always be obvious whether upper and lower control limits are necessary. For circumstances when the top limit is an issue, one-sided process charts might be created. Examples include the pH of an acidified food that must be distributed at room temperature or the retort temperature for a canned food that must be maintained above a specific minimum requirement, but too high of a temperature is resource-wasting or can produce contaminants derived from the processing. At CCPs in the food processing industry, it's common for one restriction to regulate product safety while the other symbolizes manufacturing efficiency or an appealing aspect of the meal. For evaluating or improving process control, these kinds of industrial process charts are often just as helpful as SPC charts. Control charts are helpful in modifying absence tests) and variable data in addition to monitoring established, well-established processes.

General Rules for Making Variable Control Charts

Plots of data over time, which might represent data from a single lot or data from many lots, are known as statistical process control charts. In a control chart, the x-axis represents typically the time at which the sample or samples were gathered throughout the process, and the y-axis represents the result of the measurement. Three parallel lines make up the control chart: an upper control limit, a middle line, and a lower control limit. The LCL is sometimes considered to be zero or any other pre-designated number below the lower limit of detection in situations when the lower control limit is below the lower limit of detection. Every stage of a process has some built-in unpredictability. The unpredictability of each stage adds to the system's total variability when taken as a whole. Data points often gather around a core value in a well-managed system. The mean, median, and mode are common statistical measures of central tendency, as are measures of variability like range, standard deviation, and standard error of the mean. The surrounding lines on a control chart serve as limitations depending on the computed degree of variability, whereas the centerline on a control chart is often a measure of central tendency.

Understanding the "sigma" idea as it relates to control charts is crucial to comprehend how the LCL and UCL are often achieved. The phrase used to describe the variety of batches or lots is comparable to the phrase. The distribution of the data gathered during the process is often assumed to be Normal or roughly Normal when using control charts. According to the normal distribution, almost 68% of values will be within plus or minus one standard deviation of the mean, roughly 95% will be within two standard deviations, and roughly 99.7% will be within three. Hence, "one sigma" denotes a standard deviation of one from the mean, "2 sigma" a standard deviation of two, and "3 sigma" a standard deviation of three. The

most typical range for control limits is plus or minus 3 deviations from the mean. The risk of any specific datum point going beyond the control limits by chance alone when 3- control limits are utilised is 0.3% when the process is genuinely under control. A process is considered to be out of control if values fluctuate more often than 0.3% above or below the value of 3. This is one of the primary principles of control charting as created by Shewart in the 1920s and these rules are named the "Western Electric Rules". The OCAP determines whether the process owner decides to act in response to the out of control state.

Control charts and their measurements of central tendency and variation may be used to anticipate the frequency that failures will happen even when a process is under control in addition to identifying when a process is out of control. For instance, Peleg and colleagues predicted the frequency of extremely high bacterial counts in meals using control charts in conjunction with a probabilistic model. The X R control chart will be used as an example to introduce key ideas about process control charting. The expression "X R" indicates the usage of the subgroup's mean and the range between the lowest and greatest values. Variable charting uses it often. In reality, the X R chart consists of two charts, one measuring central tendency between subgroups and the other measuring diversity within subgroups. Its foundation is the comparison of tiny subgroups, fulfilling the Normal distribution's basic purpose or precision, i.e., the comparison of means. To make it easier to utilise X and R together to determine if the process is under control, they are often placed on the same control chart. Since it shows the Mean Count for each subgroup, the X R chart is among the most practical. The log10 of the individual values is employed for evaluating microbiological population density data, converting the log normal distribution linked to microbial "concentrations" to a normal distribution of log values.

Corrections When Data Automatically Correlate

As was previously said, the majority of control charts are developed on the presumption that when the individual data points are displayed, they are randomly distributed over time according to a Normal or Lognormal distribution. Yet in real production environments, it's common for consecutive measurements of product characteristics to be connected with one another. When data are gathered at closely spaced intervals of time, auto-correlation is more likely to occur. Setting control limits may be affected by auto-impact correlation's on the pattern of data on control charts. Data auto-correlation sometimes has an impact on system control as a whole. Comprehensive coverage of auto-correlated data and control charts is provided by standard SPC references. These references provide suggestions on how to deal with data auto-correlation in control charts. Using total batch means is a standard practical strategy for overcoming auto-correlation bias.

Individual Microbial Population Density Counts: Additional Factors for Charting

Simple variable charts, like the hypothetical example, may be used to display data on pathogen or indicator organism concentrations. In this example, the amounts of indicator organisms in samples of a final product are numerically estimated. This graph illustrates normal variations in viable numbers through week 40. The same kind of swings resume after week 40, but they centre around a considerably lower midpoint. This might be the result of a modification to the raw materials or machinery, a seasonal influence, a modification to the analytical procedure, etc. Despite the change's apparent positivity, more examination is warranted since it could point to an analytical issue or provide a way to find a component that regularly improves system performance.

Charting the individual test findings is one of the easiest ways to think about a collection of quantitative microbiological data. Such a control chart, often known as an individual

measures chart, is straightforward and seldom deceptive. The criteria used to identify loss of control and signal safety issues are often unusually high counts or patterns of abnormally high numbers. On the other hand, exceptionally low counts or trends of low counts may indicate the need for an analysis protocol review or a possible process improvement. Both types of obvious patterns would be worthy of attention. Although certain indicator levels are likely to wander up and down over weeks or months and may be interpreted as random fluctuation, subtle patterns in the values for an indicator may not necessarily be viewed as trends at all. However given that they can be a forewarning of a progressive loss of process control chart, such tendencies might call for a reconsideration of the procedure. The 50th percentile and from the gaps in the percentiles of the data will be provided by any method of cumulative probability of the normal distribution, but, if that is not the chosen alternative. For instance, there should be a 76% difference between the 70th and 90th percentiles. As a general rule, percentiles higher than the 90th need to be avoided for this usage.

The first test results obtained after the first limits have been established should be carefully examined in order to ensure that the data used to create the initial limits were obtained while the process was under control. One would typically anticipate not finding any numbers at this point that are beyond the aforementioned range. If variances are seen, the data should be examined for trends, and any such trends that are found should be looked into. Investigate potential explanations, such as a change in the procedure or ingredients, a change in the sampling or analytical techniques, if any result is below - 3 or above +3, or if 3 of any 5 successive results are below - 2 or above +2. In such cases, it will be necessary to repeat the process capability study again using the updated data until the warning and stopping limit values match. These limits at X 2 and X 3 should be chosen as starting control limits for verification testing after the process capability analysis has been completed without any deviations or obvious trends.

The restrictions should be compared to the probability of type II mistakes and extended as necessary if it seems that they are producing an excessive number of "false alarms" during verification testing. Unless enough data are gathered so that the process can be effectively assessed in respect to: how really uncommon will deviations be while the process is under control, and how often control is lost, such an extension of the limitations should be done carefully with continuing or increased examination.

The first limit-setting exercise may have been excessively restricted for two reasons, to name only two.The constraints could become overly restrictive because of seasonal changes in X or. Seasonality generally won't have an impact on if it has no impact on X. If X is impacted, the charts may need to be modified by adding seasonal X lines, and the data must be examined to see if the has also changed by looking at the R or s chart. X may be fit by a curved line with -determined lines curving at constant distances above and below X, or the seasonal fluctuations in X and the control lines can create a series of zones with various straight lines. Nevertheless, the correct course of action would be to remove the seasonal variability and not change the control chart boundaries if seasonality results in an unacceptable danger to safety.

The original control limits will be impacted if the limit-setting research used just one source of raw materials and the process thereafter uses a range of sources. This introduces another source of variance. X and should be adjusted once further information is available that covers more raw material sources. Therefore, the proper reaction would be to minimize the unacceptable variability associated with the new supply of raw material rather than changing the control chart limitations if the additional sources of raw material pose an unacceptable risk to safety.

Use Care When Understanding Some Variable Charts

For process control monitoring of parameters like pH and temperature, moving range charts are helpful. These graphs show the difference in absolute terms between each result and the preceding one. The same rules apply to increases and decreases. A big decrease in microbial concentration may not be as important as an increase when discussing microorganism levels. This is due to the fact that downward trends are often nothing to be concerned about and that although a low number may appear substantial as a range, it does not necessarily make the high value any more meaningful.

General quality control books provide standard methodologies for estimating from the average of the changing ranges. Yet, pathogens and indicator organisms should also avoid doing this. Weather, seasonal variations, changing harvest zones, early or late harvests, spot markets for components, and other variables will push X and MR values upward or downward across periods of several testing in ways that cannot be anticipated with great precision. There will be some auto-correlation as a result. According to the definition given above, auto-correlation is the propensity for results to resemble those that are nearby in the test sequence more and less than those that are farther away. The distribution of differences between subsequent test results will thus often underestimate the actual variability of microbial levels in meals.

Similar considerations make it inappropriate for these variable charts to use cumulative sums of variations in the observed log10 microbial concentrations and some goal log values. As one does not want to reach a goal value of pathogens, the mean, or target log number, is not really a target value. Setting standard limits for a chart showing the mean of the prior n observations is impossible in this case due to the uncontrolled shifting of X. Yet, such charts would still be helpful for small n in terms of measuring variability that can arise as a consequence of seasonal change and demonstrating changing patterns.

Additional Control Charts for Microbiological Data

The traditional control chart procedures may be employed when process counts offer estimates of product levels. The estimated levels can be seen as variables data. There are several control charts that may be used to graph data with variables. Other charts include the Xi and MR, CUSUM, and X-bar and s, in addition to the X-bar and R chart example from above. The Xi and MR charts are part of this illustration. Plotting each outcome across time is all that the Xi chart entails. The difference between the current sample result, Xi, and the prior sample result must be taken into account while calculating the MR chart. Xi-1. Thus, for values of I = 2,..., n, the points that are shown are: MRi = Xi - Xi-1. These charts were selected because they are simple to create and are often used to track operations for which control with regard to overall levels of product measurement is sought. When the baseline data are plotted, a "sle appearing" process results. The Xi and MRi values are then plotted as they are gathered on a blank control chart once the limits have been transferred to it. Both the Xi and MR charts are examined for out of control sequences after the Xi and MRi are plotted and linked to the preceding point with a straight line. Pyzdek advises using the following illogical rules:

Attribute Charts for Testing Presence-Absence

A standard procedure is used to test an analytical unit with a known weight to see whether the target microorganism is present in the food sample or not. This kind of microbiological test may be used throughout time to evaluate how well food safety management measures are being kept up to date. Nevertheless, the technique utilised to identify the microorganism's existence, in particular its lower limit of detection, has a significant impact on how these tests

should be interpreted. So, unless both data sets were obtained using the same standard procedure that has similar analytic performance characteristics, it is often not feasible to compare or integrate the findings from one data set with those from another. Take a fictitious instance of a single microbial presence. Salmonella spp. non-detection test with 0.25 in-control detection rate. In this illustration, control is lost on Day 29 and regained when it returned to "normal" on Day 37 of the chart. This straightforward illustration shows that the "reaction time" associated with a control chart depends on the testing frequency, the established decision criteria, and the amount of time needed to examine the data. For instance, the failure would have been discovered a day sooner if the decision threshold had been consecutive positive samples. The likelihood of Type I mistakes would rise with this more sensitive criteria, however.

absence of outcomes. These charts often operate on the presumption that while a process or system is under control, some percentage "p" of sample units will provide a "yes" response upon analysis. Moreover, it is considered that outcomes from subsequent tests are unrelated to one another. Chart analysis reveals if these hypotheses are true, which might result in process improvements. For instance, it would indicate that there may be another source of variability during the day shift that may be reducible if the day shift at the same factory had a greater p than the night shift. On the other hand, the premise of the independence of observations may not be valid if a longer than anticipated run of "no" findings is seen, which may help identify ways to further enhance the process.

The frequency of discovering the target microorganism should rise if a procedure is altered that results in p rising noticeably. Tracking the sums of detection findings over some period of results allows one to confirm that p is not changing. One may establish an upper limit, designated U, that should not be surpassed by the cumulative aggregate of the samples in which the microbe is found, starting from some initial point in the data collecting process. This statistic is known as the "cumulative sum," or CUSUM, for this reason. The likelihood of receiving j positive isolations out of n tests when the probability of the microorganism's existence is p is computed using standard statistical software that is often bundled with spreadsheet applications. Before becoming involved in the procedure, it is necessary to determine the acceptable level of rarity. Hence, for every n, that criteria will decide what U is. After the process capability assessment that evaluated p, a le of values for U will be created. The CUSUM is then compared to U after each test. The statistic has not shown a loss of control as long as CUSUM does not go beyond U.

The length of closely related sequential outcomes sequentially has practical bounds. The CUSUM is also rather intricate. This makes choosing a different statistic occasionally more appealing. If the most recent n findings are summed without raising n further, there is minimal loss of discrimination after the CUSUM has been followed up to a significant n. As a consequence of the count being generated from a zone that moves along the string of observations, this number of times the presence of the microbe was identified in the prior n results is known as the "moving sum," or MSUM. Naturally, the upper limit for this fixed MSUM is equivalent to the upper limit for the nth step in the CUSUM. One may quickly discover any significant movement in p if they use a modest n for analysing the MSUM. On the other hand, the sum over a small n will not be sensitive enough to identify it if p moves slowly upward. A greater n may delay the realisation of the shift since it is more selective and as a result more sensitive to even small changes in p. The "Moving Window" assay is one of the MSUM approaches that has been extensively employed with microbial food safety verification testing.

By using several MSUMs, this impasse between rapid reaction and high sensitivity may be resolved. They provide a middle ground between the CUSUM and a single MSUM. A choice of two MSUM charts with a short period for responsiveness and a large interval for sensitivity may be quite helpful for many procedures. Due to the significant correlation between MSUMS and their predecessors, seemingly extended chains of MSUMS that are just slightly over the mean value are of no particular significance. When MSUMS or CUSUMS are getting near to their action limitations, increased testing frequency may be particularly beneficial. In the event that these thresholds are surpassed, exploratory sampling and problem-solving are required to identify the new cause of attribute variability and bring the process back under control.

The counts visible on a plate cannot easily be regarded as variable data when they are low and there is a low possibility of not finding any colony forming units, as shown in Sect. 13.6.5. A discrete distribution, such a Poisson distribution or a negative binomial distribution, might be taken into consideration in this situation to describe the distribution of counts. There are fewer instances of microorganisms that lend themselves to Poisson-like probability distributions than to binomial or normal distributions. The value of one parameter, the distribution's expected value, entirely describes the Poisson distribution. As the lowest and highest values may both be as high as they choose to be, the Poisson distribution is positively skewed and its variance is equal to the expected value. Poisson distributions appear under extremely specific circumstances, where it is permissible to assume "pure" or simple uniformity. Unfortunately, this assumption is often incorrect since a variety of variables might have an impact on the outcomes at once, making the assumption of pure or simple uniformity inappropriate. So, under certain conditions, two parameter distributions, such as a negative binomial or even a binomial distribution, may fit data effectively. The example is being offered because the Poisson distribution is significant and in certain cases could offer a reasonable match to the data.

Control Diagram for Binomial Data with a Single Sample Size

For the detection of a single pathogen in samples, qualitative studies are often carried out. A "NP" - control chart is a graph that may be used to monitor how well a process is being controlled in relation to the presence of certain pathogens in samples. Typically, these graphs may be used to identify a binomially distributed feature, like Salmonella, in samples. The categorization for which process control is monitored is the one for which the label "defective" or "positive" is given. P stands for the likelihood or percentage of "defective" units, and its size must be under control.

The number of "positive results" is plotted, and the initials "NP" are used as a mnemonic for this process. For an example of an NP chart, the expected value of the number of positive results is equal to the sample size, N, times the estimated percentage of positive samples, P. See AOAC Appendix 4 for more specific instructions on how to create this graphic. The number of positive outcomes, which is the output in this case, is predicated on a sample size of 50 product testing. A plot illustrating the number of "positive" test findings inside a sample over time is known as an NP-chart. The methods shown in the example may be used when the sample sizes differ.

The number of positive outcomes out of a sample of 50 units was shown on the binomial control chart in the earlier example that was previously provided. The set number of units per sample in that instance ensured that the anticipated number of positive outcomes each sample was the same. The sample size isn't always the same, however, and as a consequence, the anticipated number of favourable outcomes isn't always the same. Since the results would not follow a common distribution when the process is under control, the underlying assumption

for the data to be used for plotting, that the results are from a common distribution, would not be satisfied, plotting the quantity of positive results is not appropriate for a control chart. Plotting the percentage of positive findings, Pi, rather than the total number of positive results could be a straightforward alteration. However, although the anticipated value would be the same for all samples, the expected variances of the results would no longer be the same. For the reasons outlined above, such data would not be suitable for charting. Plotting Zi = SQRT, where P is the presumed real percentage of positive findings and Ni is the sample size for the ith sample, is one technique to remedy this, however. Z in this situation has an expected value of zero and a standard deviation of 12. The Zi values might be used to create CUSUMS and moving averages. Similarly, if the sample sizes were not so big, an arcsine transformation: yi = sin−1

Sample size is a discrete variable, but time is a continuous variable. When the MTBF is "small," this disparity might lead to certain issues. It's a good idea to have a backup plan in case the backup plan doesn't work. HACCP guidelines. This legislation mandated the implementation of two types of microbiological testing to confirm the efficacy of the HACCP systems necessary for the slaughter of meat and poultry. The first included commercial testing of corpses for biotype I Escherichia coli, a sign of faecal contamination. The second was the USDA's testing for Salmonella spp. Both were based on the deployment of the Moving Window MSUM control charting approach. The method was modified for stratified quantitative data in the instance of the E. coli test by utilising a limit that could not be surpassed and a warning value that could not be exceeded more than three times in a moving window of 13 tests. The m-value and M-value are particular to that product and were based on national baseline surveys of different kinds of meat and poultry. The sample rate varies depending on the commodity. Salmonella was only detected in the corpses using a presence-only approach. HACCP guidelines

Examining and gaining knowledge from previously unknown or unexpected factors or events

A HACCP programme was designed based on a hazard analysis, and HACCP verification may be seen in part as extra testing done to ensure that the circumstances and requirements indicated in the hazard analysis are still valid. Take pasteurisation of liquid egg products as an example. The heat step with essential timetime control charts and off-line microbiological data is a clear CCP for the manufacturing of pasteurised liquid egg products and would certainly allow identification of the shift. Instead, an elevated prevalence of salmonellae seen in periodic samples obtained as part of efficient verification systems warned the egg industry. This is a step in the data usage and verification process. Also, it is a component of change management that calls for the validation of processing conditions and a periodic evaluation of HACCP. Including this in the SPC programme may work as a proactive signal of change management, as opposed to the more reactive monitoring and evaluation of HACCP protocols.

CHAPTER 13

A FLATOXINS IN PEANUTS

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Chemical meolites known as aflatoxins are mostly formed by Aspergillus species in food stored in favourable circumstances. These are the most powerful known liver carcinogens and have been shown to cause cancer in every examined animal species, including humans. Aspergillus flavus, A. parasiticus, and A. nomius are the main Aspergillus species that are sources of aflatoxins. Some less frequent Aspergillus species that have the potential to create aflatoxins include A. ara-chidicola, A. bombycis, A. minisclerotigenes, A. parvisclerotigenes, A. pseudocaelatus, A. pseudono- mius, A. pseudotamarii, A. togoensis, A. mottae, A. sergii, A. transmontanensis, and A. Aspergillus flavus is connected to peanuts, maize, and cottonseed and is prevalent in tropical and warm temperate regions. Furthermore, it may be found in tree nuts, particularly Brazil and pistachio nuts. According to the available data, A. parasiticus has a smaller geographic distribution than A. flavus, which predominates in peanuts. Although being prone to misidentification, A. nomius has been identified as a significant source of aflatoxins in Brazil nuts. Nevertheless, this may understate the species' actual prevalence.

The four main aflatoxins that are formed naturally go by the names B1, B2, G1, and G2. The letters "B" and "G" stand for the blue and green fluorescent hues that thin layer chromatography plates emit when exposed to UV light, while the subscript numbers "1" and "2" denote the plates' order of elution. Only approximately 40–60% of A. flavus isolates generate aflatoxins, however these percentages may fluctuate. A. flavus solely produces B aflatoxins. Both B and G aflatoxins are produced by A. parasiticus and A. nomius, and the majority of isolates are producers. The most common testing procedures are immunochemical ones since aflatoxins are exceedingly hazardous and have global regulatory thresholds ranging from 1 to 35. Limits of detection are below 0.1 g10.1007 World Health Organization, making liquid chromatography and mass spectroscopy standard procedure for sophisticated users such large volume analytical labs or regulatory organisations. According to a Chinese research, children's daily exposure to aflatoxin from peanuts ranges from 0.22 to 0.22 ng, while it is 0.10 to 0.108 ng for adults. Population risk based on aflatox exposure is much greater in African nations. Many times, the levels are concerning enough for risk managers to think about taking action among the demographic categories most susceptible to exposure. Aflatoxin contamination affects a number of staple foods in Gambia, including maize, millet, sorghum, rice, and groundnuts. The total exposure is the sum of the exposure from all individual sources. The average daily intake of aflatoxin in Gambia is estimated to be 16 ng per person due to the country's heavy groundnut consumption. The average potency determined from the individual potencies of HBsAg+ and HBsAg- groups, in which the HBsAg+ prevalence rate was estimated to be 25%, was used to calculate the population cancer risk per year in the Gambia as a developing nation, resulting in 1.3 cancers per 100,000 people annually. For lower limit to upper bound, the dietary exposure to aflatoxins in the European Union varied from 0.93 to 2.45 ngday. Exposure was estimated to be 2.7 ngday in the United States and 0.73 ngday on average in Japan. The actual incidence rates of liver cancer in men per 100,000 people vary greatly between nations, with high rates in Middle,

Western, and Southern Africa, sub-Saharan Africa, and South Eastern Asia and low rates in North America, Western, and Northern Europe, as well as Latin America and the Caribbean. As there are additional causes causing liver cancer in people, this results do not directly link eating food tainted with aflatoxins. The disparity in regional aflatoxin exposures, however, is consistent with a greater prevalence of liver cancer.

Factors Influencing the Evaluation of Exposure

A number of epidemiological studies have showed that consumption of aflatoxins alone provides a significant risk, while others have shown that other variables, such as the hepatitis B virus, are required to cause liver cancer. Understanding the aetiology of liver cancer has advanced significantly with the discovery of the hepatitis C virus. Liver cancer incidence and HCV antigens are strongly correlated, according to the majority of epidemiological research. HBV and other risk variables have no effect on the risk associated with HCV. In nations with a low to moderate risk of developing liver cancer, such the United States, Europe, and Australia, HCV is likely to be the main contributing factor. An international team led by the IARC has examined and approved the epidemiological evidence of the carcinogenicity of HCV. Hepatitis virus is a significant global public health issue. Almost 300 million people are thought to have chronic hepatitis B infection, whereas 100 million people may have hepatitis C. The WHO Expert Committee on Food Additives estimated that the carcinogenity potency of aflatoxins for people with the hepatitis B virus to be 0.3 cancers100,000 individuals, which is 30 times higher than the potency for non-infected people, despite the fact that the evidence is still inconclusive. This estimate applies to 50-100% of liver cancer cases worldwide. More recently, a review of re-analysis of the overall evidence from epidemiological studies revealed that people with chronic HBV infection have a particularly elevated risk of developing hepatocellular carcinoma from aflatoxin exposure, and there is also some evidence to suggest that people who are exposed to aflatoxins but do not have chronic HBV infection also have an increased risk. Considering that there are more than 350 million chronic HBV carriers worldwide, many of whom reside in locations where aflatoxin is prevalent, the need to limit aflatoxin exposure is still very important for cancer prevention.

Characterization of Risk

A distinct central tendency estimated potency and ranges for hepatitis B positive and hepatitis B negative persons were selected by JECFA after reviewing the potencies of aflatoxins determined from the positive epidemiological studies. The potency levels used for positive people had an uncertainty range of 0.05–0.5 and were set as 0.3 malignancies per year per 100,000 people per ng of aflatoxin consumed per kg of body weight each day. Among those who tested negative, there were 0.01 malignancies annually per 100,000 people per ng aflatoxin per kg body weight per day, with a 0.002-0.03 range of uncertainty. Based on the aforementioned supposition, a research implies that eating peanuts increases the probability of developing liver cancer by 0.003-0.17 cancer cases per year, for instance, in China. The mean cancer risk for this group was 0.004 cancers per 100,000 people per year, assuming a 60 kg individual. Using the quantitative cancer risk assessment and national data on aflatoxin levels in food, consumption of maize and peanuts, and HBV prevalence, Liu and Wu calculated the worldwide burden of hepatocellular carcinoma caused by aflatoxin. Aflatoxin's ability to cause cancer in both HBV-positive and HBV-negative people as well as uncertainties in every other variable were taken into account. Among the 550,000–600,000 new HCC cases reported annually globally, Liu and Wu calculated that 25,200–155,000 of those cases may be linked to aflatoxin exposure. In other words, between 4.6 and 28.2% of all cases worldwide may be caused by aflatoxin. The majority of cases happen in sub-Saharan

Africa, South-East Asia, and China, where there is a high prevalence of HBV and relatively unrestricted aflatoxin exposure among the population.

Government rules that outline a maximum tolerated level of aflatoxins in peanuts or agricultural and public health actions that may be used to lower aflatoxins levels in peanuts are two ways that the danger of aflatoxins in peanuts can be reduced. A different approach to risk management has been used than what would be anticipated for bacteria or bacterial toxins since aflatoxins are a well-known chemical danger. The limits established for aflatoxins in foods in the years after their discovery mirrored the limitations of detection of the chemical tests. Its original value for importing nations was 5 kg A maximum threshold of 15 gor temperature was established by the Codex Alimentarius Committee on Food Additives and Contaminants in 1999. Aflatoxin development may be limited by sound agricultural practices, but it cannot be entirely stopped.

The fungi that make aflatoxins cannot develop in peanuts that have been properly dried on the farm and kept dry throughout transit and storage, thus no rise in aflatoxins should happen. The FAO advised storing of the dried peanut at 10 °C or below 65-70% RH and moisture level of 5-6%. A. flavus generally cannot grow in peanuts with a relative humidity (RH) of 85% or below. Aflatoxigenic fungi may proliferate and aflatoxins can continue to be produced in certain tropical regions, however, when high humidity conditions are present and storage conditions are insufficient. Marketing and distribution of processed peanuts should be controlled for moisture content at the proper RH and temperature. Depending on intended storage time, RH below 70% and temperature between 0 and 10 °C are advised for post processing storage. The storage life is increased with decreasing temperature.

Preparation FSO

Because to the lack of a no-effect threshold, it has been very difficult to establish an adequate degree of protection for aflatoxins. As aflatoxin B1 possesses a reactive meolite that directly interacts with DNA, it is considered that there is no safe level of exposure below which cancer cannot develop. One may argue that an ALOP should be set at the lowest level at which cancer in humans could be detected, such as the amount of aflatoxin in the average diet needed to cause one instance of liver cancer for every 106 people per year. On the other hand, it may be claimed that any degree of cancer caused by aflatoxins is too high, meaning that the ALOP may only be 1000 times lower. The Food Safety Objective idea is often used to describe the protection against toxic and pathogenic germs, but it has also been used to describe the maximum concentration of a hazard in a product at the time of consumption, or mycotoxins. Limits imposed by a nation for aflatoxins in foods may rationally be thought of as having the status of an FSO in the case of a chemical toxin like aflatoxins. The toxicity of aflatoxins was thoroughly reexamined by JECFA and CCFAC in the middle of the 1990s, particularly in light of more recent data on the impact of hepatitis viruses on its carcinogenicity. The maximum allowable limit for total aflatoxins in foods in international commerce, as suggested by CCFAC to Codex, has been established for peanuts for further processing in international trade at 15 gkg.

This FSO is based on several variables. First, statistical analysis of thorough surveys of aflatoxins levels found in European foods have shown the need for lowering that limit to 10 or even 5 gig for the consuming nations. Finally, according to JECFA, there is still a lack of proof that aflatoxin causes human cancer in the absence of hepatitis B virus. If definitive proof becomes available, this FSO could need to be reduced. Major exporting nations like the United States and Australia believe that this FSO is technologically feasible, but some tropical producing nations are now out of reach.

Defining and Achieving Performance Goals

The performance objective, which is the maximum frequency, comes before the food safety objective (FSO). In 2006, the Brazilian Sanitary Surveillance Agency set specific mycotoxin limits for cereals and nuts for further processing, which still require physical or other treatments before direct consumption. If the product's moisture content is kept low in this situation, aflatoxin levels won't rise and PO may be equivalent to FSO.

Transmission Point of Entry

The "Code of practise for the prevention and control of aflatoxin contamination in peanuts" published by the Codex Alimentarius offers recommendations for handling and preparing peanuts for human use. According to Codex Alimentarius, owing to condensation or leaks in the warehouse, post-harvest storage of peanuts is the stage that might contribute the most to the aflatoxin issue in peanuts. Use enclosed or sealed containers, tarpaulins, or both to safeguard peanut deliveries from any further moisture. Avoid temperature changes that might lead to condensation on the peanuts, which could result in local moisture buildup, fungal development, and the production of aflatoxin. During storage, water activity, which changes with temperature and moisture content, has to be carefully managed. Low moisture content, proper ambient temperatures, and sanitary conditions are necessary for preventing aflatoxin accumulation during storage and transit. A. flavuskg or the company's established limit. After the finished product is made, it must be carefully packed to keep water out and maintain quality.

Primary Manufacturing

To make sure the PO is met, a rigorous sample strategy must be used. Using a single 20 kilogramme laboratory sample of shelled peanuts to be collected from a lot and tested against a maximum level of 15 g53 kg for total aflatoxins, Codex Alimentarius developed a sampling strategy for total aflatoxins in peanuts destined for further processing. If no one of the three 10 kg samples from the commodities meant for direct human use has more than 4 gkg consistently, the batch is allowed. One company in Australia that shells peanuts separates them such that the mean aflatoxin concentration of samples from any one lot does not exceed 3 gkg FSO.

Process Standards

Color sorting, blanching, and roasting are the main processes for lowering aflatoxins in shelled peanuts. Several time-temperature combinations, such as 150–160 °C for 30–120 min., 170–185 °C for 8–11 min., or higher temperatures, such 290 °C for 6–13 min., are used for roasting peanuts. As a result, the amount of aflatoxins that are reduced in peanuts after roasting may vary, while tests on typical samples showed that an average level of 15 gkg was present.

Blanching for 30 to 36 hours, on average, eradicated more than 98% of all aflatoxins, demonstrating that sorting is a very successful method of lowering the aflatoxin level of Brazil nuts. Depending on the time-temperature combination used, a research on pistachio nuts revealed a decrease of 30–50% during roasting and an additional reduction of 26% during sorting.

WHO Aflatoxins

Examination of the safety of certain food additives and pollutants. Its use as a hygienic indicator during the production of powdered baby formulas is highly endorsed in the findings of the FAOWHO, Cahill et al., and ICMSF, which were prepared by the Forty-ninth Meeting of the Joint FAOWHO expert consultations. Few but severe instances and outbreaks have

been connected to Cronobacter species. A third of them have a direct connection to baby formula that is inherently contaminated. Additional studies indicated that the food had been contaminated during preparation or, in a number of instances, that PIF had not been provided to the babies. No other pathogen has received a "A" rating despite reviews that have been published by several authors in numerous studies. While Cronobacter spp. has only been clearly established as pathogenic for infants up to 6 months with neonates, particularly pre-term infants, low-birth weight infants, or immune-compromised infants, Salmonella is considered a pathogen for infants as well as for young children and of course for consumers belonging to other age ranges. The issue for follow-up formula was specifically investigated, and it was determined that there was no epidemiological evidence indicating babies older than 6 months were sensitive. Infections include eye infections, urinary tract infections, and other forms of systemic infections are reported in publications that deal with instances of Cronobacter spp. in adults and the elderly, therefore these cases are not foodborne.

Notwithstanding the uncommon instances and outbreaks, it is safe to infer that exposure is relatively minimal since Salmonella and Cronobacter spp. are seldom found in goods. Reij et al. estimated a minimal number of Cronobacter spp. that is ingested, thus corresponding to an exposure, by all infants through PIF of 7.84 log CFUWHO 2004, 2006). Exposure to significant levels at the time of consumption is primarily due to rehydrated products being improperly handled after reconstitution have been involved in outbreaks. Infants that are preterm, low birth weight, and immuno impaired are thought to be at the greatest risk for illness.

Indicator organisms and pathogens in powdered infant formula

According to Euromonitor estimates, the annual world output of PIF was over 106 tonnes in 2005, and US$41 billion was spent on milk formula worldwide in 2013. The types of items implicated in Salmonella outbreaks are often not specified. Items like follow-up formulas and those ingested by young children, who are also vulnerable to salmonella, need to be considered, which results in much greater tonnages of goods. Infants don't seem to have any dose-response data or models, but those that do exist for other age groups suggest that even extremely low quantities of salmonellae cells may cause sickness. Because to a paucity of information, FAO Processing

Pasteurization and sterilisation using direct steam injection are two common processing conditions used during heat treatment. Due to the heat sensitivity of both species, these methods accomplish reductions of around 6-7 log units for the mildest treatments and well over 50 log units for the harsher ones.Salmonella and Cronobacter spp. contamination in finished products produced in accordance with the three types of processing is caused by the calibre of the raw materials used in product manufacture as well as contamination from the processing environment, which occurs throughout the entire process for exclusively dry-mixing operations or after heat-treatment and drying in the case of wet-mixing or combined operations. It has been shown that Cronobacter spp. can endure longer durations of desiccation and may thrive in dry processing settings and dry goods. Salmonella can yet endure arid circumstances for extended periods of time, while likely not being as resilient. The primary sources of these microorganisms in the completed goods include post-process contamination after the kill stage, contaminated processed components added after the kill step, and processing environments and lines. No growth will take place throughout storage and distribution up to the end consumers. Yet, if present, Salmonella and Cronobacter spp. may persist for a long time in dry foodstuffs.

Approval Standards for the Completed Work

Organoleptic

Depending on its composition, each form of PIF has unique organoleptic properties, and some of these factors are exploited for release.Chemical, physical, and nutritional PIF must adhere to the specifications outlined in the applicable national legislation or the Codex Alimentarius Standard for these goods, as applicable. These specifications relate to the content of the nutrients on the one hand, and to standards for chemical pollutants on the other.

Microbiological

End product testing for Salmonella is often restricted and only performed for verification when producers concentrate on the deployment of an integrated sampling strategy including in-process and environmental samples. Testing of up to 60 analytical units of 25 g for release purposes may become necessary under such circumstances if positive findings of either in-process or environmental samples point to a possibility for elevated risk of Salmonella presence in the completed product. Testing of completed goods has a greater relevance and is usually used for release to ensure compliance with regulatory requirements because of the pervasive presence of Cronobacter spp. in processing settings, even though it may be controlled too low to very low levels.

Using Risk Metrics to Assess the Strictness of HACCP Systems for the Food Safety System. The processing stage where the heat-treatment is administered is regarded as CCP and must be controlled as such. Depending on the kind of heat-treatment used, critical limitations may change. Other control measures, typically regarded as prerequisite programmes like the air handling units, may be considered as operational prerequisite programmes or CCPs and managed as such given the stringency of the hygiene control measures required for control of Salmonella, and particularly Cronobacter spp.

Programs GHP

The production of PIF must adhere to severe microbiological standards outlined by Codex Alimentarius, which necessitates significantly more stringent pre-requisite procedures than were previously required for Salmonella control. The fundamental difference lays in a considerably stronger control of the presence of traces of water or humidity to avoid development of Cronobacter spp. found at low levels in process- ing settings. Cordier has provided extensive information on the history of the development of hygiene control procedures to manufacture PIF as well as the variations in the management of Salmonella or Cronobacter spp.

Listeria Monocytogenes in Deli Meats that are Ready to Eat

Listeria monocytogenes is a major foodborne pathogen which is extensively spread in nature and may be found on practically all foods, in soil, water, sewage, silage, slaughterhouse waste, milk from healthy and mastitic cows, as well as in human and animal faeces. It is one of a select few foodborne pathogens that can thrive at low temperatures, endure in food processing facilities for extended periods of time, and have a high case-fatality rate. Even though there are only 2 to 6 instances of foodborne listeriosis per million people yearly, 20 to 30% of these cases result in death.

Think of L. monocytogenes, which may develop in a range of perishable RTE foods, such as ready-to-eat deli meats, as an example of a microbiological danger. With connection to HACCP plans, the application of performance goals, performance criteria, product and process criteria, and validation will be discussed. This pathogen is psychotrophic, establishes itself in crevices, and 10.1007 cooler in this example as it does in many foods. Some businesses freeze food using cold water, sometimes with salt added to drop the temperature

below the water's typical freezing point. A brine chiller is what it is. Other eslishments may use cold air, and some use a combination of methods. Some of these goods, however, are cooked in a water bath or in a steam chamber. Following cooling, many of the goods are packed as, e.g., entire roasts, for the retail deli market, while others are sliced and packaged in retail consumer sized quantities. Many are vacuum-packaged, which helps to protect the product quality and increase the shelf-life. Adequate cooking renders meats free of *L. monocytogenes.* However, post-processing contamina- tion can occur and has been the cause of a number of large meat-borne listeriosis outbreaks, such as the Canadian outbreak associated with the consumption of deli-meats, in which 24 deaths occurred. It should be noted that multiplication of *L. monocytogenes* on cooked deli-meats is generally considered necessary for disease to occur. There is limited data on the dose-response relationship of *L. monocytogenes* in humans and, unfortunately, suile animal models of infection are still lacking.

Exposure Assessment

Exposure assessment is one of the four components of a risk assessment within the risk analysis framework adopted by Codex as the basis for standard-setting processes. Exposure assessment includes an assessment of the extent of actual or anticipated human exposure. For microbiological agents, exposure assessment is typically based on the potential extent of food contamination by a particular agent or its toxins, conditions between consumer purchase and consumption, and dietary information related to frequency and extent of consumption.

Many listeriosis outbreaks have been related to RTE meats, which are often contaminated with *L. monocytogenes.* Yet, there is a long history of safe meat production in the meat business. *L. monocytogenes* was discovered by Wallace et al. to be present in 1.6% of frankfurter packages, and USDAg) that might theoretically arise in raw materials. Also, if a product has been recontaminated between cooking and packing, "post-pack pasteurisation" may get rid of *L. monocytogenes* from the surface. Several manufacturers throughout the globe presently use this procedure for business purposes.

Contamination and Exposure after Cooking

In facilities operating with successfully developed and executed HACCP plans, the cooking process for cooked beef products can be controlled, but it is considerably more challenging to avoid contamination during chilling and packing. A key contributor to product contamination includes contamination of the working environment and equipment, as well as the spread of *L. monocytogenes* during operations including chilling, slicing, and packing. For example, Listeria spp., a well implemented environmental monitoring programme may identify potential harborage locations.

Temperature and Time within the Supply Chain

The temperature and duration of storage, which are within the control of the producer, retailer, or customer at various points in the product's life, have a substantial impact on the development of *L. monocytogenes* on deli meats. A multi-hurdle method to growth management may be used to create circumstances in which *L. monocytogenes* cannot grow on a meat product by combining product and environmental factors such pH, water activity, temperature, and antimicrobials.

CHAPTER 14

FACTORS AFFECTING DOSE-RESPONSE

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For healthy persons and high-risk groups including expectant mothers, the elderly, and immunosuppressed people, multiple dose-response models exist. Across these groups, the real risk varies greatly. According to estimates, some subpopulations may be 20–1100 times more likely to get listeriosis than the general population. examined instances of listeriosis recorded in France from 2001 to 2008 and computed risk ratios for several high-risk categories as compared with normal persons <65 years of age. For instance, the risk was 116 times higher for pregnant women. Also worth noting is that although the danger was just eight times higher for persons aged 65 to 74, it was twenty times higher for those above 74.

Strain Asymmetry

There are recognised virulence variations among the many serotypes of L. monocytogenes, with serotype 4b typically considered to be the most virulent. Differences in virulence may, however, occur even across serotypes. The development of shortened internalins has been connected to several of the strains that have been related to decreased virulence.

Dose-response

An accurate dose-response model for *L. monocytogenes* cannot be constructed using data from experimental outbreaks, human volunteer feeding trials, or animal experiments. To "correct" a mouse-derived model for the range of virulence and make it relevant to humans, FDA and FSIS employed a method based on the use of a "dose-response scaling factor," however that model changes with each iteration of the risk assessment and is neither easily reproducible nor easily characterised. Risk assessment by FAOFSIS FDA According to a WHO risk assessment, awareness of *L. monocytogenes* is especially poor among seniors, which is concerning since they represent a significant risk group. Moreover, Cates et al. discovered that pregnant women, another risk category, were unaware of the dangers of foodborne listeriosis and were not practising preventative measures. In average, people keep their deli meats in storage longer than is advised. The message to reach the vulnerable populations has increased. For instance, to prevent listeriosis, The Centers for Disease Control and Prevention advises adults 50 years of age or older to reheat their cold meat before eating. Hot dogs, cold cuts, luncheon meats, and other types of meat should be cooked to at least 165 °F, or what the CDC refers to as "steaming hot," for those over 50 and particularly over 65.

The USA has around 315 million people living there as of the 2013 census. From 2009 to 2013, the FoodNet system projected that there were 3.4, 2.8, 3.1, 2.6, and 2.6 cases of listeriosis per million people year. Around 83% of the infections and fatalities caused by *L. monocytogenes* from eating deli meat in the USA were attributed to the meat being sliced and packed at retail delis, while 17% were attributed to prepackaged deli meats. The following are the results of the survey. 17% of anticipated annual fatalities were directly related to retail-sliced food.

Data from a study by the NAFSS in which 6 of 3522 samples and 49 of 3518 samples tested positive for *L. monocytogenes* from prepackaged and retail-sliced deli meats, respectively, were used to determine the prevalence and level of *L. monocytogenes* in RTE meat and poultry deli-meats at retail establishments. *L. monocytogenes* was detected in 57 samples, yielding a prevalence rate of 0.76 percent overall. According to this data, the risk of listeriosis mortality from RTE meat and poultry items cut at retail is roughly 4.9 times higher annually than the risk from pre-packaged goods. The cause of 83% of *L. monocytogenes* mortality from deli meats is retail-sliced goods. This proportion is not significantly impacted by consumer storage time, product shelf life, or the overall number of fatalities linked to *L. monocytogenes*. Overall, there was no statistically significant variation in *L. monocytogenes* frequency amongst the various deli meat varieties.

Primary Preventative Measures

Deli meat contamination mostly happens post-heat processing, also known as post-processing contamination. Thus, it is crucial to regulate the post-processing environment in order to control L. monocyto- genes in deli meats. Moreover, as the majority of deli-meat-related illnesses and fatalities result from deli-meats that are sliced and packed at retail, cleanliness at retail is crucial, particularly with respect to deli-meat slicers, display cases, and sinks. The regulation of the cold chain and shelf-life dating are crucial elements.

Primary Manufacturing Beginning Raw Material Level Regulation

It might be crucial to handle raw materials properly throughout storage and preparation in order to reduce a number rise brought on by contamination or growth. For certain commodities, such as raw milk cheeses produced from the milk of cows with listerial mastitis, fundamental production controls may be crucial. For deli meats and fermented sausage products, excessive levels of *L. monocytogenes* in the raw meat may outweigh the pathogen's potential to be inactivated by the following fermentation.

Manufacturing

Raw ingredients, product composition, cooking, and handling procedures after cooking may all vary significantly. At each stage of the manufacturing process, these variances may modify the risk and vulnerability of the product. To achieve the necessary degree of control of microbiological risks for the specific product, its components, formulation, manufacturing technique, packaging, storage and distribution, and shelf-life, GHP and HACCP must be applied. It's essential to take into account the manufacturing processes and available controls that apply to a certain product. The following *L. monocytogenes* control methods could be appropriate.

Cooking

L. monocytogenes in the middle of a raw beef emulsion or an injected entire muscle must be completely destroyed by cooking. Goal reductions between 5 and 6 logs should be acceptable.

keeping food from being contaminated while being packaged

The period between cooking and packing may lead to recontamination in many, if not all, deli meats. For the majority of items, it is necessary to minimise recontamination between cooking and packing by using GHP procedures. Depending on the amount of control, *L. monocytogenes* may be recovered from a variety of sites in the plant environment. In industrial conditions, the organism may survive for extended periods of time in the absence of an efficient control regime. So, even if a performance goal for prepared deli meat is

achieved, recontamination of processed goods is still a possibility unless GHPs that are intended to control *L. monocytogenes* have received the required attention.

It has been discovered that certain GHP methods are crucial for the control of *L. monocytogenes* in cooked meat and poultry operations. The measures include maintaining a clean and dry environment, using enclosed steam to sanitise equipment as part of a scheduled routine procedure, and low temperature storage. They also include plant layout, equipment design, equipment maintenance, and cleaning and sanitising procedures that are specific to listeriae control.

The efficiency of a listeriae control programme is influenced by two aspects, namely regular environmental testing and the reaction to a positive discovery. Control might be difficult to evaluate in the absence of an environmental monitoring programme. In addition, remedial steps should be taken to stop the source of contamination in the case that a positive product contact sample is found, lowering the risk of product contamination. Plants should put in place an environmental monitoring programme for *L. monocytogenes* or an indicator species like Listeria innocua or Listeria spp. to verify control. Each plant needs a customised programme that specifies the regions to be sampled, the analytical technique to be used, the sampling frequency, and the course of action to be done when Listeria spp. are found.

The following tactics should be taken into account by a monitoring programme that is successful in evaluating management of the environment for cooked products:

Avoiding the recruitment of *L. monocytogenes* in areas or situations where they could contaminate RTE food, putting in place a routine of sampling that can quickly determine if the RTE environment is under control, responding as quickly and efficiently as feasible to each positive product contact surface sample. That the issue has been fixed and giving information to enable both short- and long-term assessments of control.

Experience in the production of cooked meat and poultry suggests that niches are a prevalent source of contamination. These are the locations where the *L. monocytogenes* get eslished and multiply in the cooked product environment. The areas are generally enclosed by machinery and are difficult to access and sanitise using standard cleaning and sanitising techniques. In actuality, the processing area often seems orderly and organised. The locations operate as a reservoir from which the disease spreads and contaminates surfaces where products come into touch. Testing for microorganisms is required to find the niche.

It is advisable to take the following into account when responding to a positive product contact sample. The main source of contamination is a niche, supposing a successful control programme is in place. In most cases, pollution moves like a river through the process flow as it moves downstream. The location of the source is crucial. The easiest way to do this is to plan out the locations of the cooked product rooms and the equipment configuration. Each piece of equipment's sample findings, including both good and negative outcomes, should be included on the map. The next step is to look for patterns in the layout map. Which websites that can affect a product's safety are more commonly positive? Where do the first positives occur in the process flow? While looking for the source, it's crucial to study each environmental sample individually rather than as a composite. Moreover, sampling should be done more often and in more places during the course of the operation. Sadly, niches are seldom discovered till the machinery is in use and the product is being produced. The possibility that a niche may not be implicated must be taken into account when trying to identify the source of contamination. The following stages may be successful after the source has been found to be equipment. When the apparatus is being disassembled, samples of any questionable material should be taken. Second, while the equipment is being put back

together, repair any visible damaged components and clean and sterilise it. If this process is unsuccessful in removing the source of contamination, it might be necessary to heat the equipment, remove any sensitive electronics, and remove grease and oil. The apparatus may be heated to an interior temperature of 71 °C with high humidity to achieve this. As an alternative, the apparatus may be covered with a tarp and heated inside with steam until it reaches a temperature of 71 °C. Strategically positioned thermocouples may be used to measure internal temperatures.

The following are examples of known causes of contamination in activities involving cooked meat:

1. Doors and other brine cold system openings are sealed with rubber gaskets.
2. Insulation on pipes supplying refrigerant to the brine chill system has become saturated
3. Before packing, peelers that remove artificial casings
4. Methods for removing casing
5. Conveyor rollers that are hollow
6. Ion cutting apparatus

Cross-contamination from display cases to open deli-meats, tools including knife racks, cutting boards, scales, workers, and en diacetate to prolong the lag phase and slow the development rate of *L. monocytogenes* are some causes of contamination in retail operations for deli-meats. According to a risk assessment conducted by Pradhan et al., the number of human listeriosis fatalities associated with ham and turkey would be reduced by and nine times, respectively, by reformulating deli meats with growth inhibitors. Another technique that manufacturers might employ is starting cultures to limit the development of *L. monocytogenes* in cooked meat products. In fact, several bacterial strains, such as Carnobacterium maltaromaticum, have already been given the go-ahead to be used on meat products.

There is a lot of ambiguity around the consumption of deli meat, but in the past ten years there has been a noticeable decline as more businesses use high-pressure processing or growth inhibitors as additional control measures. The FDAor has reached an advanced stage. GHP and HACCP programmes that are specifically designed to manage *L. monocytogenes* must be used at all phases of production, storage, transportation, and retail in order to accomplish these objectives. Research should also go on to create new barriers to stop the spread of *L. monocytogenes* in deli meats. It is now understood that individuals regularly consume small amounts of *L. monocytogenes* from meals. Hence, the following food safety goal has been suggested based on epidemiologic and prevalence data:

L. monocytogenes concentration in cooked deli meats shouldn't be more than 100 cfu.100 CFUor concentration of a hazard in a product at a specific phase in the food chain before the time of consumption that gives or contributes to an FSO or ALOP, as applicable, do not offer a better degree of protection than the WHO's risk assessment for *L. monocytogenes* in ready-to-eat foods. Performance targets are often used at stages when risks may be either decreased or increased.An illustration of how to calculate a PO from the FSO may be found in the parts that follow. The risk of listeriosis is greatly influenced by a number of processes, including cooking, post-pack pasteurisation, and the use of antibacterial chemicals.

To arrive at a PO for the control measures needed to meet an FSO for cooked deli-meats, the equa- tion from Chap can be used:

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

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

Where:

FSO = Food safety objective Ho = Initial level of the hazard

ΣR = the total reduction of the hazard from processing, etc.

ΣI = the total increase of the hazard

FSO, Ho, ΣR, and ΣI are expressed in log10 units, ΣR is expressed as the number of log cycles of reduction achieved and ΣI is the log cycles of increase that occur as a result of both growth and recontamination.

The equation is then rearranged to solve for Ho.

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

Since the ΣR and ΣI apply only to the process subsequent to the point at which the PO is defined, PO effectively is the Ho value for the process between the PO and the FSO.

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

With the exception of the FSO, it is significant to note that the values above have distributions, which are supposed to be represented by the mean log concentration and its standard deviation. The calculations used to determine the POs in the examples below guarantee that the FSO will be met with 95% certainty.

After Cooking, Manufacturing Recontamination

Unless solutions have been developed to stop the development of L. monocytogenes, every re-contamination incident has the potential to result in a scenario where *L. monocytogenes* may proliferate to reach large levels during the following distribution and storage. Unless there is in-pack pasteurisation or re-heating before to consumption, high quantities may be eaten.

According to experience, the most frequent cause of *L. monocytogenes* contamination in deli meats is post-processing recontamination, which occurs after cooking.

In order to deal with recontamination, the manufacturer may need to make a calculated choice about how much it contributes to the total effect of I. The amount of the pathogens after cooking would be increased by the logarithmic rise of the recontamination if it occurs after cooking but before growth.

This would act as the starting point for any future growth-related increases. For instance, it would be reasonable to convert to arithmetic values, add them, and then reconvert to log numbers, i.e. 1000 cfug = 1100 cfug = 3.04, if the level of *L. monocytogenes* was lowered to log10 = 3 after cooking and then the meat was recontaminated to a level of 10 cfug.

The FSO would really be surpassed 50% of the time if we take the example of deli meat that has been recontaminated after cooking at 10 cfug with an SD of 0.8, the SD for R is 0.5, and the SD for I is 0.8. But, under the same circumstances, if one wished to have a far higher probability of reaching the FSO, one could also increase the lag phase by, for example, lowering the Ho to 1 log cfudiacetate. Considering the same scenario, it would be natural to anticipate POafter packing 2 + 0 - 0 POafter packing 2 Preparationg or less. The potential for *L. monocytogenes* to multiply when raw meat and poultry are kept and made ready for cooking should be taken into account.

The results collected from Combase by water submersion for about 10 min should produce a 2- to 4-log inactivation of *L. monocytogenes* on the product surface, which further puts the probability of multiplication during preparation into perspective.

Criteria for Processes Thermal Processes

The cooking process uses thermal procedures, and post-packaging pasteurisation is an optional step. Each producer must identify the conditions for heating that will offer the required product quality, cost, and, in this case, ensuring the performance target is reached for cooked goods. Challenge tests utilising surface-inoculated cooked beef might be used to evaluate product characteristics that are meant to limit growth after packing by determining the likelihood of a rise in *L. monocytogenes* before the advised use-by date. For example, commercial products infected and packed in a lab with a combination of five isolates of *L. monocytogenes* kept at temperatures the product would be exposed to throughout storage and distribution may be used in challenge experiments. While carrying out such investigations, several elements should be taken into account. The behaviour of the pathogen and its potential for growth or inactivation may also be estimated using predictive modelling.

If the goal of the product criterion is to prevent the development of L. monocytogenes, consideration must be given to the formulation's level of safety and the level of accuracy needed for process control to guarantee that each manufacturing lot complies with the specifications in every way. Finding the critical limits required to stop growth and establish product criteria may be done with the use of sensitivity analysis and process control techniques.

Shelf-Life Boundaries

Limits on shelf life might be established in terms of product accessibility to customers or by limiting L. monocytogenes' growth potential. Without in-pack pasteurisation, it's conceivable that the food may become dangerous before it loses its organoleptic properties. The duration of the lag phase may need to be matched with the organoleptic accepility of the product if antimicrobials are employed to stop *L. monocytogenes* from growing. There isn't a standardised process in place to confirm code-dating techniques and guarantee the security of perishable items with long shelf lives. As described in Chap. 3, a variety of elements must be addressed when verifying the efficacy of one or more stages along the food chain. While conducting a validation study for code dating, the following elements should be taken into account:

1. The inoculum's physiological condition
2. Technique for immunization
3. Degree of inoculum
4. Origin and variety of strains
5. Storage-temperature range
6. Competitive natural flora
7. product development
8. Organoleptic

Depending on its composition, each variety of deli meat has distinct and recognizable organoleptic features, and some of these factors are employed for its release. Cooked meat and poultry must adhere to any applicable national laws or the recommendations made by the Codex Alimentarius Commission. These specifications relate to the nutritional content on the one hand and to standards for chemical pollutants on the other.

Microbiological

It is not advised to regularly sample deli meats for *L. monocytogenes.* Nonetheless, sampling for *L. monocytogenes* would be appropriate if the regular use of GHPs and HACCP is in doubt. Codex Alimentarius's suggestions should be followed when creating sample strategies for *L. monocytogenes.* More particular, sampling of deli meats should be taken into

consideration when there is a possibility of contamination with L. monocytogenes, such as when positive findings for food-contact surfaces or the efficacy of remedial activities has not yet been confirmed. Sampling the finished product when it is known that it has been post-pack pasteurised adds little value and is not advised. Testing the finished product has minimal relevance for facilities that use a validated kill step for cooking and where an efficient environmental monitoring programme shows that the risk of re-contamination is being minimised. The cause is that a thorough management system may keep contamination frequencies to around 0.5%. Under these conditions, the frequency of flawed units is too low to be picked up by any workable sampling scheme.

End product testing may be necessary when a product is travelling across international boundaries and little is known about it or the production process. The suggested sample strategy for deli meats encouraging growth would be n = 5 25 g, with a c = 0 and m = absence in 25 g. At the time of final manufacturing to the point of retail sale, the Codex microbiological standards are in effect. These parameters change depending to whether the product is capable of sustaining the development of L. mono- cytogenes. A 2-class plan is used in both situations; for products that support growth, a qualitative method for the absence of *L. monocytogenes* in 5–25 g is anticipated, whereas for products where growth won't happen, a quantitative standard of 100 cfug and an analytical standard deviation of 0.25 log cfug are used. The quantitative approach takes the ISO 11290-2 approach as a given. A lot of food with a geometric mean concentration of 93.3 cfug would be identified and rejected based on any of the five samples over 100 cfug and up to 45% of the samples being above 100 cfug, according to this sampling strategy, which assumes a log-normal distribution, with 95% confidence. If there are worries that too many samples are grouped close to the 100 cfuGL 61-2007 threshold.

Factors for Dose Response

The amount of information is inadequate to establish a trustworthy dose-response2 connection for the likelihood of infectious sickness.When several dose-response functions were examined and fitted to epidemic data for EHECs, it was found that the Crockett et al. model was covered by the outbreak models' boundaries. The "best fit" and "median" models of Strachan et al. and the Beta-Poisson models reported by Cassin et al. were reproduced and directly compared using parameter values. The ID50 for Shigella infections, according to the Cassin et al. model, is about 2900 cells. 0.9 log cells were reported as the standard deviation by Cassin et al. The ID50 predicted by the Strachan et al. "best fit" model based on EHEC epidemic data is 200,000 cells, and their "median" model was employed to lessen the impact of data that seemed to be out of the ordinary. The Cassin et al. model is more comparable to the Strachan et al. "median" than the Strachan et al. "best fit" model, which forecasts an ID50 of 300 cells.

Cleaning after Consumption

It is unlikely that customers' further washing of packaged RTE leafy vigils would increase safety. Any safety advantage that further washing could provide may be outweighed by the danger of cross contamination from food handlers and food contact surfaces utilised during cleaning. Palumbo et al. suggested that leafy vegetable salads in sealed bags labelled "washed" or "ready-to-eat," made at a facility inspected by a regulatory body and run in accordance with GHPs, do not need extra washing at the time of consumption unless expressly instructed on the label.

Achieving an ALOPor level of hazard concentration in a food at the moment of ingestion that is thought to provide an adequate degree of health protection. Determining a reasonable level

of risk is difficult since, although "zero risk" from food would be ideal, this is presently not technologically possible for many minimally processed ready-to-eat items. As a result, some countries suggest risk reductions that are gradual as food safety objectives. By 2020, the US Office of Disease Prevention and Health Promotion hopes to have reduced the incidence of illnesses brought on by E. coli O157:H7 by 1.2 cases per 100,000 people, or 50%. For the sake of this example research, leafy vegetables in the United States are subject to the same public health objective. Nevertheless, in order to convert this objective into a food safety objective, it is necessary to convert the observed incidence of EHEC infections from produce into risk per serving of leafy vegetables. This goal effectively limits the number of illnesses per 2.08 million servings of leafy salad vegetables, or 50% of the present rate, to an ALOP. This objective must be translated into a quantifiable quantity in order to provide industry and regulators with useful guidance, such as a maximum frequency and 205,000EHEC cells g, i.e., from the desired FSO the average contamination level in leafy vegetables at the point of consumption must be 5.31 log cfu. WHO 2010), the likelihood of sickness will be inversely related to the number of cells at these low EHEC levels. Due to the average potential for growth, the tolerance for contaminated bags at the conclusion of processing must be lowered by a factor of four in order to retain the FSO. No more than one 340 g bag in 777, or a contamination level of less than 1 EHECg, may have EHECs in order to fulfil the FSO. The product's performance goal after processing is to have this amount of contamination.

The product should be distributed and marketed in such a way that the rise in contamination levels does not exceed 0.6 log cfu, taking into account the consumer's circumstances and the anticipated holding period.Gateway of Entrance the Performance Goal for Port of Entry inspection should be the same as for the processor of the product because to the high perishability, short shelf life, and short supply chain of these items. Thus, the same Performance Goal as for processing would be the most practical and logical choice as there is no additional production involving these items.

Primary Manufacturing

To put the PO determined into perspective, an estimate of H0 is necessary; if present on-farm management techniques are enough to dependably attain the PO, no processing to lower EHEC levels would be necessary. Alternately, extra processing for inactivation or elimination of EHECs will be necessary if EHEC levels on raw materials are greater than the PO.

Here, we take into account the levels of EHECs discovered on commercial items after harvest but before to processing, as well as the possibility that EHECs may be inactivated or removed during processing based on commercial data. The effectiveness of different processing aids that are now in use and others that have been suggested is examined in laboratories.

As mentioned earlier, leafy vegele testing results may be used to determine H0. The prevalence is estimated at 0.16% based on data collected in confidence from a number of leafy vegetable producers and processors. Out of 184,000 tests conducted over a one to two year period, 290 were found to be positive for EHECs or E. coli O157:H7 before processing. In contrast, out of 90,400 samples that had undergone processing, had tested positive for EHEC or E. coli O157:H7, with a prevalence of 0.019% and demonstrating a roughly 10-fold decrease in prevalence.

The average contamination level in entering product is thus roughly estimated to be 1 cell per 476 kg following processing. The processor also uses a second, less intense sampling strategy that uses analytical units of 60 leaves, or 150 g. The average contamination before processing from the prevalence of positive samples is calculated as 4.98 log cfug after processing, if that less severe approach was used.

Additional information received from the USDA gives an overall picture of agricultural cleanliness for all farmers. Among 4664 samples of conventional or organic lettuce and bagged spinach, there were 34 EHEC detections by PCR, however only 0.4% of the samples were positive on culture. Compared to either variety of lettuce, spinach had a far greater detection rate. After processing, samples were obtained, each weighing 450 g. This leads us to the conclusion that there is typically 1 cell every 113 kg of pollution.

Enter numbers for faulty units, where a bag is deemed defective if it even includes one cell. To approve the manufacturing batch and show that it fulfils the FSO, 570 analytical units must be analysed and proven to be free of EHEC and Pa = 0.05. For a batch to meet the FSO with a 95% confidence level, 7750 tests would need to be run if the analytical unit was 25 g. Product testing at the point of consumption is not possible and, as shown, calls for excessive volumes of the product. Instead, the Food Safety Goal has to be transformed into a Processing-specific Performance Objective. But first, we must determine how much the levels of danger have changed between the time of preparation and the time of consumption.

Escherichia Coli Enterohemorrhagic on Fresh-cut Leafy Vegetables

A testing regime's dependability may be evaluated using the binomial distribution function. As an example, one producer checks 300 leaves every batch of food they deem "high risk," such as spinach and Romaine. The causes of these classifications are unclear, but they presumably have to do with how often outbreaks of specific sorts of leafy vegele occur. E. coli are harder to get rid of from the underside of spinach leaves, according to Zhou et al. This is most likely because of the ultrastructure of the underside of the leaf. A batch weights around 182 kg, but a spinach leaf only weighs about 2.5 g. The leaves are enhanced and composited, and the enhanced leaves are examined for EHECs. The highest sensitivity of the test procedure may be assessed assuming that the test technique is flawless, i.e., that the approach will identify a single EHEC if present in the 750 g composite sample. Again, adopting a 95% confidence level, we can evaluate Eq. 17.2, as follows:

Using a 95% confidence level once again, we may assess. 17.2 as follows: P = n 0.05 = 300. By finding the value of p and solving for it, we discover that p = 0.00994, meaning that just 300 leaves will be tested in order to identify contamination in 95% of instances if more than one in around 100 leaves are contaminated. In other words, the approach will accurately determine if there is more than one EHEC per 250 g with a 95% accuracy. This leads us to conclude that the testing regime's sensitivity limit is 2.39 log cfug. Only "high-risk" items are tested at this level; batches of other product kinds simply have a small number of leaves taken and composited. A procedure using composites of 60 leaves is employed for "reduced risk" goods.

Reducing the Product's Potential for EHEC Growth

The majority of RTE green vegetable products are marketed without preservatives or packaging that inhibits microbial development or residual plant metabolism. According to the study shown above, lowering the temperature would be necessary to cut the anticipated increase in the distribution system by around 0.27 log cfu. This may be accomplished by avoiding higher temperatures, where the growth rate is more rapid, or by lowering the average temperature throughout distribution and storage. By using the growth rate model created by Danyluk and Schaffner and assuming a product has a 10-day shelf life after it is received by the retail store, we can put into perspective the size of the temperature reduction needed to achieve the desired reduction in growth. For example, if temperature is below 2.628 degrees Celsius, growth rate equals.

Although E. coli ordinarily cannot thrive at temperatures below 7 °C, distribution chain temperatures often vary, perhaps allowing for periods of growth. The FSO would be attained at the projected average storage temperature linked to a 0.6 log cfug over a 10-day period. Average storage temperature would have to be 5.9 °C in order to achieve the FSO; a decrease of 0.7 °C in average storage temperature is predicted to do this.In the third instance, which had an average product, temperature control alone was not sufficient to accomplish the FSO; further steps were needed to lower the H0, enhance inactivation, or remove EHECs from the product after harvest. an additional 0.76 log cfu decrease for physical inactivation72,800. There are 300 opportunities to choose one of those leaves if high risk sampling is employed, compared to 60 opportunities if "standard" risk testing is used. Applying Eq. 17.2, the likelihood of discovering the contamination prior to processing is 2% for the "high" risk sampling strategy, compared to 0.4% for the "normal" risk product. The faeces contain 6 log cfuml if we suppose that each of these 5 leaves is contaminated with 0.2 g of faeces. When sanitizer is not present in the wash water, testing the wash water after a concentration stage may be a way to determine if EHEC is present in a batch without testing the product after processing. If a sanitizer is a part of the washing system, care must be taken to deactivate it during the water sampling in order to prevent an unnaturally low result brought on by sanitizer action on the EHEC in the time between sampling and sample processing. In this case, the presence of any EHEC would suggest that the whole batch could have been contaminated. The EHEC concentration after washing is 0.549 cfuleaf and should be detectable by "normal" or "high risk" sample techniques, assuming the remaining 10% of EHEC from the five originally infected leaves is evenly dispersed across all leaves that pass through the wash system. Although frequent product post-processing testing is normally not advised, it is possible to anticipate that the washing process may disperse EHEC from a few highly infected leaves more evenly throughout all leaves in the system. This would increase the likelihood that highly concentrated but rare contamination would be found and that the test findings were indicative of the overall contamination in the batch.

Several scenarios were investigated. EHEC on leaf after Processing may indicate worst-case situations that are not anticipated in the day-to-day operations of RTE leafy vegele processing. Based on many inputs, including the number of infected leaves, the quantity of faecal matter per leaf, and the concentration of EHEC in the entering faecal matter, the likelihood of identifying EHEC using "normal" or "high risk" sample techniques. In this case, the need of post-process testing is also shown since the corresponding sampling techniques for all but one scenario or "high risk" scenario are 6.84% and 16.2%. These low detection probability emphasise the significance of cross-contamination avoidance in washing systems, crop exclusion from the processing stream, and contamination prevention in the field.

Stochastic Methods

Deterministic computations often provide single values or risk estimates that are not indicative of the range of potential outcomes, which may result in poor risk management choices. Nauti spoke about and provided an example of this. Relevant to this case, Danyluk, Schaffner, and Pérez Rodrguez's studies were based on Ecosure data, which provides mean temperatures and temperature ranges for retail back room refrigerators, retail display refrigerators, and residential refrigerators.Both studies calculated the increase in that portion of the farm-to-fork chain as 0.6 log cfumaing using the distribution of temperatures and periods of storage at those phases of the retail-to-consumption parts of the supply chain. As was taken into consideration in Sect. 17.4.2.5, the standard deviation is typical of the very inhomogeneous distribution of pollutants that may be predicted on green vegetables. The percentage of samples of various sizes predicted to contain one or more EHEC cells and that might be identified by an enrichment approach may be calculated from that distribution. We

take into account sample sizes of 25 g and 100 g for this example. Around 0.267% of samples are anticipated to possess a level over 2 log cfug for a normal distribution with mean 5.9 and standard deviation 1.4. Similar to this, it would be estimated that 1 in 1538 samples weighing 25 g would contain a cell, and 1121 samples weighing 100 g would need to be tested and proven to be free of EHEC. At the same degree of certainty with 750 g samples, 194 samples would need to be analysed.

An illustration of the predicted cell count dispersion in a batch of RTE green vegetables. The average concentration in the distribution is 5.9 log cfug, which is seen as typical of an inhomogeneous product. The cumulative distribution, which shows the percentage of samples predicted to have concentrations below those stated, is also shown. To illustrate the percentage of samples that would be anticipated to include one or more cells in a sample of that size, the three dotted lines represent various sample sizes.

Enterohemorrhagic E. Coli on Freshly Cut Leafy Vegetables

We use the case of a few severely infected leaves in a batch of generally clean leaves as another illustration of the advantages of a stochastic method. Imagine a batch of 5 leaves that are infected with 0.2 g of faeces each that contain 1000 cfug for the purpose of the demonstration. The batch has a total of 182, 000 g and 181, 987.5 g105 cfu g = 1002 cells. The average harvest procedures thus equal 2.26 logcfu. The significance of GAPs for avoiding initial contamination and subsequent amplification of pathogenic microbes has been amply shown by outbreak investigations, field studies, and laboratory-based research.

CHAPTER 15

REGULATORY REQUIREMENTS AND CRITERIA FOR VIRUSES IN OYSTERS

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There are currently no globally standardized methods for determining the microbiological safety of freshly cut leafy vegetables. The 2011 Food Safety Modernization Act, which was passed into law in the United States, strengthens the ability of the Food and Drug Administration to impose particular safety standards on growers, harvesters, and facilities that produce, process, pack, or hold food products, including fresh-cut leafy vegetables. The Produce Safety Rule's requirements are based on the FDA's previous recommendations for GAPs and Good Handling Practices for fresh fruits, vegetables, and nuts. They also include detailed metrics regarding the use of raw manure, biological soil amendments, and the microbiological quality of agricultural water that comes into contact with the harvested surface of the fruit. Operations using freshly cut green vegetables are likewise covered by the FSMA's Preventative Controls for Human Foods Regulation. According to the PC regulation, every facility must create a food safety plan that includes a hazard analysis to find and resolve any problems that need to be prevented, as well as a recall strategy in the event that problems are found.

Oysters with Viruses

In especially in coastal areas and island countries, seafood is a vital component of the human diet. The average annual per capita intake of fish and shellfish worldwide in 2013 was 18.98 kg, according to FAO. Between 1961 and 2013, the amount of shellfish produced increased significantly, from 1.9 million metric tonnes to 17.5 million. In 2013, the United States produced 1,057,882.44 tonnes of mollusks, with a 3.31 kgh per capita consumption. These filter feeders consume bacteria and viruses that may be present in the aquatic environment in addition to the suspended algae that serves as their main source of nutrition. It has been shown that relative to their surroundings, bivalves may bioaccumulate and concentrate viruses up to 100-fold. Most shellfish are grown in coastal and estuary waterways, which may be contaminated by sewage. The majority of foodborne outbreaks involving shellfish are linked to eating shellfish from waterways that have been contaminated by human waste or by toxic microalgae blooms. Contamination of the food may also be brought on by infected or asymptomatic food handlers.

Oysters with Viruses

The magnitude of the problem increases because many shellfish are generally eaten raw or only partially cooked, and the two commonly used methods to make the bivalves safe for consumption, i.e. relaying and depuration, are not effective in purging out the viruses as compared to faecal coliforms and pathogenic, enteric bacteria.

NoV is one of the top three causes of gastroenteritis, according to a research on the prevalence of foodborne illness in New Zealand. According to data from Australia, the Netherlands, the United Kingdom, and New Zealand, viruses are responsible for 31.6%, 10-20%, 8%, and 14.4% of cases of foodborne gastroenteritis, respectively. Asian nations have

little information accessible. At 48.2% of all cases recorded, viral agents are the most common cause of foodborne diseases, according to the Japanese Ministry of Health, Welfare, and Labor. With a single episode of projectile vomiting, which is often linked to norovirus illness, roughly 30 million virus particles may be released into the environment. This is particularly important for those who handle food and gather seafood.

Differences in susceptibility between SE+ and SE- persons, variations in NoV strains, and apparent disparities in levels required to generate an infection vs levels to cause sickness challenge the development of dose-response curves for NoV. For instance, study of data from outbreaks associated to oysters showed that the likelihood of infection from a single genome copy of NoV GI and GII was 0.29 and 0.40, respectively, whereas the likelihood of disease was 0.13 and 0.18, respectively, for SE+ people. Those who were SE-, on the other hand, were well protected. According to Teunis et al., the likelihood of sickness increases with dosage, with an estimated ID50 for illness being about 6000 genome copies. Due to NoV's tendency to aggregate, it is difficult to determine reliable dose-response curves, which may lead to an order of magnitude underestimating of the likelihood of infection and sickness. According to EFSA, exposing human volunteers to successive dilutions resulted in a dose-dependent likelihood of becoming sick that ranged from 0.1 to 0.7. Teunis et al. also calculated that for disaggregated NoV delivered in human challenge experiments, the dosage necessary to produce sickness ranged between about 1000 infectious units or 1 million particles. In a U.K. investigation, a statistically significant variation in norovirus RNA levels in oyster samples was discovered that was highly associated with norovirus or sickness of the sort normally observed in regions of commercial production. Moreover, none of the outbreak-related samples had less than 152 copies of the digestive tissue from shellfish. It is reasonable to assume that a lower dosage extrapolation would be linear given the strong infectivity projected at levels as low as one genome copy per dish.

Influenza A

Hepatitis A has an incubation period that ranges from two to six weeks, with average lasting 28 to thirty days. The incubation stage and the first two weeks after the beginning of symptoms are when the virus sheds its virions. Hepatitis A infection symptoms include fever, appetite loss, nausea, vomiting, and abdominal pain, followed by jaundice. Symptoms normally last fewer than 2 months, while some individuals may be unwell for as long as 6 months. Infections are often minor or asymptomatic in newborns and kids under the age of five. Severe hepatitis A symptoms are more common in older adults and those with chronic liver disease. Those under 60 years old had the highest case-fatality rate among cases reported by U.S. national surveillance, whereas older hepatitis A patients have a higher incidence of fulminant hepatitis. Hepatitis A immunity is often permanent. According to reports, some mutations in the HAV genome at the 5′NCR or the VP1X2A and 2C regions are linked to full-blown hepatitis and increased virulence in tamarinds, respectively. With hepatitis A, the infectious dosage is thought to be between 10 and 100 virus particles. Similar to norovirus, the virus is highly concentrated in faeces even before symptoms appear in infected people. HAV is known to linger on fomites for a long time. The combination of these two factors, as well as the prolonged shedding time, makes secondary spread a crucial component of the virus' infectivity.

Factors Influencing the Evaluation of Exposure

Contamination Sources and Pathways for Oysters

Untreated wastewater and faeces storms are two major causes of contamination of oysters at the location of production or harvest. Peak norovirus titer levels were seen in a volunteer

norovirus infection research at 2–5 days following inoculation, with viral shedding initially identified by reverse transcriptase–PCR at 18 hours and persisting, on average, 28 days. Another element linked to the subsequent dissemination of the virus is viral shedding in asymptomatic individuals. NoV was found in 5.2% of healthy controls in the general population and in 19% of healthy controls in epidemic settings, according to a research conducted in the Netherlands. HAV begins to shed the virus 10–14 days before symptoms appear, possibly exposing a broad population of vulnerable people to the risk.

Oyster Virus Prevalence and level

Many studies have been done across the globe on the frequency of NoV and HAV in oysters and other molluscan shellfish. The rates of prevalence vary from location to location and season to season. Reports on the quantity of viruses in oysters, however, are few. Data on the presence of human enteric viruses in shellfish from European markets was gathered by Boxman; 0 to 90% of the samples of shellfish had enteric viruses. A two-year systematic investigation from 2009 to 2011 in the UK revealed that 39 oyster producing regions had norovirus contamination in 76.2% of oyster samples. NoV or HAV were found in around 5% of retail oysters in a comparable study carried out in 9 states in the United States in 2007.

Seasonal Impact

The bulk of gastroenteritis cases linked to shellfish happen in the winter and very seldom in the summer. In Europe, the months of October through March saw the greatest number of viral discoveries in oysters. During November and January, about 78% of the shellfish-related illnesses that occurred in the US between 1991 and 1998 did so. This may be partially attributable to climate occurrences like increased rainfall from January to April, which raises the possibility of sewage entering coastal habitats. The reduced physiological activity of shellfish below certain temperatures is another possible cause. Shellfish pump much less when the temperature is below 2 °C and more when the temperature is between 8 and 28 °C. When the temperature is over 35 °C, the shellfish pump less and stop feeding. The virus removal from the filter feeders may suffer from the decreased water exchange.

Consumption and Consumer Practices

The ingestion of raw or undercooked seafood is the main cause of outbreaks connected to shellfish. The enteric viruses have a strong tolerance for heat and an acidic pH. HAV was discovered to still be infectious 5 hours and 90 minutes after being exposed to pH 1 at room temperature and 38 °C, respectively. Hepatitis At 85–90 °C for 1 minute, a virus is inactivated; boiling in water for 3 minutes is preferable to steaming for 3 minutes. The majority of viruses may be effectively inactivated by heating food at 85 to 90 °C for 90 seconds, however doing so may make shellfish unfit for human consumption. The oysters are often heated until the shell opens, however this is inadequate to inactivate viruses.

Virus Characteristics, Behaviour, and Survival

Being non-enveloped, enteric viruses are very resilient to the effects of disinfectants, severe pH levels, drying, radiation, etc. Since noroviruses cannot be cultured using the current cell lines, information on their infectivity, persistence, and inactivation is not accessible. Model surrogate viruses have so been used. They include the murine norovirus and the feline calicivirus. As the latter is a respiratory infection, it may not be an appropriate substitute for the human norovirus. Because of its ability to survive acidic pHs better than FCV and other viruses, the MuNoV is often employed as a stand-in for the human norovirus. Norovirus is more heat-resistant than vegetative pathogenic bacteria and can withstand pH 2.7 for three hours at room temperature. Moreover, the virus is unaffected by free chlorine concentrations between 0.5 and 1 mgL. Compared to enteric adenovirus and poliovirus, the HAV is often far

more resistant to ordinary food preservation techniques and disinfectants. It has been shown that it is more resistant to heat, desiccation, pH fluctuations, and ionising radiations than other enteric viruses. According to studies employing artificially contaminated finger pads, 10% to 50% of HAV may spread to other foods and other surfaces. High relative humidity promotes the survival of enteroviruses, but HAV and rotavirus are the opposite. The HAV remained contagious in dried faeces for 30 days at a temperature of 25 °C and a relative humidity of 42%. According to Mbithi et al., 16–30% of HAV may be recovered after 4 hours of room temperature drying on the finger pads. An illustration of the long-term survival of the virus on inanimate items under challenging settings is the recurrence of outbreaks of a similar norovirus strain on cruise ships despite extensive treatment. NoV could be extracted from polyvinyl chloride and stainless steel for 4 and 8 weeks at 20 and 4 degrees Celsius, respectively. In dried faeces, the hepatitis A virus may persist for up to one month. Treatment of mixed human and animal waste for 7 days before to disposal only lowered virus titers by 1 log10 at 37 °C. The virus may stay contagious for months after being introduced to an aquatic environment and has been linked to marine sediment.

The enteric viruses often interact with clay and other sediments in marine water. At acidic pHs, viral binding to marine sediments increases. Enteric viruses may remain longer in the marine environment if there are contaminants present in the water. Because of heat inactivation, pH fluctuations, sunshine, and other water-based microbes, viruses are rendered inactive. HAV has been shown to remain infectious in saltwater for a number of weeks, with the time lengthening in cooler water temperatures. River water, ground water, and tap water all preserved their HAV infectivity for 48, 12 weeks, and 60 days, respectively. In a study on the persistence of human NoV in ground water, the virus was detected by real time reverse transcriptase PCR after storage for 1266 days in groundwater, with no significant reduction after 622 days and only a 1.79 log10 reduction by day-1266. Enteric viruses are quickly absorbed by shellfish, with maximal absorption occurring 6 hours after storage in contaminated water and viral detection as early as 1 hour after exposure. As noted earlier, the viral load of the shellfish can be considerably higher than the surrounding water.

Methods for Detection and Their Impact on Exposure Assessment

For the detection and quantification of the viral load in the samples of shellfish, RT-PCR and RT-qPCR are often utilised. Low virus concentrations in muscle tissue, the effectiveness of the extraction process, the presence of PCR inhibitors, and variations in viral sequences, particularly NoV, all affect the efficacy of PCR tests for enteric viruses. Moreover, a positive PCR result merely shows that the target gene sequences were present and intact and does not necessarily mean that infectious virus particles are present.

It has been discovered that shellfish have viral populations that are sufficiently high yet below the detection thresholds of these techniques to infect vulnerable human populations. Methods for viral particle extraction and detection must be sensitive enough to pick up even small amounts of viral particles. The viruses have reportedly been shown to cluster in the digestive diverticulum of shellfish and to bind to N-acetylgalactosamine receptors found in oysters' digestive tracts. Increased method sensitivity may result from using the digestive tract rather than the whole mollusk. The fact that this tissue makes up just one tenth of the weight of the clam makes processing simpler. The use of RT-PCR, RT-qPCR, or the combination of these PCR techniques with viral capture systems, such as the use of antibodies or cell receptors to separate virions from the environmental matrix, currently provides the most practical method for the analysis of the viral genome and quantification of viral levels. This is because of all the aforementioned factors and the lack of secure cell culture systems for the detection of norovirus.

The lack of internationally defined and established procedures for the detection of viruses in foods is a significant barrier to the use of literature data to undertake exposure assessments. A standard RT-qPCR technique has been established by the European Committee of Standardization for quantifying HAV and NoV genogroup I and II RNA from foods or food surfaces. The measurement of viral particles with less than 100 genome copies, however, is not possible using this approach. Included in this should be sporadic contamination brought on by occurrences involving the treated sewage. On average, 103-104 genome copies are often encountered throughout the relaying process (clogging by sediments, physiological stress, shell damage). Moreover, it is difficult to guarantee the water quality in relaying regions and recontamination owing to severe rains and related land runoff is a risk.

Ways to Manage the Post-Harvest

There was no evidence of heat treatment. For the total inactivation of HAV, which is thought to be more heat resistant, internal temperatures of 85–90 °C for 1 minute are required. Similar to this, no infectious HAV could be found at an internal temperature of 88 °C. As the clam only has an internal temperature of 70 °C at this point, which is inadequate to inactivate all the harmful viruses, the practise of heating until the shell opens is not seen to be suitable.

Post-harvest methods have been implemented by a small number of oyster suppliers to lower the levels of foodborne pathogens, notably Vibro parahaemolyticus and Vibrio vulnificus. Individual rapid freezing, Heat-Cool Pasteurization, and High Hydrostatic Pressure are some of these intervention methods. These technologies will probably have little effect on enteric viruses, however. Commercially, a patented method of prolonged low temperature heating followed by quick cooling is utilised to lower Vibrio levels; nonetheless, the method is insufficient to render NoV or HAV inactive. Only heat treatments like roasting, boiling at 100 °C for 30 minutes, and baking can effectively thermally activate NoV. The food matrix affects how viruses are inactivated by heat in shellfish. Heat penetration is hampered by the structure of the muscle tissue and the abundance of viruses in the digestive diverticula. Setting criteria for cooking durations and temperatures is challenging because to the variability of shellfish sizes, their virus makeup, and cooking circumstances. Pinto et al. calculated that boiling shellfish for an additional 5 minutes after the shells opened would further lower attack rates in their quantitative risk assessment of shellfish-borne HAV infections based on outbreak data. They came to the conclusion that although cooking decreased the incidence of HAV infections, it did not completely remove it.

Atomic Radiation

A radiation treatment of 3 kGy resulted in a 95% decrease in HAV in shellfish, but it also changed the product's organoleptic characteristics. It has been discovered that human and murine norovirus-like particles are resistant to gamma irradiation, with a dosage of 5.6 kGy only achieving a 1.7- to 2.4-log decrease in virus on fresh food. It is often thought to be impracticable to employ irradiation to get rid of viral contamination.

Hydrostatic Pressure is High

In the shellfish sector, high hydrostatic pressure has been utilised to inactivate Vibrio organisms without affecting the organoleptic qualities of oysters. HP was able to reduce HAV by three logs at 400 MPa for one minute at nine degrees Celsius, and murine NoV-1 by four logs at five degrees. According to Chen et al., lower temperatures allowed for a 4- to 5-log drop in FCV, as opposed to a 0.3 log reduction at 20 °C and a pressure of 200 MPa. Kingsley et al. found similar results, noting a 1.2-log decrease at 350 mPa at 30 °C as opposed to a 5.6-log reduction for MNV-1 at 5 °C. HP is not always effective against viruses; for instance, the

poliovirus is particularly resistant since it has huge capsid proteins. HP inactivation of HAV in buffered growing media with salt was examined by Grove et al. to aid in their preservation.

Handwashing and Post-Harvest Handling Procedures

While unintentional contamination of shellfish should be avoided, post-harvest contamination is not a significant route of transmission. After harvest, a HACCP plan should be followed for all processing procedures. Shucking, packing, shipping, etc. should all be carried out in a clean environment. The ideal temperatures for live and shucked shellstock are 10 and 7.2 degrees Celsius, respectively. Pole water and chlorine-based disinfectants should be used to thoroughly clean any surfaces that come into touch with food. Water should be of excellent quality or capable of being treated with UV light when it comes to cleaning and producing ice.

The efficiency of ethanol as a disinfectant for NoV and its surrogate viruses, FCV and MuNoV-1, has been the subject of several investigations. It has been discovered that ethanol does not totally render NoV inactive. One study observed reductions of 2 log10 after a contact period of 8 min, whereas other researchers at different ethanol concentrations reported reductions of 0.5 log10 and 2.2 log10 following contact times of 30 sec and 5 min, respectively. Research conducted in long-term healthcare facilities have shown that alcohol-based hand sanitizers are inefficient at stopping the spread of NoV, with the incidence of NoV being six times greater in facilities employing ABHS than in facilities utilising traditional hand washing. A 60% ethanol and quaternary ammonium compound spray with a pH of 10.8 fully inactivated FCV, according to Whitehead and McCue, in 1 minute. The NSSP advises using hand-washing water with a minimum temperature of 43 °C in all shellfish handling facilities. The shellstock should be properly tagged to provide for tracking in the event of an epidemic.

The HAV vaccine is very effective and offers long-lasting protection in adults and children older than 1-2 years.

Educating and Communicating Risks

Systems for delivering and exchanging information throughout the farm to le continuum depend heavily on risk communication and education. Food hygiene training is crucial for ensuring that all staff members are aware of their responsibilities and roles in preventing food contamination. Consumer education on the possible health dangers of eating raw shellfish as well as how to prevent shellfish-borne viral infections by completely cooking shellfish and avoiding cross-contamination after cooking are important steps in the prevention of foodborne disease.

Public health initiatives promoting food safety, such as the Fight BAC® national public campaign in the United States and the Food Safe Partnership national public campaign in New Zealand, are reliable sources of information. The U.S. FDA 2009 Code requirement, which permits food service establishments to serve, raw or undercooked foods upon customer request, provided that the customer is informed of the risks associated with consuming such foods and the customer is not a member of a high-risk group, includes raw shellfish. On American menus, the phrase "Consuming raw or undercooked meats, poultry, seafood, shellfish, or eggs may raise your risk of foodborne disease" is featured prominently. Quick identification of infections in individuals who seek medical care for sickness after consuming raw or undercooked seafood is crucial for proper testing and early treatment for doctors and lab technicians. In order to quickly execute control measures and lessen the effect of possible outbreaks, rapid reporting of cases to public health authorities is essential for identifying both contaminated seafood and problematic harvest regions.

Increased Consumer Protection Levels

FS

Since the ID50 is uncertain, pre-harvest environmental measures are ineffective, post-harvest treatments do not significantly lower exposure, and the majority of servings are eaten raw, OFSO for NoV or HAV in oysters is challenging. The FSO and PO for the undamaged oyster would be equal in this case since human viruses do not spread via shellfish. Consumers of raw oysters, who make up a significant fraction of the overall consumer population, often eat many oysters at a time. With a serving size of 360 g, a concentration of 1 genome copy, or 2.56 log10 genome copies, is assumed. FSO for enteric viruses is difficult, especially for NoV. The EFSA came to the conclusion that eating raw oysters is now unlikely to be compatible with an FSO-based approach. Instead, achieving public health objectives would probably require using warning labels to reduce the number of people who ingest raw oysters. FSO Performance Standards The needed result of one or more control measures at a step or combination of stages to achieve an FSO is known as a performance criteria. Performance criteria are often used at points when risks may be either minimised or increased. The ICMSF conceptual model may be used to determine a performance requirement for the control measures required to fulfil an FSO for viruses in oysters:

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

where:

FSO = Food safety objective Ho = Initial level of the hazard

ΣR = Total reduction of the hazard

ΣI = Total increase of the hazard

FSO, Ho, R, and I are expressed in log10 units, R is negative and I positive.

It is required to choose an ALOP that can be converted into the FSO and from which Performance Objectives and Performance Criteria can be deduced in order for this strategy to be effective. A universally acknowledged ALOP for NoV infection from foods does not exist, however. Without such an ALOP, we provide an example based on the current prevalence of food-borne illnesses from all foods in developed countries, which is around 1 per 10,000 meals. The risk of disease per meal is currently the same for all food-borne infections, however. As a result, for the sake of example, we will use the assumption that the ID50 for sickness from NoV is 1 million infectious particles and that the risk of illness from NoV is 1 per 1 million servings of bivalve shellfish. This ALOP translates to: If all shellfish were evenly polluted.

$$Ho - \Sigma R + \Sigma I \leq -2.10 \log 10 \text{ g}$$

Beginning Raw Material Levels Controlled

The most crucial control method is lowering the initial norovirus concentrations in shellfish, and this may be done by raising shellfish in clean water. The harvesting of shellfish is often halted if levels of pathogenic enteric bacteria, such as Salmonella, microbiological markers of faecal contamination, or pathogenic Vibrio, are higher than predetermined thresholds in the locations where it is being done. Nevertheless, due to the weak association between bacterial indicators and NoV and HAV, monitoring for viral contamination may not be managed by this activity. This also illustrates how even tertiary wastewater treatment has little to no impact on viral populations. Although it is technically possible to keep an eye out for viral genomes in ambient waters or shellfish, this preventative approach has not yet gained much traction. By looking through the records of contamination, it is possible to pinpoint times

when there is a higher danger of viral infection. After such occurrences, cultivated areas may be monitored more closely in order to avoid harvesting during such high risk times or following unintentional contamination from unexpected sources. With the advancement of new environmental sensing technology and testing procedures, this is becoming more and more practical from an economic and technological standpoint.

After Harvest, Avoid Contamination, and before Cooking

To stop the spread of the norovirus, proper personal hygiene habits are crucial. The best strategy to lessen norovirus infection on hands is to wash them well with soap and running water for at least 20 seconds. Excluding infected individuals from the food chain is advised for a period of time during and after active signs of an infection is considered to be a practical way to stop virus transmission in light of the highly contagious nature of NoV and HAV and the significance of person-to-person transmission in their epidemiology. Nevertheless, the efficacy of this control may be restricted owing to shedding of the virus before and after overt symptoms and the likelihood of asymptomatic carriers.

Lowering NoV Levels by HHP

Infectious virus titers can be reduced by up to 1000-fold using commercial high pressure processing at 275–300 MPa of pressure for 3 min. Thus, Ho values of ≤ 0.90 log10 cooking is an important post-harvest step to reduce virus levels in molluscs and thus reduce the risk of foodborne infection, even though such treatments may not guarantee total inactiva- tion of viruses. Using HAV as an example, cooking at low temperatures until the shell opens has been estimated to produce a 99.46% reduction in HAV titers, whereas cooking to an internal temperature of 90 °C for 90 s reduces HAV levels by 99.86%. Thus, Ho values of ≤ 0.48 log10 genome copiesg) would be expected to be reduced to ≤ −1.79 log10 genome copiesg and current limit of quantitation for NoV from oysters is 100 RNA genome copiesabsence sam- pling affects the results due to the potential for overestimating risk with pooled samples and the potential for false negative results when individual oyster testing. The presence of infectious vs. non- infectious viral particles also needs to be considered when setting microbiological criteria.

HACCP Plans

The NSSP-guide to the control of molluscan shellfish describes the measures that are currently recommended to ensure safe production of shellfish.

Programs GHP

Proposed Codex guidelines on ‘The Application of General Principles of Food Hygiene to the Control of Viruses in Food’ provide advice to governments for the control of human enteric viruses in food, especially NoV and HAV, with a view towards protecting the health of consumers and ensuring fair practises in food trade. The guideline also contains an annex on specific measures for the control of HAV and NoV in bivalve molluscs.

Regulatory Requirements and Criteria

There is presently no internationally harmonised approach to shellfish testing for human viruses. While long recognised that the use of bacterial indicator microorganisms is a poor predictor of contamination of bivalve molluscs with human viruses, these indicator bacteria continue to be the primary means for assessing growing waters and shellfish. For example, in the U.S. the standards for molluscs are centred on the detection of coliforms and Escherichia coli in the shellfish growing waters.

CHAPTER 16

CAMPYLOBACTER IN CHICKEN MEAT

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The worldwide commerce in chicken flesh is enormous. 10.8 million tons of goods were exported and 8.9 million tons were imported globally in 2016. With 4.1 million tons exported annually in 2016, Brazil was the world's biggest producer of chicken meat. Beginning with the hatchery where chicks are raised, chicken meat is produced. They are placed in chicken farms, where they are raised to the necessary weight. Organic farms dedicated to "free range raising" and intensive, contained chicken houses where chicks are ready for slaughter at around 5-7 weeks of age are both used to produce chickens. With line processing rates surpassing 175 birds per minute, the subsequent processing phases of slaughter, dressing, and chilling are highly automated. Distribution and retail sales conducted under chilled or frozen conditions come next. There are several chances for pathogenic germs to infect chickens and chicken meat throughout primary production and processing. The main pathogens to be concerned about are Salmonella and Campylobacter, with the latter being the subject of this article.

In many nations, thermotolerant Campylobacter is the most common bacterial gastrointestinal illness in humans. The environment, water, interaction with animals, and food are all potential sources of infection. Between 42% and 80% of instances are thought to be caused by diet, according to estimates. It's been a while since I've done this, but I think I've finally figured out how to do it. According to estimates, this infection pathway is responsible for 20–30% of human cases of campylobacteriosis in the European Union, while the chicken reservoir as a whole may be responsible for 50–80%.

This is meant to serve as an example of how the principles for setting a food safety objective, associated performance objectives, and where appropriate microbiological criteria can be applied towards the control of Campylobacter contamination of raw chicken meat throughout production and processing as well as preparation and consumption in the home. This is not meant to be a thorough analysis of the information that is currently accessible or earlier research in the field. To put the issue in perspective and illustrate the processes required for applying these risk management measures to the danger presented by Campylobacter on raw chicken, however, adequate information will be supplied.

Campylobacter are spiral-shaped, gram-negative bacteria that do not create spores. They move by means of a polar flagellum in a corkscrew-like manner. Rather of fermenting or oxidizing carbohydrates, campylobacter uses meolism of amino acids or intermediates produced by the tricarboxylic acid cycle to provide energy. The genus has around 20 species, however there is still disagreement about this in the literature.

The danger of infection in poultry is greater for thermophilic species. The optimal growth temperature for thermophilic Campylobacter is 41.5 °C, and it thrives between 37 °C and 45 °C. It is too cold for them to grow. Moisture-rich settings are ideal for thermophilic Campylobacter development, which peaks at a water activity of 0.997 with a lower limit of 0.987. They do not grow beyond pH 9.0 or below pH 4.9, and their ideal pH range is between

pH 6.5 and 7.5. They are categorized as microaerophilic and sensitive to oxygen and oxidizing radicals; they thrive in an atmosphere with 10% carbon dioxide and 5-6% oxygen.

Since 2005, campylobacter has dominated the list of bacterial causes of gastroenteritis in Europe. The incidence rate for confirmed cases in 2014 was 71 cases for per 100,000 people. Based on FOODNET data from 10 U.S. locations, the incidence rate for Campylobacter in the United States in 2013 was 13.8 cases per 100,000 people. In Australia, there were 113 incidences of Campylobacter infection per 100,000 people in 2010. In 2010, New Zealand recorded a 168.2 percent Campylobacter notification rate. Nonetheless, under-reporting rates of between 10 and 100 times have been proposed, indicating that the population's burden of sickness is far higher than these data imply. Wild and domesticated birds and animals are the primary hosts of the Campylobacter bacteria. Cattle, pigs, poultry, and sheep are the major sources of food animals. For up to three months, Campylobacter may live in wet areas and water sources. These species are most suited for colonization and development in the chicken gastrointestinal system since the usual body temperature of hens is 40.5 °C to 42.5 °C. Infections related with the intake of chicken are caused largely by the species C. jejuni, followed by C. coli and, to a considerably lesser amount, C. lari.

Hazard Identification

Illness Signs and Symptoms

Human campylobacteriosis is caused by campylobacter and is the most common bacterial cause of gastrointestinal sickness worldwide. Nowadays, C. jejuni and C. coli are the most common germs that cause campylobacteriosis in people. Human enteritis is brought on by the C. jejuni infection. Common symptoms include watery or bloody diarrhea, fever, cramping in the abdomen, and nausea. The incubation phase may last from 1 to 10 days, although it usually lasts between 2 and 5 days. Symptoms often last 1 day to 1 week or longer. The bacterium is usually self-limiting and excretes in the faeces for 2 to 3 weeks on average. The assault rate may go up to 45%. Similar to C. jejuni enteritis is C. coli enteritis.

Systemic infections including bacteremia and post-infectious consequences like Guillain-Barré Syndrome are also linked to campylobacter jejuni. GBS is an acute demyelinating illness of the peripheral nerve system that affects 1-2 people per 100,000 people in the United States each year. It is very uncommon, having an incidence of 1 per 1000 infections. After contracting certain Campylobacter serotypes, the chance of acquiring GBS rises. GBS may develop one to three weeks after gastroenteritis, and 20% of those who have it end up disabled and 5% die away.

Cholecystitis, pancreatitis, peritonitis, and severe gastrointestinal bleeding are examples of local consequences of Campylobacter infections that may develop as a result of direct dissemination from the gastrointestinal tract. Rarely, Campylobacter infections may cause meningitis, endocarditis, septic arthritis, osteomyelitis, and newborn sepsis as extra-intestinal symptoms. Bacteremia is seen in just 1% of Campylobacter enteritis patients, and it is more common in immunocompromised patients, children, and young people in their 20s, with a greater frequency in men. Campylobacter has also been linked to Reiter's disease, which occurs in around 1% of all campylobacteriosis cases, while other bacteria that cause foodborne illness may also have a similar impact.

Antimicrobial Resistance

According to several investigations on the antimicrobial resistance of C. jejuni and C. coli species, the most isolates are resistant to ciprofloxacin, tetracycline, nalidixic acid, amoxicillin, and colistin. Smaller percentages of isolates exhibited resistance to gentamicin but not to streptomycin, neomycin, erythromycin, chloramphenicol, or ampicillin. Other

research, however, has shown only little gentamicin resistance. Certain isolates have the ability to withstand two or more different antibiotics.

Threat Factors

Important virulence factors in campylobacteriosis include flagella-mediated motility, which is linked to adherence, invasion, and colonization, adherence to intestinal epithelial cells, invasion, and survival in the host cells, as well as the ability to produce toxins, particularly cytolethal distending toxins.

Models of Dose-Response

The conditional likelihood of eating a dosage of Campylobacter, the conditional chance that these organisms will survive transit through the stomach, and the conditional probability that sickness will manifest after a person is infected all contribute to the probability of campylobacteriosis. The virulence and survival characteristics of the bacterium influence the spread of infection. The likelihood of infection and disease is also affected by host characteristics, such as immune system function, general health, and stomach contents.

Salmonella in Chicken Meat

The FAOWHO 2009 has a fantastic debate on Campylobacter dose-response. Here, the topic will only be briefly explained and is based on this knowledge. Dose response modeling is the use of a mathematical formula to determine the likelihood that a person will get infected after ingesting a certain amount of harmful germs. The underlying information may come from epidemiological investigations, or more often, feeding tests on animals or, ideally, people. The attack rate and ingested dosage must be provided if epidemiological data are to be utilized for dose response modeling. As determining the infectious dosage is not the main goal of epidemiological research, the latter is seldom compiled. While these data do not permit modeling, a dose-response connection between intake of liver pâté and infection with Campylobacter from a U.K. epidemic has been described. Data from feeding trials may also be limited since the provided dosages are often excessive compared to the actual number of organisms consumed via food. Also, participants for feeding trials are only allowed to be healthy people and are not always representative of the general population. Also, it is challenging for such trials to duplicate the variety of pathogenic organisms that normally make up the pathogen population.

For Campylobacter, only feeding experiment data are provided and these data are from a single study. Two strains of C. jejuni that have been linked to human sickness were employed, and healthy volunteers in the USA were given milk containing the strains. In order to represent this data, it is necessary to make one of two assumptions: either that infection cannot occur below a certain dosage, or, alternatively, that each Campylobacter cell has a chance of inflicting sickness, and that chance grows with the number of germs present. The Campylobacter dose-response relationship has been described using the latter model type.

By using a Beta-Poisson model, the FAO

N is the ingested dosage, and = 59.95 and = 0.21 are the dose-response parameters. Since there was no obvious connection between the feeding trail data and the model, it was not possible to determine the likelihood of disease at a particular dosage. Of of the 89 persons who were infected, 29 people developed illnesses, according to the combined statistics. According to reports, cattle constitute the main non-poultry reservoir. Early in the manufacturing cycle, contamination of hens is caused by Campylobacter entering from the environment. Once present, Campylobacter is shed in the feces of birds, which colonizes the flock within a few days. As a result, efficient agricultural biosecurity is crucial. In addition to

colonization of other species, Campylobacter has been discovered in or on puddles, water supplies, and agricultural machinery outside of the chicken buildings. Campylobacter has been discovered in drinkers, anteroom surfaces, and the sheds between production cycles in chicken sheds. The entry of Campylobacter into chicken buildings may also be significantly influenced by pests; flies in particular have been noted as a problem throughout the spring and summer. The trucks, catchers, and in particular the boxes and modules used to carry the chickens to the slaughterhouse are sources of Campylobacter as well as the equipment used for catching and transporting. In this regard, it has been determined that the technique of thinning, which entails the partial depopulation of flocks, poses a specific danger for the spread of Campylobacter into chicken houses. Age of chicken house, rodent control, age of chicken at introduction of whole wheat in the chicken feed, age of chicken at mass murder, storage of whole wheat, number of chimneys upon that chicken house, having one vs. more chicken houses on the farm, and location of the chicken farm in relation to cattle density" were listed in order of importance in an analysis of the relative importance of primary production factors that affect Campylobacter infection of flocks.

The majority of chicken flocks are found to be Campylobacter-colonized at the time of slaughter, according to data gathered from several research conducted in a variety of nations. For instance, a research conducted in the USA in 2001 discovered that 28 out of 32 flocks tested positive for Campylobacter. In 789 batches of chicken from 214 farms, a UK research indicated an average 35% incidence of Campylobacter in flocks that had not been reduced. Another UK investigation on 1174 mixed batches of thinned and unthinned chicken delivered for slaughter found an average frequency of 79.2%. In Japan, 67 of 142 flocks were found to have Campylobacter infections. Comparable levels of Campylobacter were detected in French chicken flocks at the time of slaughter (77.2% with an average value of 8.04 log cfug). EFSA carried out a thorough baseline investigation on Campylobacter in chickens in Europe in 2008. A frequency of 72.1% was discovered after sampling 10,132 chicken batch samples from 561 slaughterhouses across 26 countries in the EU.

Research on Campylobacter contamination of carcasses during chicken slaughter have uncovered a number of crucial procedures. A systematic review by Guerin et al. examined the findings of 32 studies that were published in the scholarly literature. They discovered that Campylobacter prevalence and concentration on corpses decreased during the "scalding" step, in which feathers are released by submerging deceased birds in hot water. The prevalence decreased more dramatically when the scald tank was heated to 58 °C as opposed to 55.4 °C. A quantitative through-chain study was carried out by Duffy et al. on 4 flocks that were processed at 2 slaughterhouses in Australia. The scorching stage, they discovered. Yet, 4 poultry slaughterhouses in the USA have found Campylobacter in the chilloutside bird washers on naturally contaminated bird corpses. While tests revealed that the primary decrease was obtained after the first washing, each facility employed between 2 and 3 washers sequentially. Reductions in mean Campylobacter concentrations varied between 0.26 and 0.63 log10 cfucarcass and an acidified sodium chlorite spray led in a decrease in Campylobacter of 1.26 log10 cfuWHO 2008). According to Guerin et AL assessment's for emersion cooled carcasses with water temperatures including free available chlorine levels of 4.2 °C 3.5 ppm, 6.9 °C 1 ppm, 4.4 °C 2.7 ppm, and 7.0 °C 1.0 ppm, the reduction in Campylobacter levels varied between 0.8 and 1.7 log10 cfucarcass. Campylobacter inactivation levels did not correlate with chlorine concentration or chill temperature.Just 2 experiments had pre- and post-air chilling decreases that varied between 0.2 log10 cfug, according to their findings.

An extensive international study shows the variation in Campylobacter contamination on chicken at the point of slaughter. Significant differences were detected across EU member

states in a baseline assessment of Campylobacter levels on chicken skin at the conclusion of slaughter. The proportion of samples with skin in them for the entire EU ranges from 10 to 99, 1000 to 10,000, and more. As opposed to this, in a different research, Campylobacter inoculation onto chicken carcasses decreased by 1.88 and 2.33 log10 cfucm2 after 7 days and by 1.07 log10 cfu1000 g chicken with an average reduction throughout the five flocks of 1.77 log10 cfu1000 g chicken. In conclusion, it would seem that any reduction in Campylobacter during cool storage in the retail distribution chain is incompatible with the disparities in findings between chicken meat that has been inoculated and chicken meat that has been naturally infected. Packaging gas type also affects reductions, with greater reductions occurring in oxygen-rich environments. It indicates that frozen storage reduces the amount of Campylobacter found in chicken flesh. An extensive nationwide research may be used to examine the diversity in Campylobacter contamination on chicken towards the end of retail. In the UK, the Campylobacter infection on whole chicken skin at retail was observed for a whole year. This investigation looked at the levels of Campylobacter on 4011 chickens across all retail locations between February 2014 and March 2015. Examination of the raw data2 indicated a mean log-concentration of 1.83 log10 cfug). This indicates the wide range of Campylobacter concentrations seen in chicken sold at retail levels, with 5.5% of samples having concentrations below the WHO's recommended guideline of 1 cfu in 2009. Hence, the FSO may be determined by rearrangement.

FSO equals log

Based on the total number of people who became ill given the total number of people infected across all trials, the average probability of illness given infection can be calculated as 0.325843. The values of beta and alpha, which are the dose response parameters for Campylobacter, are 59.95 and 0.21, respectively. In other words, around 1 out of every 3 people who eat a Campylobacter cell will get unwell. The result of Pill and Pinf is the likelihood of sickness given infection, which is 0.00113, implying that, typically, 1 in 885 persons who ingest a single Campylobacter cell will acquire campylobacteriosis.

An FSO of - 4.15 log10 cfug) cooked chicken meat as eaten is obtained by substituting the values for the number of servings per year, the mass of a serving in grams, the likelihood of sickness given consumption of a single Campylobacter cell, and the goal ALOP.

Calculating a Public Health Objective

In order to enhance public health, public health agencies from all over the globe work to lower the incidence of foodborne diseases among their respective populations. In this context, they often describe a public health objective in qualitative terms, such as lowering the incidence of foodborne disease. Yet, a risk management strategy that can achieve a numerical public health objective is made possible by the risk management metrics embraced globally. The public health objective that will be used here is a 50% decrease in the ALOP for illustration purposes. So, it is necessary to lower the maximum number of campylobacteriosis cases per million people to 424. The poultry production business is required to satisfy an FSO of - 4.45 log10 cfug) cooked chicken meat as eaten by following the procedures outlined in Sect. 19.3.2 and switching the ALOP with the new public health aim.Control Techniques to Reach a Public Health ObjectiveSeveral control strategies have been proposed, as mentioned. It is evident that the technique of thinning violates biosecurity and makes Campylobacter more common on chicken at the time of slaughter.

Three crucial procedures at the moment of slaughter stand out as possible sites of control for the decrease of Campylobacter on chicken carcasses. Before to being eviscerated, the chickens are subjected to a scalding process that decreases the number of organisms on them;

the magnitude of the reduction depends on the temperature of the tanks. If antimicrobials are applied, the washing process may provide even further decreases. Last but not least, chilling via submersion in water that contains antimicrobials will decrease the amount of Campylobacter on the chilled carcasses. While cool distribution temperatures and periods in air appear to have little effect, chilling may aid to minimize the amount of Campylobacter on carcasses during retail distribution and storage.

The consumer's thorough cooking is a crucial control strategy for Campylobacter in fresh chicken meat. Unfortunately, there is a lot of variation in this control measure, and cross contamination from handling and preparing raw meat in the home kitchen can negate the control that cooking exerts, either by transferring Campylobacter to the cooked meat or by transferring Campylobacter to other ready-to-eat foods.Setting Performance Goals across the Chicken Production Chain to Take Control Measures into Consideration Performance goals are benchmarks at certain points in the food supply chain where food companies may make sure their processing control systems are adequate to achieve the FSO. The food industry may use any number and combination of control methods as long as the PO is met, making them adaptable. Hence, POs may encourage innovation in process control while also enhancing public health.

The public health target FSO of 4.45 log10 chug decrease in Campylobacter would be reached throughout the meat is shown in examples in the next sections. Risk managers may establish POs at various stages in the chicken production chain to achieve this goal. As there is no development of Campylobacter in the consumer's house prior to cooking, I is minimal and should be disregarded. The maximum concentration of Campylobacter that may be tolerated on raw chicken as it enters the consumer's house, H0-c, can be calculated using, and would still make it easier to accomplish the public health target FSO.

H0c FSO R 4.45 + 7 2.55 log10 cfug. The public health goal FSO of 4.45 log10 cfu) would be met at this level, where: C = log increase in concentration due to cross contamination; R = log reduction due to cooking; Tr = transfer rate of Campylobacter added towards the food by that of the cross contamination event; p = proportion of cross contamination events happening in the population of all preparation events.It is important to alter the basic ICMSF equation as given in order to compute the net impact of the cooking and cross-contamination stage.

$$H0{-}c - \sum R + \sum C \leq FSO$$

The extent of Campylobacter contamination transferred from the raw chicken to the cooked chicken through the use of the chopping board and knife using the transfer rate described by Luber et al. shows the effects of various starting log concentrations of Campylobacter on raw chicken in the example illustrated in this section within which cooked chicken is cross-contaminated after cooking by bacteria originating upon that raw chicken.

FSO calculation in several cross contamination situations (a, b)

Fixed price

$$\sum R \ 7.00 \ Tr \ 0.001133$$

Cross Contamination Prevalence

H0-c. By clearly outlining safe handling recommendations on the package of raw chicken, food firms may help customers handle the food more effectively. As chicken is a recognized source of Campylobacter that may spread around the kitchen, this should have a warning not to wash it. With national education efforts, the government may also influence consumer behavior to improve when it comes to handling and preparing chicken at home. To cope with

a high concentration of Campylobacter on the raw chicken, it is doubtful that education and labeling alone could reach the low incidence of cross-contamination required.

Making sure customers don't come into touch with raw chicken is one approach to get a very low cross-contamination rate in the house. This may be accomplished by food companies wrapping chicken in heat-resistant packaging that allows the chicken to be cooked within the pack without handling. There is equipment available to accomplish this with complete chicken carcasses, and several industries in Europe are doing it. If prescribed cooking guidelines are followed, this control measure would result in a very low prevalence rate of cross-contamination, which would mean that the capacity of the cooking route to lower the amount of Campylobacter on the raw chicken would meet the public health aim FSO. The PO at the conclusion of retail distribution with this packaging approach might be as high as 2.55 log10 cfug.

Using a reliable microbiological criteria, batches of chicken may be confirmed to be in compliance with the POr. Nevertheless, testing for Campylobacter on chicken is normally done by measuring the numbers on the surface of the carcass. Either a complete carcass rinse approach or a 25 g mass of chicken neck skin taken for microbiological testing are used to accomplish this. Instead of per milliliter of carcass rinse or per gram of skin, the PO number refers to the concentration per gram of chicken. As a result, in order to operationalize the PO, the PO concentration must be converted to a concentration per milliliter of carcass rinse or per gram of skin. 988 g of flesh and skin comprise a 1.5 kilogram whole chicken. Hence if all of the Campylobacter are assumed to be made available on the skin then a chicken meeting the POr of 1.495 log10 cfuml carcass rinsate or 2.56 log10 cfuml carcass rinsate, the batch mean would need to be 1.092 log10 cfug chicken, therefore 988 g of meat and skin would contain 30,886 Campylobacter cfu. If everything is on the skin, log 10.

In contrast, from the point of slaughter to the consumer's house, Campylobacter counts are reduced through frozen retail storage and distribution channels. The severity of the effect described in reports on the impact of freezing on Campylobacter varies, and it also seems that the source of the bacteria—natural contamination or injected laboratory strains—has an impact on the outcome. The research conducted by Georgsson et al. offers a sound foundation for quantifying the impact of freezing on Campylobacter. According to their findings, hens from five flocks were frozen for 31 days at -20 °C, resulting in log-reductions of 0.65 to 2.87 on average across the five flocks. Using this intervention, the slaughter plant's raw chicken may enter the retail frozen distribution chain with a greater concentration of Campylobacter while still meeting the POr requirements at the conclusion of retail distribution. The ICMSF equation may be used to compute the acceple POs, POs = H0fd POr R 2.49 + 1.77 4.26 log10 cfu ml of Campylobacter on chicken at the point of slaughter intended for sale in frozen form at retail; POr = Performance Objective at the point of retail; and R = the total of log reductions as a result of distribution in frozen form. A POs may be established for raw chicken leaving the slaughter plant, which is identical to the H0-fd estimated, if frozen distribution at retail is selected as an intervention to enable the raw chicken to meet the POr at the conclusion of retail distribution. This means that for a batch of raw chickens that fulfills the POs at the conclusion of slaughter for chicken intended for frozen retail storage and distribution, the batch mean would have to be 2.86 log10 cfuml.

A possible risk management strategy by the retailer, working with the slaughterhouse, could be to test batches of chicken at the end of slaughter using the MCs suggested here. If they don't meet the POs for chill distribution, they are frozen and diverted to frozen distribution—providing they meet the POs for frozen distribution—if there is consumer demand for both

chilled and frozen chicken. This would depend on whether it was possible to store carcasses while awaiting the results of microbiological testing.

Control Procedures during Processing and Slaughter

It has looked on how various slaughter and processing interventions affect the amount of Campylobacter in chicken. The total of log-increases and log-reductions in Campylobacter based on a series of processing stages done, as well as controls on the level of Campylobacter on the birds received from the farm prior to the scalding phase in ICMSF notation, may all be utilized in combination with each other. Depending on the method of slaughter used and the retail distribution chain chosen, Defeatheringg (or ml) for birds before scalding for chicken carcasses preceding slaughter.