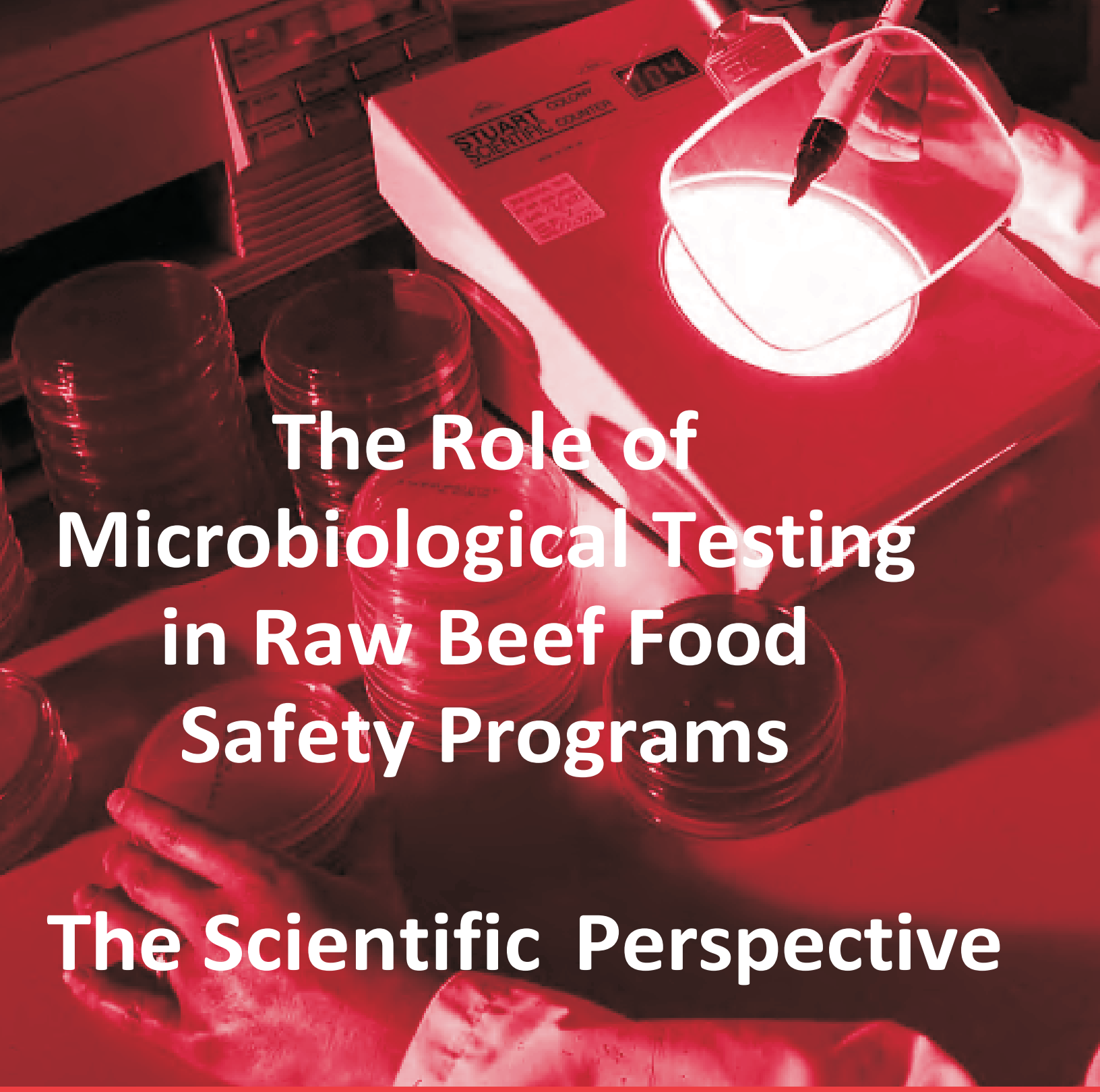

The Role of Microbiological Testing in Raw Beef Food Safety Programs

The Scientific Perspective

The American Meat Science Association

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Foreword

In 1999, the AMSA Board organized a symposium and invited over 35 experts from academia, government, and the meat industry to discuss the role of microbiological testing in a beef food safety program from the scientific perspective. The goal of the symposium was to document the science behind the sampling process and to present clear recommendations for the development and evaluation of testing programs.

The current document is the result of an effort initiated by AMSA and BIFSCo to identify and charge a committee of experts with various backgrounds and experiences to review the symposium document and update/add to it as necessary to make it current. The goal for the updated document was to provide a current scientific perspective on the role of microbiological testing in raw beef food safety systems to supplement other documentation already available through other sources such as BIFSCo best practices documents that also were recently updated.

The concept that you cannot "test your way to 100% safe food" is almost universally accepted today. This is at least partly because pathogens in raw beef occur sporadically and at low concentrations and are not uniformly distributed, so short of testing 100% of the product, you cannot guarantee no pathogens are present. This does not mean that microbiological testing is not an important part of a food safety system. The decision to conduct microbiological testing as part of a company's food safety system will be based on a variety of factors. This document provides the scientific basis as a part of that decision process. Microbiological testing is but one component of a food safety system and should not be viewed as a stand-alone approach to food safety. The relative value of microbiological testing for pathogens should always be re-evaluated in light of changes in legislative, legal and public relations concerns as well as advancements in science such as improved detection technologies. Much progress has been made regarding effective food safety systems for beef and how to use microbiological testing. It is imperative to understand the purpose of all testing, how to interpret the results, and how to react to that interpretation. This document is intended to complement other resources on implementing food safety systems.

1.0 Introduction

The role of microbiological sampling in commercial beef production has evolved as the industry has collaborated to share research and learning. This open sharing between companies, researchers and academia has been the foundation of continual improvement. The philosophy of microbial testing in the beef supply chain is based on the fundamental principle of taking all microbiological data generated by the system and applying process change. Commercial facilities must listen and react to all the data being generated from their production facility in order to understand if their respective process is in control.

The science of microbiological sampling has evolved within sampling plans and analysis platforms. There are three pillars that are paramount to success. The sampling plan must be statistically based, must target where microorganism will be present and laboratory analysis must have both sensitivity and specificity to ensure reliable detection even if the microorganism is there in very small numbers. Using these core principles, the beef industry has continued to drive continual improvement in process hygiene and intervention. This has led to the continued improvement in the safety of beef products and positively impacted public health.

The philosophy of assessing the entire system based on data generated by the Food Safety System has led the industry to improvements in pre-harvest treatments, hygienic harvest, scientifically validated process interventions, chilling, sanitation and processing. These data range from microbial mapping during harvest, post-harvest carcass data, finished product pathogen analysis and Food Safety System verification.

It is a proven fact that guaranteed safety cannot be 'tested' into a process. It is also a proven fact that random uncontrolled testing does not provide meaningful system feedback nor does it improve the safety of products. Utilizing our historical knowledge we must design appropriate sampling plans for the identified hazard and react to all the data generated.

2.0 Food Safety Systems

The system approach is imperative in order to effectively reduce risk of microbiological contamination in beef processing. A Food Safety System, as the term applies, is the process of taking individual food safety actions and incorporating them into a larger comprehensive system that can achieve food safety objectives. In general terms, the Food Safety System should incorporate four Major Impact Areas: 1) microbiological preventive practices, 2) mechanical interventions designed to effectively apply antimicrobial treatments, and 3) microbiological testing to demonstrate validation and verification of the system, and 4) data analysis, trending and reporting. If any one of the four Major Impact Areas of the Food Safety System are not applied, the entire system may be at risk. Although all four Major Impact Areas are of equal importance, the remainder of this section will focus on the microbiological analysis within the Food Safety System.

An effective Food Safety System must include multiple steps that when looked at collectively, have the ability to prevent and reduce microbiological contamination to below detectable levels for pathogens and within process control levels for indicator bacteria, e.g., Aerobic Plate Counts or *Enterobacteriaceae* counts. When looking at microbiological sampling within a Food Safety System, both environment sampling and product sampling should be evaluated. Although the role of testing is different between product and environmental sampling, each have their place in the Food Safety System. Regardless of the process or products, the role of microbial analysis in a Food Safety System should focus on validation and verification of the system. Although microbial testing can play a duplicate role in 1) lot acceptance/rejection and 2) verification and validation, the latter of the two incorporated into a Food Safety System will prove to be the most effective in ultimately controlling microbiological contamination and protecting public health.

Different areas of the industry will incorporate microbial testing in different ways. For example, beef slaughter and further processors in a raw, non-cooked process, should perform environmental microbiological analysis using indicator bacteria. Microbial sampling of the environmental should be designed primarily to 1) verify sanitation and 2) evaluate general bacterial activity of product contact surfaces. Product lot sampling and microbial analysis at the end of the process (finished product sampling) will then include analyzing for the presence of pathogens to verify the microbial prevention and intervention process was effectively implemented, and if necessary, divert contaminated products lots to a process with full lethality. However, in a ready-to-eat environment, the microbial sampling takes on an additional role of verification of pathogen absence in the environment as well as in the finished product lots.

It is important to note that when pathogenic microbiological sampling within a Food Safety System occurs, it must be known what the results of the individual sample represents. When product is sampled, all microbiological independent lots must be controlled until a negative result is received.

3.0 Sampling

The sampling plan used is critical to the success of microbiological testing. The definition of lots and lot size are also considerations in developing a sampling plan. Before defining the sampling plan, a rationale for defining lots must be implemented. The lot definition strategy must consider all testing needs as well as the practicality of holding products until pathogen test results are available. Generally, lot sizes should be as small as can be justified. For example, a lot can be a quantity of production defined by cleanup to cleanup or can be broken into smaller units defined by time (1 hour of ground beef production) or quantity (a 2000 lb combo of trimmings), etc. The product within a lot should be as substantially identical as possible, *i.e.,* homogeneous, made from the same ingredients, by the same process continuously on the same production line. A lot may be a single batch or multiple batches made by a continuous batch process or product made with a continuous process over a period of time. There should

be a scientific basis to support the microbial independence of the lots.

Samples taken from the lot must be representative of the lot. The manner of sampling as well as the number and size of samples are considerations in determining a sampling plan that is representative of the lot. An additional consideration is the purpose of the sampling. For any given number of samples, a smaller sample size may be suitable for testing for microbiological indicators such as aerobic plate count, or coliform count. However, a larger sample size is desirable when testing for microbial pathogens such as *E. coli* O157:H7 that may be present in low numbers. Statistics of population probabilities based on the expected incidence of the organism(s) of interest should be used to determine the number and size of the samples. It is likely that pathogens will be present in low numbers and be non-randomly distributed and this makes it difficult to use statistical methods that require a normal distribution. However, larger numbers of samples and larger sample sizes proportionately increase the probability of detecting pathogens within a lot. Generally, pathogen sampling plans are designed with the largest number of samples per lot and the largest sample size that is practical to implement.

Sampling should be designed to enhance the detection of organisms of interest. For example, when testing pathogens in beef trimmings, the sample should target pieces that came from what was once the carcass surface because the surface is the likely site of contamination. Sampling for statistical process control should focus on obtaining samples that will be representative of the whole process on an ongoing basis. Sampling for sanitation verification should focus on locations difficult to clean and those most likely to harbor organisms that could contaminate product. Detailed information regarding statistical concepts of population probabilities and sampling, choice of sampling procedures, decision criteria, and practical applications in food microbiology can be found in the book Microorganisms in Foods 8, authored by the International Commission on Microbiological Specifications for Foods (ICMSF, 2011. Springer. ISBN 978-1-4419-9374-8). Implementation guidance can be found in Guidance Document for Lotting and Sampling of Beef Products for Pathogen Analysis (BIFSCo, 2016).

When testing for STECs, or any other pathogen for which there is no tolerance for a positive result, the product from sampled lots must remain under positive control so that product disposition can be handled based on test results. Also, it is important to maintain the integrity of lots. For example, for raw meats one should avoid adding rework from a previous lot(s) to subsequent lots as this practice can undermine the independence of a lot and expand the amount of product affected by a positive test result.

4.0 Microbiological Components and Analytical Methods

Management of microbiological food safety is largely based on knowledge of products and good design of procedures. Finished product testing may be considered as a control measure at the end of the production process, however testing gives only very limited information on the safety status of a food. If a hazardous organism or pathogen is present then actions must be taken, but the absence of a positive test in a limited number of samples is no guarantee of

safety of a whole production lot. Processors need to establish microbiological criteria for their products that adequately address their needs for safety and hygiene. Multiple testing methodologies are available for use to help reach these goals.

Criteria that consider the microbiological components of beef should address those organisms widely accepted as relevant as pathogens (such as *Salmonella*, *E. coli* O157:H7), indicator organisms (such as Generic *E. coli*, *Enterobacteriaceae*) or spoilage organisms (such as *Lactobacillus* genus). Microbiological components of beef may include bacteria, viruses, yeasts, molds, parasitic protozoa and helminths and their toxins or metabolites. Organisms whose significance in beef is doubtful should not be included in a criterion. Where pathogens can be detected directly and reliably, consideration should be given to testing for them in preference to testing for indicator organisms. If a test for an indicator organism is applied, there should be a clear rationale (for instance by setting limits for satisfactory, acceptable, and unsatisfactory) to use the test results to indicate unsatisfactory hygienic practices or a health hazard. Suitable indicator organisms such as aerobic plate count (APC) or total viable count (TVC) bacteria, *Enterobacteriaceae* (EB), *E. coli* (generic or Biotype 1) and coliforms are those whose presence indicates the likelihood that faulty manufacturing practices, or failure of control processes, occurred and adversely affected the safety or quality of the product. It might also show a higher risk for the presence of pathogens or toxins. The significance of indicator organisms as food contaminants can be understood only by having a thorough knowledge of the microflora of the ingredients, the usual source or reservoir of the indicator, the production environment and the process, and by recognizing that the point of sampling may influence the meaning and/or the validity of the results.

The types of testing generally fall into two categories: (i) specific detection tests for particular organisms or toxins of interest (usually pathogens) and (ii) non-specific tests that enumerate large populations of bacteria (indicator bacteria). Specific tests usually target the organism of interest using (i) molecular methods that detect unique genetic elements or the organism, or (ii) target the organism in a physical assay such as an antibody-based assay. Most specific tests are rapid, providing results within 12 hours or less. Non-specific tests for population of bacteria like APC/TVC, EB, *E. coli* and coliforms are usually based on directly growing the target population on a plate and counting the number of colony forming units (CFU) present. These tests require 24-48 h for proper growth of the group of organisms.

Testing may be applied to samples throughout the beef chain from animals and holding pens, through harvest steps to finished products. By far the most common sample type is that of finished beef products, boneless beef trimmings in particular. When samples are collected for testing, how the results of the microbiological test on the specific sample type will be used need to be known beforehand. For instance, beef trim is sampled to ensure satisfactory hygienic practices, that a health hazard is not present and to meet customer requirements for COA, while sponge surface samples may be collected from a carcass during processing to collect data on the efficacy of specific process treatments or intervention strategies. Sample collection must be well defined as to the method used (excision or swab samples for carcass testing, N60 or

cloth swab sampling for beef trim, swab for environmental monitoring) as well as the requirements for proper test performance and data analysis. Test portion weights or volumes, and sampling areas need to be defined and standardized for testing. Any testing method shall also be proven to perform adequately for the test portion weights or volumes used. Environmental testing also involves the collection of surface samples from equipment, typically post sanitation, and from food contact surfaces or areas that may harbor biofilm growth. Sample collection frequency should be suited for the specific needs of each user and can be statistically based. Process steps need to be carefully considered as to where environmental samples are best collected and the sort of testing to perform, non-specific colony count (APC/ TVC or *E. coli*) or specific pathogen (*L. monocytogenes* in a cold room vs. *E. coli* O157H7 from kill floor surfaces).

The presence of specific pathogens can be implied through various rapid molecular tests for molecular markers common to that pathogen or through antibody-based assays for particular bacterial epitopes/proteins, however, only culture isolation can definitively confirm a pathogen is present. If indicator organism levels are observed to increase over time indicating a potential process or manufacturing practice may be failing, then the potential for pathogen contamination is likewise increased. In absence of indicator bacteria counts, other molecular markers may also be enumerated and monitored over time to indicate potential system failures. Many rapid molecular screening assays identify multiple markers that must all be present to indicate the likely presence of a pathogen, but the presence of any subset of recurring markers (pathogen index targets) can be monitored to identify times at which processes may be trending towards unacceptable limits.

Microbiological testing is a valuable tool for verifying an establishment's process management system is under control. Testing must be supportable and fit for the intended purpose and the establishment is ultimately responsible to ensure the methodologies and practices followed by the laboratory meet the company's food safety needs. A test method that is "fit for purpose" implies it is appropriate for its intended purpose. For example (as described by FSIS) if an establishment is collecting beef trim samples for purposes of product disposition decisions and providing a Certificate of Assurance (COA) to downstream customers, it is important to consider whether the chosen method is equivalent to the FSIS MLG method. A rapid in-plant testing kit may meet AOAC or ISO rapid method certification standards, but if it does not ensure that the detection capabilities are at least as sensitive as the FSIS MLG method, screen testing of the product may not be considered sufficient for the intended use. In practice, the negative results may not meet a customer's purchase specifications or an agency regulatory requirement if the screening method was not "fit for purpose". Users of microbiological testing methods shall check with their method supplier for the support data available for a given application, the limitations of use, and the official method certification study report.

Establishments or end users of test results need to understand the results, how it was generated and the analysis that lead to the final result. They also need to consider lab operator education in order to guarantee that the method is properly implemented and final testing

results are reliable. The Establishment shall also develop their action plans for product disposition in case of positive testing results or on results of presumptive positive screening results in the absence of confirmation testing. Knowing this allows one to "listen to their data" recognizing where manufacturing practice, or process control failures are occurring. All test results or data need to be reviewed in a timely manner, if a test or its data becomes irrelevant and of no purpose, then that test should be discontinued. Further, data and test results should not be used in a way that is inconsistent with its intended use. A series of increased *E. coli* counts does not mean a pathogen is likely present, just as a series of negative pathogen tests do not mean *E. coli* or other indicator bacteria counts are within acceptable limits.

FSIS Foodborne Pathogen Test Kits Validated by Independent Organizations available at: *http://www.fsis.usda.gov/wps/wcm/connect/f97532f4-9c28-4ecc-9aee-0e1e6cde1a89/Validated-Test-Kit-Spreadsheet.pdf?MOD=AJPERES*

FSIS Microbiology Laboratory Guidebook of current protocols for analytical tests used by FSIS available at:
http://www.fsis.usda.gov/wps/portal/fsis/topics/science/laboratories-and-procedures/guidebooks-and-methods/microbiology-laboratory-guidebook/microbiology-laboratory-guidebook

5.0 Recommendations for Application of Microbiological Criteria

In September 1980, the National Marine Fisheries Service, the U.S. Department of Agriculture, the Food and Drug Administration, and the U.S. Army Natick Research and Development Center requested that the National Research Council (NRC) assemble a panel of experts to develop principles for the establishment of microbiological criteria for food. A report was prepared that provided detailed information on the application of microbiological criteria to 22 groups of food and food ingredients (NRC, 1985). At that point in time, microbiological criteria were not recommended for raw meats because such criteria would neither prevent spoilage nor foodborne illness. According to the committee, microorganisms of public health concern are often present in small numbers as part of the natural microflora of live animals, and current production and processing procedures cannot eliminate those microorganisms from raw meat. Therefore, it would be impractical to set limits for microbiological pathogens in raw meats as it would be impossible to comply consistently with the limits. Rather, the NRC committee stated that to reduce the health hazards from raw meats the following needs to prevail: (1) a recognition that low levels of pathogens may be present on raw meats, (2) strict adherence to good food preparation practices, (3) application of new processing procedures designed to reduce the presence of pathogens, (4) education on food handling practices, and (5) implementation of HACCP. Most of these recommendations have been addressed by the beef industry in the United States.

To define microbiological criteria three terms have been used. Microbiological criteria includes: (1) microbiological standards, (2) microbiological guidance, and (3) microbiological specifications (NRC 1985; Jean-Lous Cordier, 2004). A microbiological standard is defined as part of a law, regulation, or ordinance and as a result is a mandatory microbiological criterion. Microbiological standards are applied by the regulatory agencies at a point the food chain where it is expected to result in improved consumer protection from the microbiological hazard. A microbiological guideline is a criterion used to assess microbiological conditions during the food production and processing chain. This is used as an advisory criterion to provide information to food producers and processors on microbiological levels that can be achieved with optimal process controls and best practices. The microbiological specification is often used as a purchase specification between buyers and suppliers. The use of purchase specifications is most useful for buyers when controls for microbial contaminations are most effectively applied by the supplier.

The implementation of microbiological criteria for beef products has occurred since the review conducted by the NRC published in 1985. Microbiological criteria are being used within the beef industry by processors and regulatory agencies to improve public health, to improve sanitary processing conditions, and as a communication tool between buyers and sellers. The following are examples from the beef processing industry where microbiological criteria are being used. In addition, the discussion of these examples addresses why the beef industry is using microbiological criteria.

NRC (National Research Council). 1985. An Evaluation of the Role of Microbiological Criteria for Foods and Food Ingredients. Washington, D.C., National Academy Press.

Jean-Louis Cordier, 2004 Micrbiological criteria – Purpose and Limitations. Presented at the 36th Symposium of the Swiss Society of Food Hygiene, Zurich, 8 October 2003, Mitt. Lebensm. Hyg. 95: 28-31.

5.1 Supply Chain including Supplier Programs and Customer Requirements

Microbial criteria through the beef processing chain are not stand-alone entities to control foodborne pathogens. However, they are very important to verify that food safety programs are properly implemented and controlling foodborne pathogens. While many regulatory requirements exist for microbial testing through the beef chain to address the presence of indicator bacteria, *Salmonella,* STECs or other pathogens, additional requirements may be in place as required by the customer. Some suppliers have higher standards to open new markets or to provide additional documentation of the effectiveness of food safety programs. Testing protocols for products at any point in the supply chain before it is delivered to the customer should be contained in a comprehensive written testing program with details to address the actions taken if criteria are not met. Each segment of the supply chain will be discussed with

regard to testing programs and microbiological criteria.

Two types of testing will be discussed including the presence of indicator bacteria and the presence of pathogens. Both types of testing are beneficial under specified conditions. Testing for indicator bacteria such as Generic *E. coli*, Coliforms, and total Aerobic Plate Counts (APC) are useful to assess process control and testing for pathogens can be useful to make decisions on the use of the product. Testing for indicator bacteria has an advantage over pathogen testing in that it is usually possible to detect some amount of the indicators in the product while finding a low prevalence pathogen may require more testing and could be more costly. Even with the increased cost and testing requirements, under certain conditions, it is beneficial for pathogen testing to be conducted. It is important to note that pathogen testing must be conducted under well-designed testing programs and the absence of the pathogen does not indicate that all of the product is pathogen-free. Testing for indicators and pathogens will be discussed for each point in the supply chain.

5.11 Carcass Testing: Currently microbial testing of beef carcasses is focused on meeting regulatory requirements for indicator bacteria. Testing for indicators is done on a daily basis at specified intervals that are determined by the number of carcasses produced in the facility. In addition to the testing for indicators on a daily basis, *Salmonella* testing is also conducted by the FSIS. Within a given day, a "set" of samples will be taken from a carcass to verify process control.

Most carcasses produced in the US are transferred to fabrication within the same facility and therefore the entity that produced the carcasses would be the supplier to themselves to the fabrication area. There are few test and hold type programs for carcass production regardless of the testing done. There could be times in a facility when additional carcass testing is conducted such as when an intervention is being validated but at this time, testing beyond regulatory requirements is not commonly done.

5.12 Testing of Subprimals: Subprimals generated during fabrication are generally not subjected to extensive microbial testing. As with carcasses, some testing may occur in plant to validate interventions, but a comprehensive testing program for food safety is not commonly used. Some criteria could be implemented when subprimals are destined for export, especially when an extended shelf-life is desired. Testing for indicators such as APC, coliforms, generic *E. coli*, psychrophilic bacteria, and Enterococci could be conducted to inform the supplier of the potential shelf-life. The customer may also request the testing to ensure an adequate shelf-life of the product. There is an assumption made that subprimals are not intended for the production of ground beef in the scenario that excludes pathogen testing. Beef trimmings are generated during the production of subprimals and pathogen testing programs are focused on beef trimmings testing.

5.13 Testing of Beef Trimmings: Testing for verification of food safety programs is most common in beef trimmings. Many customers request specific pathogen testing of beef trim and many suppliers of trim choose to conduct testing prior to shipping. The most common testing

programs focus on testing for the presence of the regulatory adulterants *E. coli* O157:H7 followed by the other 6 STECs. Testing for *E. coli* O157:H7 is done much more extensively that testing for the other 6 STECs and the USDA guidelines for N60 sampling are followed in testing programs. Some processors choose to implement more stringent testing programs than the USDA recommendations for N60 sampling. Trim can also be tested for the presence of *Salmonella*. The handling of a positive test result or presumptive positive test result varies depending on the target pathogen. With any positive STEC sample, the product must be diverted to a facility with a validated protocol for cooking to destroy the pathogen and cannot be used to produce raw ground beef. For *Salmonella* positive trim, the product can be used to produce ground beef from a regulatory perspective, but the customer and/or supplier must decide if this product will be used to produce a raw final product.

Beef trimmings can also be tested for the presence of indicator bacteria. The presence of indicators can inform the customer and supplier on the potential shelf-life of the product.

5.14 Testing of Raw Ground Beef: As previously stated, there are regulatory requirements that address the presence of STECs in ground beef as they are considered adulterants. The presence of *E. coli* O157:H7 is most commonly the target of testing of raw ground beef as a final product process step while testing for the other 6 STEC is also done. Testing can be conducted by the USDA or by the processor on a voluntary basis. Regardless of who conducts the testing, when positive for STECs, the product is considered adulterated and can't be sold raw to the consumer. Regarding sampling programs for raw ground beef, fewer samples per lot are required relative to the number needed for trimmings due to the more homogeneous nature of ground beef compared to the trimmings. When ground beef tests positive for *Salmonella*, no regulatory action is required at this time and decisions must be made by the supplier about final product use. As with testing for beef trimmings, testing of ground beef for indicator bacteria can also be conducted as an indicator of shelf-life determination.

5.2 Process Control in Raw Meat Processing

5.21 Introduction: The International Commission on Microbiological Specifications for Foods (ICMSF) has published an entire book on the "Use of Data for Assessing Process Control and Product Acceptance". This book "Microorganisms in Foods 8" should serve as the key reference for process control. Since that resource is readily available, this short communique will offer some examples of process control data gathering by a surface rinse method.

Having the capability to take numerous non-destructive samples for indicator organisms is a powerful tool for understanding operational process control. The "whole-portion" or "whole-muscle rinse" offers that tool. In addition to evaluating process control, the method is very suitable for supplier Baseline Data (incoming raw material), Shelf Life Studies, Intervention Validation and Verification, and Trouble Shooting (line-sampling).

5.22 Methodology: The basis for the method is an entire surface rinse of the portion (cube, small steak, chicken breast, chicken tender, sliced or diced meat, etc.) or muscle (various sub-primals) and then enumerating the selected indicator microorganism (Aerobic Plate Count (APC), coliforms, *Enterobacteriaceae*, Lactic Acid Bacteria, etc.). APCs are good indicators of overall sanitation and temperature control. *Enterobacteriaceae* are good indicators of sanitary dressing and temperature abuse. The results can be reported as Colony Forming Units (CFU) per total portion, CFU/gram or CFU/cm^2. Typically, one would use 100 ml of rinsate for portions and 250 ml for whole muscles. Different volumes could be used depending on sample size and anticipated CFUs. Distilled water is suitable if the time between sampling and plating is not more that 24 hours.

Portions: In this example, the sample is an 8oz vacuum-packaged Choice Top Sirloin Steak. With an alcohol swab sanitize the area of the package to be cut open. As aseptically as possible, remove the portion and place in a Whirl Pack or Zip Lock bag. SPECIAL NOTE: the packaging material will account for 30-40% of the bacteria at the package-meat surface interface. For the most accurate count, rinse the package purge with the rinsate before pouring the rinsate over the removed steak. Massage the steak while shaking the bag 25 times in the arc of a foot for 15 seconds. (Make sure the rinsate bag is sealed!) Prepare appropriate dilutions and plate to desired media. One milliliter of rinse-ate is the 10^2 dilution for the entire steak. An APC count on that plate of 200 would represent 20,000 CFU/steak or Log 4.30 CFU/steak. By estimating the surface area, one could divide that area into the 20,000 to convert to CFU/cm^2. The count could be converted to CFU/g by dividing the 20,000 by 227grams (8oz). The latter is not recommended as surface bacteria are the major concern in these tests and the bulk of sterile, deep-muscle will distort the counts.

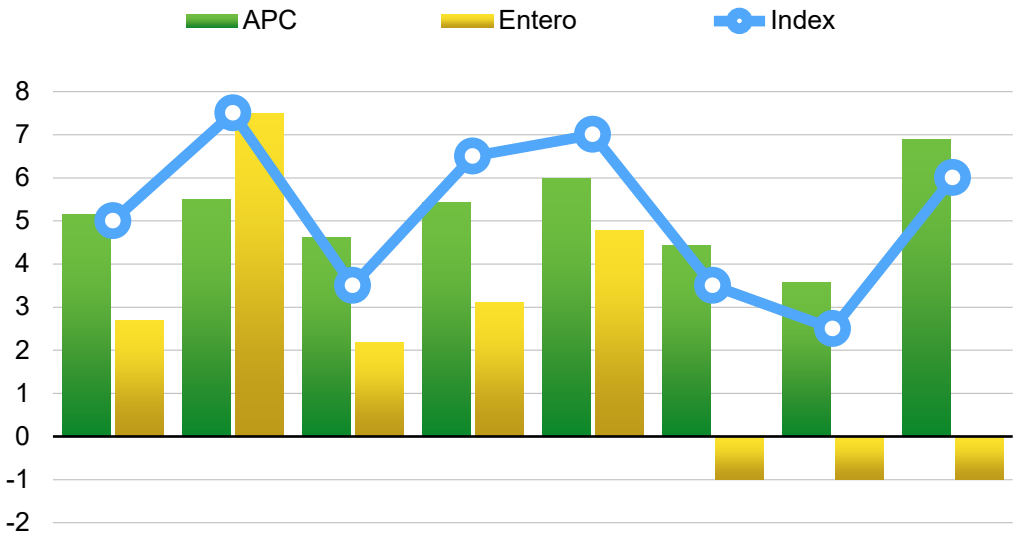
Whole Muscle: The sample is a 12-pound vacuum packaged Select Beef Tenderloin. Because of the size of the sample, a modification in sampling technique is required. Sanitize the surface to be cut. Open the package over a clean tote and with a sterile gauze wash the surface of the whole muscle with the 250 ml of rinsate. Pour purge into tote, leave gauze in the tote and pour contents of tote into sample bag for shipment to the laboratory. Through the sample bag massage gauze in the rinsate and prepare appropriate dilutions. One milliliter of rinse-ate now represents 1/250th of the whole muscle. The countable plate of 200 is multiplied by 250 for 50,000 CFU/muscle or Log 4.70 CFU/muscle.

Some trial runs will probably need to be conducted to determine appropriate volume of rinse-ate and dilutions for countable plates. The key is that a lot of data can be collected from the processing line with little disruption and no destruction. Explain to USDA-IIC that the rinsate is distilled water and the product can go back to the line.

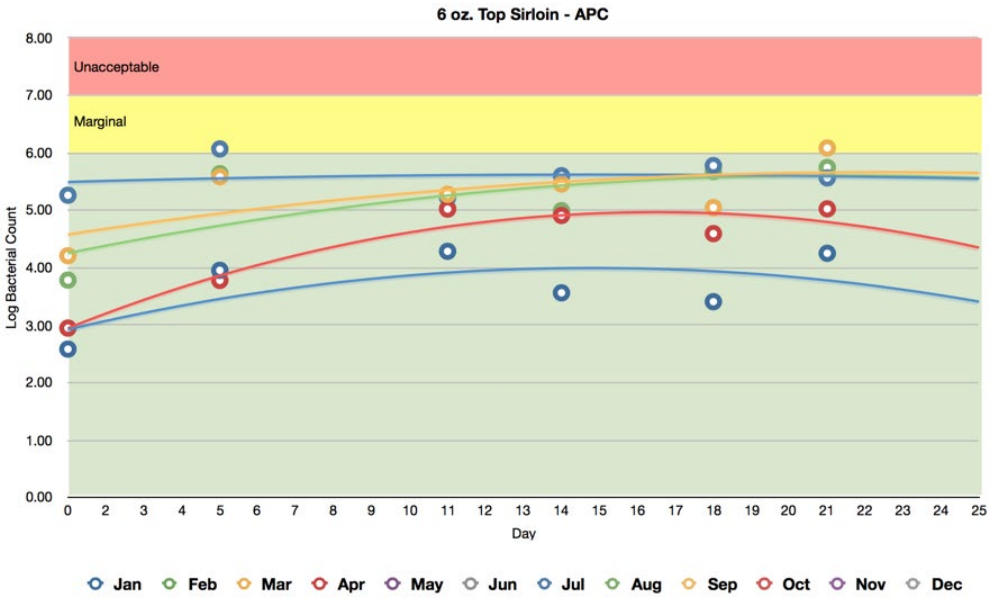
5.23 Baseline Data: Baselines can be developed to monitor raw material or finished product. The goal is to establish levels of indicator bacteria that best represent the process being under control. Once these levels have been established then the numbers can be included in product specifications. The example shown below is baseline data collected on incoming raw material (vacuum-package, refrigerated sub-primals). Each data point represents the average of ten (10)

individual samples per pack date. Averages must be determined from whole number counts before converting to Log count. After reviewing this data, it was observed that there was as much variation within an establishment number as there was between establishment numbers. The first goal before setting any limits would be to reduce the variation within a single process and establish best practices for process control.

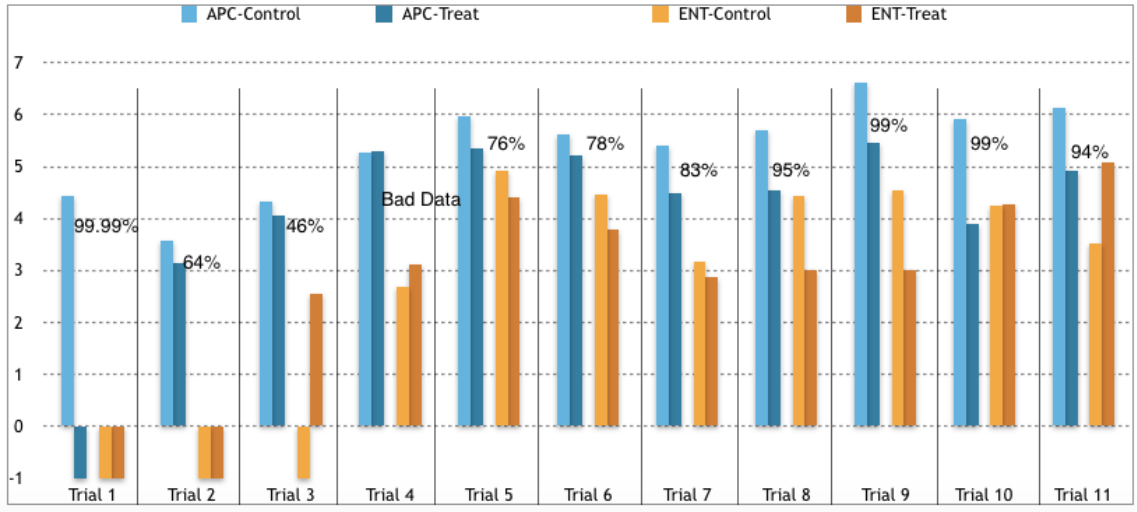
5.24 Shelf-life Studies: Shelf life studies can provide valuable information on process controls.



The goal is to predict how a product will perform under various conditions in processing and distribution. Comparing data over a period of time also allows for some understanding of seasonal variations. The example below shows the Aerobic Plate Count of portion rinsed 6 oz Top Butt Steaks stored at 36F. Each data point represents triplicate samples (n=3). Note the difference between the bottom blue line (January) and the upper yellow line (September). To better understand product performance under "field" conditions the latter stages of the shelf life can be run at slightly elevated temperatures.



5.25 Validation, Verification and Trouble Shooting: The surface rinse method can be very useful in validation and verification of interventions since most of these are meat surface related. Validation and Verification are sections covered separately in this document. The graph below shows data on packaging and surface exposed to an Ultraviolet Light Intervention. Each before and after data point are averages of ten (n=10) non-inoculated samples. Log APC and Enterobacteriaceae are shown on the Y-axis.



5.26 Summary: The surface rinse method can be a valuable tool in Process Control. Data can be generated for raw and finished baselines, shelf life parameters, validation and verification of

interventions, and in trouble shooting (i.e. line sampling for sources of contamination). The method is non-destructive, and product can be returned to the process after sampling. In most cases, the method deals with normal, non-inoculated samples that truly reflect the microbiological environment of the product.

5.3 Validation Testing

There are at least two fundamental approaches to the concept of validation. The first one focuses on achieving previously established parameters known to successfully control pathogens while the second one utilizes implementation of in-plant equipment and procedures to determine the potential of the process to reduce pathogens.

Generally, there is published scientific or supporting documentation from scientific sources, or equipment and antimicrobial suppliers, providing information on the level of pathogen reduction. For instance, documentation may indicate cooking beef patties at a certain temperature for a specified duration has been shown to reduce pathogens. Therefore, a processor of cooked beef patties could use this information to support their process by determining the temperature and time-at-temperature during cooking to ensure they meet the temperature and time combinations established in the supporting documentation. An example of the first concept is measuring the amount of time and temperature achieved in beef patties passing through an oven (time-at-temperature), or the final temperature as the patty exits the oven. The cooking process could be validated by comparing published time and temperature parameters to those achieved during cooking. If the previously established parameters solely indicate final temperature the processor could use this as a comparison temperature to determine if the process has met the published temperature for lethality. Albeit this is a validation of the process to meet parameters based on published results, it is not as rugged a validation concept as necessary to confidently identify its ability to eliminate pathogens. Using established time-at-temperature or final temperature to validate assumes every particle of the target product reaches the established parameters. In order to rely on this approach, one must assume the formation of each patty and application of heat to each patty is consistent among all patties on the same line and across different lines – this may not consistently happen. Therefore, another approach is to perform a validation study directly measuring microbial reduction. This can be accomplished using a known level of appropriate microorganism, inoculating the product, exposing the product to the treatment, and measuring the actual reduction. This method determines the direct effect based on the equipment and process used.

It is important to determine a level of microbial reduction to definitively identify the capability of the process being challenged. The first approach may not completely provide assurance that the process destroys pathogens because it does not measure pathogen reduction – only the achievement of parameters. Pathogens are destroyed by temperature, or any process, only where that process is applied to the pathogen. The temperature must be achieved in every particle of beef patty for it to be effective because any particle could contain a pathogen. It is assumed that a patty of uniform thickness and diameter, evenly spaced on a consistently paced conveyor, and exposed to consistent heat will generally cook the same. Inherent changes in the

formulation such as actual connective tissue, moisture, and fat may affect actual patty temperature within a patty due to unintended variations in patty formulation. Equipment settings that form the patty may also impact the uniformity of the patty. As such, it is necessary to know the level of reduction a process or antimicrobial can achieve.

Theoretically a process or antimicrobial properly applied at a certain level (temperature) or concentration (ppm or %) will reduce pathogens by a certain amount. This identifies the maximum incoming load of the target organism the process can eliminate. Generally, the incoming pathogen level is not consistently known, and furthermore it has the propensity to vary significantly based on cattle source (feedyard, farm), gastrointestinal tract status (feed withdrawal, access to water), transportation (trucking company, distance of travel), weather (temperature, rain), holding time at the plant (optimal, extended), and animal handling (stress associated with loading at origination, unloading at plant, movement from home pen to knock box). If the incoming level of pathogens is higher than what the process or antimicrobial can control, pathogens will survive, and contaminated product has the potential to reach consumers if it goes undetected.

Conducting a study of the process or antimicrobial using surrogates or pathogens is generally a preferred and more rugged approach to validation because results can identify the level of reduction and the level of pathogen that can be controlled in the exact conditions they are used. Studies can be performed using a pathogen, or a surrogate for the specific pathogen if one is available depending on the location of the study. Using pathogens for a validation study is generally restricted to the laboratory to prevent introduction of pathogens into the production environment. Using non-pathogenic surrogates for a pathogen allows the study to be performed in the plant setting, using the same equipment and procedures as in normal production, and without the risk of introducing the pathogen to the production environment. When planning to perform the study in the production environment there are many considerations.

The first consideration is determining the pathogen(s) of interest. Refer to the hazard analysis to determine the pathogens associated with the incoming raw material. Once the pathogen(s) have been identified, and it is determined the study will be conducted in the production environment, search the literature to identify a suitable non-pathogenic surrogate for the process being validated. These reports may be obtained by searching the Internet, contacting a laboratory service provider, other beef processors with a similar process, food safety experts, USDA-FSIS' askFSIS, process authorities, equipment providers, or antimicrobial providers. The reports must support the use of the surrogate in the same type of process or product. If the process being validated is fermented beef sticks or beef jerky, it may not be appropriate to use a surrogate that has been compared to pathogens in studies for beef carcass interventions. There may be more than one pathogen, and this may necessitate more than one validation study if for any reason they cannot be combined in a cocktail. A lone study may be performed if a single surrogate has been identified suitable for each pathogen risk. This can be determined using the published studies that identify the surrogate as suitable for the pathogen of concern. It is important to identify the correct surrogate for the study based on its specific characteristics

and the goals of the study. The microorganisms must be at least as hardy as the pathogen of interest.

Determine the concentration of surrogate microorganisms to use. The concentration of surrogates should generally be high in the inoculum. Enough organisms must be present to ensure a clear determination of the capability of the antimicrobial process. For example, if an inoculum of 3-log is used in a study to validate the efficacy of oven cooking, the cooking process may be able to destroy 5-log, but because only 3-log was used all the results were non-detectable. This means that the process easily destroyed 3-log, but the full capability is unknown – how much more is this process capable of destroying. The concentration of the inoculum also must be determined specifically when performing a study where the inoculum will be mixed with product as opposed to dipped on subprimals or sponged onto carcass surfaces. When preparing for a study where the inoculum will be mixed with product it is important to determine how the volume of inoculum will replace any added water or ice that is part of the formulation of the product. This will help ensure the formulation of the product is not changed during the validation study and that enough inoculum (concentration of surrogates) is present in each gram of product. When dipping subprimals or sponging the inoculum onto carcasses, it is still important to identify the required starting concentration of surrogates but generally application onto a surface will provide about 1 log less of concentration. The key with this type of study is to have enough inoculum at the desired concentration to cover all the surfaces of the target product used in the study.

Schedule to transport the surrogate microorganisms using appropriate methods and timing. If the inoculum must be shipped it is important to schedule it for arrival on the day of or the day before its use. The inoculum should be shipped with enough ice packs to maintain the storage temperature, but not too many that it will freeze. Determining the number of ice packs is also dependent on the weather. Shipping in hotter weather may require more ice packs compared to shipping in colder weather. The inoculum should be placed inside of a leak-proof container. The container may also be sealed at the cap with plastic wrap and then placed inside of a sealed plastic bag to keep it contained if it were to leak. It should be shipped in an insulated container.

Inoculum should be applied using the appropriate method. Application can be performed by dipping, spraying, mixing, or rubbing/dabbing with a sponge. Method of application may depend on how the samples will be collected which will also determine the unit of measure – so make sure to plan out this particularly important aspect of the study because if cfu/g is the desired unit of measure for the results, sampling with a sponge is not appropriate. A few examples of application include the following. When performing a study to determine the efficacy of hot water on beef carcasses the inoculum will be applied to a vertical surface. The appropriate ways to apply inoculum on a vertical surface is spraying or brushing with a sponge. Samples for this kind of study can be collected by excising or sponging the inoculated surface. The inoculated surface area can be identified using a template and visually designated using carcass ink. A simple way to designate a surface area is to use a plastic disposable template like the one used for Biotype I *E. coli* sampling of carcasses. Surface areas can be placed side-by-side, or above and below one another. Placing sample sites above and below one another

requires special consideration. Designate the bottom site as the “before” sample to sample it first - immediately prior to going through the hot water cabinet. The reason for this is so a sample is not collected from the bottom surface area where the contents of the top surface area have run into the bottom surface area during the application of hot water. Use a premoistened sponge with 10 mls of Buffered Peptone Water or appropriate diluent, add five mls of inoculum and apply to the designated carcass surface. It is good practice to evaluate the procedure used to ensure the volume of inoculum added to the pre-moistened sponge is enough to provide the desired concentration of surrogates on the surface of the carcass. Perform test runs by applying the inoculum to a few sample sites and then sampling. Test the samples to determine if an appropriate concentration is achieved. As a reference, the concentration collected from the inoculated surface area will be approximately half to one log less than the concentration of the starting inoculum.

Another example of applying inoculum a different way is to mix the inoculum with the product such as that necessary when validating oven cooked beef patties. To determine the efficacy of the cooking process, a reduction in pathogens must be determined throughout the entirety of the beef patty. Therefore, it is necessary to mix the inoculum with the ground beef and achieve a thorough microbial distribution. Mixing the inoculum with the ground beef requires attention to detail and special considerations. The inoculum cannot simply be poured onto the trim prior to grinding or poured into the grinder all at once during grinding. The inoculum must be distributed as evenly as possible by slowly pouring or spraying the inoculum throughout the ground beef during mixing. It is a good practice and a conservation of resources to determine the most appropriate way to achieve thorough distribution of the inoculum using the available mixing equipment. Introduction of the inoculum can be done using a smaller offline grinder and mixer instead of the primary equipment used during production. Determining the amount of inoculum to achieve the necessary concentration requires calculations. For instance, when raw ground beef patties require a concentration of 10^7 cfu/g of surrogates and the amount of ground beef to inoculate will be 300 pounds ($300\ pounds\ x\ 454\frac{g}{pound} = 136{,}200\ g$), the following formula can be used to determine the concentration of the starting inoculum to add to the 300 pounds to achieve a concentration of 10^7 cfu/g in the target product.

$$10{,}000{,}000\frac{cfu}{g}\ is\ required = \frac{how\ many\ cfu\ in\ the\ starting\ inoculum\ is\ required\ for\ 300\ pounds}{136{,}200g}$$

$$10{,}000{,}000\frac{cfu}{g}\ x\ 136{,}200g = \frac{cfu}{g}\ or\ \frac{cfu}{ml}\ in\ the\ starting\ inoculum$$

$$1.36\ x\ 10^{12}\ \frac{cfu}{ml} = required\ concentration\ of\ the\ starting\ inoculum\ to\ achieve\ 10^7\ cfu/g\ in\ the\ target\ product$$

Once the inoculum has been applied to the surface it should be allowed to sit for approximately 15-20 minutes to allow some level of attachment to the product. Inoculum in ground product does not require this additional attachment time since it has been incorporated into the fat and protein during mixing. Collect samples to represent the starting concentration of the surrogates. This will represent the microbial status of the surface or ground product prior to

any treatment and will be the starting point for calculating a reduction. Samples representing the starting point should be taken at a location in the process immediately before the treatment. Samples taken from carcasses should be taken immediately prior to the carcass entering the hot water cabinet and ground beef patties should be collected immediately after patty formation prior to their entry into the oven. The inoculated surface or product should be sampled after the treatment but when using a chemical antimicrobial, it is critical to allow the chemical treatment to drip from the treated surface to prevent accumulation of antimicrobial in the bagged sample. This is important with both sponge and product samples. Pre-moistened sponges contain a buffer used to neutralize the activity of the antimicrobial, but it may not completely prevent further activity especially in samples where the antimicrobial has accumulated in the bag. Sampling with a sponge immediately after the treatment will collect the liquid from the surface of the product. Similarly, excision sampling immediately after the treatment does not allow enough time for the antimicrobial to drip from the surface and much of it will be included in the bag along with the sample where it can continue to be active. The antimicrobial adheres to the surface and lodges along the ridges of the irregular surface, and for this reason it is recommended to allow some time for most of it to drip from the surface to prevent the sponge from absorbing it and to prevent its inclusion with the excised sample. Another technique to minimize the activity of the chemical antimicrobial is prior to collecting the sample and removing the sponge from the bag, squeeze out the diluent so that it remains in the bag. This is done so that after the sample is collected and the sponge is placed back into the bag the diluent is available to buffer the antimicrobial activity of the chemical antimicrobial collected with the sponge. This liquid is also needed for the lab to remove the aliquot necessary to perform the test.

Using a sponge to collect a sample requires a consistent and systematic technique. The procedure should account for type of diluent, volume of diluent, number of passes, direction of passes, and amount of pressure to apply on the sponge. A familiar example of this is to use a pre-moistened sponge with 10 ml of Buffered Peptone Water (BPW) and perform 10 passes vertically and 10 passes horizontally while applying enough pressure to remove dried blood – the same as carcass sampling for Biotype I *E. coli*. Collecting samples from ground beef patties should account for sample size, number of specimens, size of specimens, and type of bag. An example of this is to collect three patties per sample (each patty is a specimen), each specimen is approximately 90 grams, and a filter bag is used to place the patties. It is crucial to collect enough samples to allow the results to define the efficacy of the treatment. There are many ways to determine sample size, but the general concept is collecting enough samples to allow for a greater level of confidence in the results. An exception to this is when using an inoculum with a known concentration. The reason for this is application of an inoculum creates samples with consistent concentrations of microorganisms, and this generates results with low standard deviation. Results with low variation allow for studies to be conducted with small sample sizes because results should be consistent. An example of determining sample size is to use a statistical program such as Minitab to calculate the sample size. Although in theory there should be low variability in the population when applying surrogates, it is not uncommon for results to have some level of variability. And for this reason, it is good practice to perform the study with a greater number of samples compared to a lower number of samples.

When testing a sample, the entire sample should be tested. Therefore, it is important to collect meaningful samples. This eliminates extra work in sample collection, waste of product, and supports efficiency in the laboratory by eliminating subsampling from a larger volume of sample to create a manageable sample for testing. Another example of what is meant by meaningful samples is identified when collecting excision samples. When applying the inoculum to the surface of the product and collecting an excision sample it is important to collect an excision sample from the top layer (no more than 1/8" thick) where the inoculum was applied and minimal sample from the area below the surface. By including more of the area below the surface it will create a diluting effect and the results will be skewed towards a lower recovery, therefore potentially increasing the calculated reduction.

Reporting results should be planned with the laboratory to provide the correct units. The units that are in the most basic form are either cfu/ml or cfu/g. Knowing this allows for a calculation to be performed if a different unit is required, such as cfu/sponge or cfu/sample. This unit can be calculated by using the volume of the sponge or the weight of the sample submitted to the laboratory. An additional format for reporting results is to convert the results to log cfu/ml or log cfu/sample. The primary reason for performing this conversion is to make the interpretation of the results easier. Microbial results generally have sizeable differences when compared on a cfu basis and this can hinder the ease in interpreting the results. For example, if there are 20 results and the cfu values range from 101,500 cfu to 890,100 it may be easier to interpret the results when they are in the log format of 5.0 log cfu and 5.9 log cfu. Regardless of the units and the format it is important to maintain consistency when comparing results across studies or when communicating the level of reduction.

Identifying the success of an antimicrobial process can sometimes be viewed as subjective, especially when a level of reduction has not been established. Validation studies are necessary for supporting the process from a regulatory perspective, but they should be viewed as necessary to learn about the capability of the process and to have compelling information to support the microbial safety of the finished product to customers and to consumers. The parameters to consider for determining if a treatment is effective are incoming pathogen load, number of treatments, regulatory requirements, and customer requirements.

Pathogen reduction from a treatment should be based on the highest potential pathogen load established by the incoming material. Whether raw material is incoming cattle or incoming beef trim it is good practice, and necessary, to have information about the incoming microbial load. This should be the basis for determining the minimum reduction of pathogens from any one or a combination of treatments. If there is one treatment it should be able to control the incoming microbial load, but if there are multiple treatments the cumulative reduction should be able to control the incoming load. Another consideration is to determine the microbial load that must be controlled immediately before the treatment(s). For example, if the incoming microbial load on cattle hides is 7 log cfu/500 cm^2 and the first treatment to validate is a pre-evisceration cabinet, the minimum incoming load that the treatment must control will not be 7 log cfu/500 cm^2 because removal of the hide and trimming have removed most of this microbial

contamination. The minimum microbial population that needs to be controlled by the treatment(s) should be determined immediately prior to the first treatment.

The number of treatments will also determine if a reduction can be considered successful. If a single treatment reduces the microbial population on the finished product by 1-log, but the incoming load is 3-log then the treatment may be effective, but it does not provide the necessary control to eliminate the incoming microbial load. If there are 3 treatments and each one reduces the microbial population by 1-log, then cumulatively this 3-log reduction is enough to eliminate the identified incoming load.

Sometimes it is necessary for treatments to control a regulatory limit of pathogens such as when producing the shelf stable product beef jerky which requires a 5-log reduction of *Salmonella*. In this setting the effectiveness and success of the treatment(s) is established.

Customers may also identify a minimum microbial log reduction prior to approval as a supplier. These customer requirements have grown from the necessity to control risk to their brand by gaining a more thorough understanding of the supplier's process. This has compelled suppliers to better understand the efficacy of their treatments by performing validation studies. During these studies suppliers have gained more knowledge about the application, equipment, suppliers, parameters, and monitoring of the treatments. These requirements have been primarily identified by retailers indicating a minimum reduction prior to approval as a supplier.

Overall, the validation of a microbial treatment is a necessary consideration in the production of microbiologically safe beef. There are many appropriate ways to perform validation studies and interpret the results, but ultimately the goal is to identify if the microbial treatments are effective and successfully reduce the incoming microbial load during the production of the finished product.

5.4 Verification Testing

Verification is a key part of the process to check if a certain activity has been done correctly. According to FSIS's regulatory language, verification is accomplished but not limited to, reviewing a previously completed record, observing the performance of an activity or a corrective action, or calibrating an instrument. Ultimately verification means to check.

Including verification activity in a food safety plan is not only a regulatory requirement, but it also adds to the level of confidence in the completion of activities during food production and ultimately in the production of beef that is safe for consumers. Another way to view verification in the context of microbiological testing in beef food safety programs is to check its microbiological status. Beef is produced using the steps identified in the production flow chart, and one or more of those steps is a critical control point that has been validated and determined to eliminate pathogens. Although the steps have been validated, the process of beef production has some level of variability because of many factors including, but not limited to the following: human error, clogged nozzles, empty antimicrobial container, inefficient

boiler, broken pipe, misaligned nozzle, incorrect titration, incorrect thermometer, etc. Therefore, it is important to verify, or check, the performance of the process and its outcome. One way to do this from a microbiological perspective is to sample the raw material and/or finished product to verify the process prevented contamination.

There are many considerations related to the effective microbiological verification, and all of them fall into two basic categories: (1) sampling and (2) testing. Proper sampling ensures a representative and powerful sample is collected. Proper testing determines the presence of the target pathogen and the results ensure a confident decision in the disposition (release or diversion) of the product. Many justifiable sampling programs have considered the following list of items, but there are some that have not considered each of these variables and therefore may not be as effective. It is important to note that the sampling program developed for a specific process must be customized based on how the variables below are defined by the process and that one sampling program may not exactly fit another process. The variables to consider are:

1. Lot size
2. Specimens per sample
3. Distribution of specimen location and time
4. Specimen size
5. Type of specimen
6. Sample size
7. Test methodology
8. Interpretation of results
9. Impact of results on disposition

5.41 Lot Size: Prior to sampling raw material or finished product, a mass of product must be identified from where to sample. This is defined as the lot size. Lot size varies from a trailer load, set number of carcasses, combo bin or multiple combo bins, a pallet or multiple pallets of boxes, or a gondola of trim to name a few examples. Lot size is generally constant for each daily process unless the process changes or there are special considerations such as when a high number of positives is identified in one day (high event period) and having to determine additional follow-up sampling for the lots that were negative (other combo bins) or those items that were not sampled (subprimals).

As an example of the constant nature of lot size is the daily production of trim. The processor may capture veal trim in a gondola which is then boxed. The processor may designate one gondola of veal trim as their lot size. Therefore, each gondola of veal trim is a lot. If the processor produces thirty gondolas of veal trim in a production day, they will have thirty samples to submit for testing. The test result for that sample will have bearing on the gondola of veal trim from where it was collected and potentially from other gondolas.

Deciding the size of the lot must take into consideration the amount of product from which to sample and the impact the results will have on the entirety of the lot. If the lot is designated as

a trailer load (40,000 pounds), one must determine if this lot size is practical from a sampling and disposition standpoint. The questions to ask are: "Can 40,000 pounds be effectively represented by collecting one sample or will multiple samples need to be collected?" and "What happens to all 40,000 pounds if the result of a single sample is positive for the target pathogen?" Maintaining significant resolution from a lot size of 40,000 pounds will require many specimens to account for the large volume of product. Using the entirety of the 40,000 pounds as the sampling lot in most scenarios would not allow for good sampling resolution, therefore the confidence in the results would be questionable; and if the result is determined to be positive the entire 40,000 pounds would be jeopardized when only a portion of the lot is positive. And one could assume that either the product representing the lot is highly contaminated or by chance at least one contaminated specimen was collected in the sample.

5.42 Specimens Per Sample: The number of specimens per sample is determined based on known or assumed level of contamination. If the level of contamination is high and distributed through most of the lot, theoretically a smaller number of specimens would suffice to find the contamination. But if the level of contamination is low and distributed in only a few areas, theoretically a larger number of specimens is required to have sufficient likelihood of selecting product where contamination is present. Since this information is realistically unknown, assumptions must be made based on a combination of historical data and initial assumptions. Because of this, it is important to summarize daily results into an easily interpreted and retrievable report of the history of the process. This is part of the information necessary to justify and determine lot size and specimens per sample. Additionally, the number of specimens per sample are ultimately justified by the amount of acceptable risk – which should always be low risk when pathogens are the microbial target of verification.

The beef industry historically has had a low percent positive rate for *E. coli* O157:H7. This low rate necessitates a powerful sample with a higher number of specimens to account for the low level of contamination and the non-uniform distribution of pathogens within the lot. The beef industry has adapted the use a specimen count of 60 from N60 two-class sampling methods from ICMSF (2002) with much success. Other sampling methods have been developed which contact a higher proportion of product or utilize a higher number of specimens. This has increased the confidence in results. For confidence to increase in a sample result, the number of specimens collected, or the amount of product contacted for the sample must increase so that a higher proportion of the lot is represented.

ICMSF (International Commission on Microbiological Specifications for Foods). 2002. *Microorganisms in Foods 7. Microbiological Testing in Food Safety Management.* Kluwer Academic/Plenum Publishers, New York, New York.

5.43 Distribution of Specimen Location and Time: Considering where to collect specimens, when sampling from a fixed volume container; or when to collect specimens when sampling from moving volume of product is an important consideration for collecting a powerful sample to ensure that the entire volume of the lot is represented.

Fixed volume containers can be effectively managed using many techniques but the one that is efficient to each operation must be identified. When sampling beef trim from a combo bin it has traditionally occurred after the combo has been filled. This type of scenario allows for specimens to be sampled from only the upper layers of the combo bin – approximately one-third. Unless the trim is manipulated so that the trim below the upper layers is sampled, a large volume of trim goes unrepresented. However, the adaptation of N60 sampling to 2000 lb combo bins has been successful because it represents an intermittent sampling of a continuous process since every combo is sampled. Some sampling techniques to increase representation include sampling as the combo is filled using a continuous cloth sampling technique, or using a sampling tool to enter the mid and lower depths of the combo bin.

Lot sampling from a moving volume of product that can be selected and weighed such as ground beef have intrinsic properties that facilitate high resolution sampling. Ground beef specimens can be selected as the ground beef is being made and the size of the specimen is quickly formed by adding or removing ground beef from the specimen. This facilitates the quick and effective collection of high-resolution sampling compared to sampling beef trim from a combo bin. Ultimately, being able to collect specimens from a greater volume of the lot provides more representation of the lot and improves the likelihood of selecting specimens that contain the target organism if it is present.

5.44 Specimen Size: The size of the specimen is dependent upon the sample size and the number of specimens per sample. Examples of specimen size are weight, volume, and surface area. Sample size is limited by test methods which have been validated using a certain sample size. If the sample is much larger than this, it may require splitting a single larger sample into multiple samples; or changing the lot size; or accepting multiple samples per lot. If the target sample size is 375 g and the target number of specimens per sample is 60, the specimens should each weigh 6.25 g. If each specimen weighs more than this, then the sample size in weight will exceed the target and must be split into two samples – each tested separately and each result bearing on the disposition of the product. Another aspect of specimen size is the surface area. If the total required surface area per sample is 180 in^2, it is divided by the number of specimens to identify the target surface area of each specimen.

The size of the specimen should also be viewed in relation to how the specimens are collected. If specimens are collected at a high frequency during the production of ground beef, then the size of the specimens may be smaller because more of the volume of the ground beef lot is sampled. But if the same lot size is sampled at a lower frequency, then the size of the specimen should be increased to account for the minimal volume of the lot that was sampled. To put this into perspective if a lot of ground beef is 10,000 pounds and a specimen is taken every 30 seconds, the resolution of this sampling plan is much higher than if the same lot size is sampled every 3 minutes. Much more product will have passed unsampled within the 3-minute time frame compared to the amount of ground beef that passed between sampling with a 30 second interval. If 10,000 pounds of ground beef is produced in 9 minutes and the sample size is 375 g, sampling every 30 seconds generates 18 specimens weighing approximately 21 g; while

sampling every 3 minutes generates 3 specimens weighing approximately 125 g. This example shows that collecting specimens at high frequencies allows more contact with a larger volume of the lot compared to sampling less frequently. The collection of specimens at shorter intervals improves the resolution of the sample and increases the likelihood of selecting specimens that contain the target pathogen if it is present.

5.45 Type of Specimen: The type of specimen is determined by the product being sampled and how the product is generated. Carcasses are sampled using a sponge or excising surface area. Trim is sampled by excising surface area while the container is being filled or from a filled container, collecting trim with a sampling device from a filled container, or contacting trim with a sampling device that then becomes the sample. Ground beef is sampled by grabbing certain weight specimens immediately after grinding or from sealed packages.

An important consideration when taking excision samples is in how the specimen is removed from the product. Trim or subprimals have a surface area that becomes contaminated during dressing of the carcass and further fabrication of the primals and subprimals. That contamination lies on the surface of the product. Excision samples should be removed by excising the surface area rather than cutting a chunk from the product. By primarily removing the surface area, it creates a meaningful sample because the majority of the sample is from the surface where contamination is found. This lessens the amount of internal tissue and allows for more surface tissue to be included in the sample. Internal mass of the product is generally not considered to be contaminated. Including more internal tissue as part of the sample lessens the power of the sample because a higher percentage of the sample weight is internal tissue instead of surface area that was excluded because the sample weight had already been reached.

The nature of ground beef does not allow distinction between surface or internal tissue and for this reason grabbing ground beef is acceptable. The comminution of ground beef mixes the trim facilitating sample collection.

Sample collection using a sponge to determine the presence of pathogens should have some level of standardization. For sampling trim with a sponge where piece size and number can vary, a standard surface area should be the goal. Not all types of trim can be sampled using a sponge. Small pieces of trim generally are more difficult to sample, although not impossible. Sponge sampling trim is possible, but the size of the lot may dictate whether it is practical.

5.46 Sample Size: Once the size of the lot, number of specimens per sample, and size of the specimens are known, the sample size can be determined. The size of the collected sample will be a factor of the number and size of the specimens, but the tested sample will be dictated by the test method. Therefore, if the collected sample is two pounds (908 g) but the test method only allows a sample of up to 400 g, the collected sample will have to be split into three tested samples.

5.47 Test Methodology: Many test methods exist today for analyzing beef samples for *E. coli* O157:H7, non-O157 STEC, and *Salmonella*. Many of these methods can simultaneously analyze for multiple pathogens, such as *E. coli* O157:H7 and non-O157 STEC. All test methods must have external validation performed to ensure the method meets ISO standards for its intended use and be fit for purpose. The successful completion of this validation is accompanied by a certificate which can be requested from the laboratory service provider or the test kit manufacturer as supporting documentation. Certificates are also available for download from the certification body website. Test methods should be able to detect various levels of pathogen and have minimal false positive and false negative results. Time to report results should also be considered based on testing method so that it fits the production and transportation schedule of the operation.

5.48 Interpretation of Results: Test results are reported as negative, potential positive, presumptive positive, or confirmed positive. Each one requires more time to reach the result status. Determine what stage of positive a decision will be made about the disposition of the product. There is generally no further consideration required if the results are all negative, but if there are some results that are potential positive a decision must be made to continue testing to identify if the results are presumptive positive or confirmed positive; or if the result is truly negative. The consideration for this is based on confidence of the result at that stage of testing. This decision making can be supported by determining the level of false positives at each stage. This information should be available from the test kit manufacturer. Another way to gain confidence in the result at each of the stages is to continue the testing when a potential positive is determined. The results of the ongoing study can be summarized to determine if potential or presumptive positive results ultimately confirm. Depending on the confirmation rate of each stage result, this data can be used to determine which stage can confidently be used to decide on disposition. Additionally, if time to report is crucial because of transportation concerns and the confirmation rate is acceptable at the beginning stage of testing, then the disposition decision can be made at one of the beginning stages of testing.

5.49 Impact of Results on Disposition: Each result has bearing on the disposition of the product. The most straightforward set of results is when all samples tested negative for the pathogen of concern. This means that all the lots should be acceptable to release into commerce, assuming that the sampling of the lots was powerful enough to collect specimens representative of the lots.

When one or more results are positive at the stage that the lot will be diverted from raw production, the type of product and timing of production should be considered to determine if disposition of other lots is necessary to maintain a level of confidence and minimize risk of releasing contaminated product that was identified as negative. One example of this is when one lot is positive for the pathogen of concern. There are certain factors that need to be considered such as the type of product from which the sample was produced, the timing of production for the positive lot, and the common equipment used to produce the lot. There are many ways to rationalize the disposition of this and related product – some more conservative than others, but ultimately the goal is to prevent contaminated product from reaching the

consumer. One such disposition example would be to divert only the lot that was positive giving no consideration to adjacent or similar lots of the same product type or to other product types such as subprimals from which the trim was removed. Another example would be to scrutinize these lots. For instance, the lot of similar product type produced before and after the positive lot; the subprimals packed at the same time as when the positive trim was being produced; and trim from other lean points that shared the same conveyor. These same considerations must be given when multiple lots are found positive for the pathogen of concern.

These are all factors that the food safety team must investigate to verify the microbiological status of the product. Each scenario will be different, but the concepts are the same – collecting sufficient specimens to create samples with enough power to generate confident results for microbial verification.

5.5 High Event Period

In a raw beef product, it is common and expected to have low level occurrence of a pathogen. This normal and expected level must be handled through appropriate product disposition. For a zero-tolerance pathogen like *E. coli* O157:H7 in beef, common practice is to use pathogen analysis to verify the Food Safety System.

When the testing system provides an out-of-control signal (higher than expected number of positive results) we must react accordingly to control both the products and the processes. This signal is commonly referred to as High Event Period (HEP). FSIS has a compliance guideline that establishes parameters that can be used in determining if the situation is localized or systemic. Although these are sound guidelines, there must be people that are closely familiar with results from a specific facility to determine necessary product actions. These are the personnel that are closely monitoring and listening to their data.

When an HEP is identified it is critical that all affected product is retained. This includes other tested products that may have tested negative and potentially untested product (subprimals). The positive results must be reviewed for product type affected and scope. Tracking and traceability of materials is imperative to piece the event back together to ensure all affected product is captured. These traceability data also provide key system diagnostics to aim the investigation in the right direction (e.g., chuck related).

5.6 Sanitation

Application of microbiological testing can be utilized in accessing the effectiveness of sanitation in beef plants. Environmental sampling and testing programs may be designed to assess Sanitation Standard Operating Procedures (SSOPs), verify the efficacy of a particular sanitation program or evaluate the effectiveness of sanitation chemicals or sanitation personnel. The objectives of all microbiological sampling must be pre-determined. The sampling and testing program must provide a framework within which results may be interpreted and tied to

appropriate actions. Ongoing environmental monitoring is typically used as a verification tool and while targeted sanitation sampling is used to problem solve and trouble shoot. Tracking and trending environmental data over time is essential. Often the environmental testing results are compared to a historical baseline, yielding to subjective conclusions as to acceptability of sanitation practices. Typically, indicator organisms are used for these types of testing.

Environmental microbiological monitoring may be conducted both pre-operationally and/or operationally. Most microbiological testing of the plant environment is directed towards pre-operative sanitation. Of particular use pre-operatively is the identification and control of microbiological niches where pathogen survival and growth are possible. Pre-operational sampling should include evaluation of all inputs (i.e., air lines, water, and filters). The presence of pathogens pre-operationally is unacceptable and indicative of inadequacies in the sanitation program. Collection of environmental samples at various times during production is a strategy frequently used when trouble shooting spoilage or pathogen contamination issues. Microbial mapping of the production floor during operation can be very useful when trouble shooting.

Sample collection methodologies include: swab, sponge, gauze, and direct-contact sampling. The number of environmental samples collected and analytical methods varies widely between plants. Pre-operational sampling may include both food contact surfaces and non-food contact surfaces. The sampling of hard-to-clean areas are likely to provide the most useful information.

5.7 Compliance Sampling

Compliance sampling is that sampling conducted by the regulatory agency, most often the Food Safety and Inspection Service (FSIS) to detect pathogenic STEC in raw ground beef and ground beef components to verify process control under HACCP. The original objective of this program was to stimulate industry testing and other actions to reduce the presence of the pathogen in raw ground beef. FSIS has continued to modify the compliance testing programs and now co-analyzes all raw beef trim samples for *Salmonella* and STEC. The data from the sampling programs is also used to estimate national prevalence of these organisms in the commodity tested. FSIS considers compliance sampling one of the tools used to verify the effectiveness of an establishment's HACCP systems.

In terms of the considerations by the establishment, compliance sampling should receive the same consideration as any sampling by the establishment. When FSIS conducts random sampling of the products, the establishment is required by regulation to hold all of the product represented by the sample until the results are obtained. FSIS expects the establishment to take into consideration all source materials used at the time of sampling. FSIS does permit alternative approaches by establishments to minimize the impact of the sampling event. Accordingly, FSIS permits two alternative sampling procedures. Under both alternatives, the establishment must randomly select the lot to be sampled in terms of day, shift, time, and product. Instead of drawing the sample on the line at that time, the establishment can use one of the following alternatives.

An establishment may produce the commercial blend of ground beef on a smaller grinder provided the establishment has written procedures for such sampling. The smaller batch must be representative of the establishment's production process, and the batch must be at least 50 pounds. Alternatively, an establishment may run the selected commercial product at the start of operations, then, following sampling, stop and conduct a pre-operational sanitation. When resuming production after a complete sanitation step, the establishment would use different source material than the materials represented in the sample. Under either alternative, the establishment must hold all products, the finished ground product and all the lots of the source material used to produce the product.

Since compliance sampling is intended to verify the establishment's process control, any positive test results found by the Agency may lead to additional regulatory scrutiny, including follow-up testing. Further, the Agency will conduct follow-up sampling at the establishments that provided the source materials as applicable.

5.8 Investigative Sampling

Investigative Sampling is focused sampling of a specific process or environment for target organism(s) to discover new information to identify the root cause of a past problem or to investigate potential solutions to mitigate a potential future risk.

More specifically, investigative sampling is performed as the result of an undesirable event that has occurred in the past and is used to obtain additional information that is needed to better understand what might have happened to cause the event. The ultimate goal in investigative sampling involving historical failures is to be able to find a target organism(s) in the same environment or process that was the root cause of the undesirable event. Identifying a root cause is often very difficult to accomplish because 1) due to the infrequency of which many undesirable events in beef processing occur it is hard to demonstrate repeatability and 2) beef processing is very dynamic with many moving parts so it is unrealistic to assume an investigative sampling plan will cover all areas of potential contamination. As a result, investigative sampling more often provides companies with information about the production process and environment which allows them to implement "process improvements" designed to mitigate the reoccurrence of the undesirable event, but truly benefit the overall food safety system as a whole.

An example of investigative sampling would be if an establishment was notified that higher than normal levels of *Listeria* spp. were identified in trimmings destined for raw ground by their grind customer. The producing establishment would typically start by collecting samples from various trim products to determine a prevalence of the target organisms in the products at the plant. Once prevalence data has been collected, the producing establishment would then focus its sampling on the production environment to try and locate an area of the plant that is the source of the contamination. As mentioned previously, pinpointing the exact location of the

source of contamination is
often extremely difficult and there will be times that no root cause is ever found. Nonetheless, the benefit of investigative sampling is that although the data collected may not provide you with a root cause, it will provide you with useful information regarding where to implement process improvements that will help improve the total food safety system.

6.0 Microbiological Sampling Methods

This section describes various sampling methods to determine the microbiological status of a particular step in beef production. A given sample can be tested for specific pathogen (*E. coli* O157:H7), a number of pathogens (*E. coli* O157:H7, non-O157 pathogenic *E. coli* and *Salmonella*) or indicator organisms (Aerobic Plate Count, Generic *E. coli*, Coliforms and *Enterobacteriaceae* Count). It is important that enough samples be taken that leads to sound decision making. Insufficient number of samples could easily lead to an erroneous conclusion. It is a good idea to use an expert with statistical background to evaluate the soundness of the sampling plan including the number of samples to be taken.

Cattle hides, carcasses, and all products originating from carcasses such as subprimals, trim, and offal items as well as environmental (food contact and non-contact) surfaces can be sampled to meet specific microbiological objectives. Below are sampling guidelines which can be used to collect samples from various types of products.

Hides are sampled using a moistened sponge. It is important to collect the sample from an appropriately sized surface area to prevent the overloading of the sponge with bacteria since hides are known to have a high microbial population. Sampling a hide surface area of approximately 500 cm^2 is ideal. Samples can be processed for indicators as well as pathogenic bacteria. Hide samples give information about the incoming microbial load and if the sampling process remains consistent over time, the results can be compared and used to support a position about the incoming microbial population.

Carcasses are sampled as part of process monitoring, verification activities, or intervention validations usually with a moistened sponge. Now that the hide has been removed and with it most of the microbial population, it is appropriate to sample a larger surface area. It is recommended to sample approximately 4000 cm^2. Sampling a large surface area is necessary to reduce the variation in microbial load. Sampling 4000 cm^2 compared to 100 cm^2 is not only expected to better reflect the level of contamination on a given carcass but will reduce the variation allowing for a better assessment of microbial contamination.

Subprimals can be sampled with a moistened sponge when the result is for internal informational purposes only. If the results need to be used for regulatory support, then excision sampling is the recommended method.

Offal, depending on the type, can be sampled like subprimals. Those offal items with larger surface areas such as hearts, cheek meat, and livers can be effectively sampled using a sponge. The smaller offal items are not excluded from being sampled using a sponge, but this method may be more cumbersome and require greater finesse to effectively collect the sample. Generally, it is an easier process to collect product specimens from smaller offal items such as head meat and weasand meat.

Trim sampling for pathogen detection is typically done to meet regulatory compliance or customers' requirement. To market trim and other raw ground beef components, lotting, sampling, and testing are requirements. Without the appropriate Certificate of Analysis (CoA), any product used to manufacture raw ground beef cannot be used.

There are various sampling methods that can be used to collect an effective trim sample. The most well-known trim sampling method is that which is historically used by FSIS and many beef processors. N60 excision has been considered the gold standard trim sampling method because statistically it collects a powerful sample by selecting 60 individual specimens from different pieces of trim. It is the position of FSIS that while N60 excision is the standard sampling method, other methods have been proven to perform similarly or better than N60 excision and may be used. Each method requires validation by each facility in which it is used to demonstrate its equality as implemented in that plant to N60 excision for microbial recovery.

6.1 N60 Sampling

There are several characteristics that must be considered for the N60 excision sample method to be considered valid:

1. The first of these is that the sample must collect 60 individual specimens that are 1 inch wide by 3 inches long by 1/8 inch thick. This is done to standardize the surface area collected and to minimize the amount of internal tissue included in the sample.
2. The second part is to ensure that each of the 60 specimens are collected from 60 different pieces. The purpose of this is to create distribution of the specimens within the lot rather than groups of specimens coming from the same pieces of trim.
3. The third requirement is to collect the samples from the surface. Since the contamination associated with trim is found on the surface of the carcass, specimens collected from the surface of the trim will have a greater likelihood of being contaminated compared to specimens collected from interior muscle. Similarly, the amount of interior muscle connected to surface samples will influence the power of the sample. Since samples should weigh within a range, if the amount of internal muscle associated with surface samples is high then that means that most of the sample is from internal muscle and not surface area. For this reason, when collecting surface samples, the surface sample must be thin to prevent internal muscle from accounting for most of the weight range, in essence diluting the power of the sample.
4. Beef trim samples need to weigh approximately 375g with a range of 360g – 400g. There are many reasons for this including maintaining a consistent sample weight submitted

to the laboratory, preventing large samples from being submitted to the laboratory which must then be split into two samples, but the primary reason is for consistency in samples. It is a good practice to request from the laboratory weights and piece counts (if applicable). This can be performed on a certain percentage of the samples at some frequency during the week. This will allow for verification of the sampling procedure.

6.2 IEH N60 Plus™ Sampling

The IEH N60 Plus™ trim sampling method has been granted a Letter of No Objection from FSIS. Below are the requirements when using the IEH N60 Plus™ sampling method to obtain a valid sample:

1. Each sample must be collected using five insertions of the sampler into the combo bin. Four of the insertions must be in the corners of the combo bin and the fifth insertion must be in the middle of the comb bin. This is done to ensure distribution of the collection of specimens from the combo bin rather than collecting them from one or two locations.
2. The second requirement is the head of the sampler must be filled. Sampling using the IEH N60 PLUS sampler requires the entire volume of the sampler head to be filled. This will generate a specific sample weight that must be determined during the necessary onsite validation study. Once the weight is determined, it is established as the minimum sample weight. The minimum sample weight and the five insertions generate a complete sample. A demonstration of the sample collection can be found at the following URL: https://www.youtube.com/watch?v=vSr1Yqprfio&feature=youtu.be

6.3 MicroTally™ Continuous Sampling Device (CSD)

The MicroTally™ CSD has been granted a Letter of No Objection from FSIS. The CSD uses a stainless-steel bracket attached at the end of the trim conveyor line to hold a two-piece plastic cartridge that clamps and holds the MicroTally™ cloth. The CSD is positioned at the end of the conveyor so that the trim pieces rub against the sampling cloth as they fall from the conveyor into the combo bin. The bracket must be mounted at a 45-degree angle to the conveyor or chute that delivers the trim to the CSD. One MicroTally™ cloth is used to sample one combo. Preloaded cartridges are prepared for quick change out for a new combo. Individual plants will need to develop procedures for cleaning, preparing, and storing cartridges; and for processing the MicroTally™ cloth after sample collection. Detailed protocols can be obtained from Fremonta, Inc. Daily verification activities for CSD can include verification and documentation that the bracket is properly mounted, and that trim is properly contacting the MicroTally™ cloth by recording and monitoring cloth weights after sampling. For additional information and how to use CSD go to:
https://www.youtube.com/channel/UCJ8KH5yFHHbwp0p7_Zu71Dg?view_as=subscriber

6.4 MicroTally™ Manual Sampling Device (MSD)

The MicroTally™ MSD has been granted a Letter of No Objection from FSIS. The MSD uses the MicroTally™ cloth to sample all trim on top of the combo bin by hand after the combo has been filled with trim. Proper MSD sampling includes sanitizing gloves prior to handling the cloth, using both hands to vigorously rub the cloth across all the trim on the surface of the combo including down in between pieces using one side of the cloth for one-half of the combo surface for at least 45 seconds and the other side of the cloth for the other one-half of the combo surface for at least another 45 second. Daily verification activities for MSD can include video monitoring, a readily visible timer, or a second person to monitor the timer and ensure proper sampling technique and duration and recording and monitoring cloth weight after sampling. For additional information and how to use MSD go to: https://www.youtube.com/channel/UCJ8KH5yFHHbwp0p7_Zu71Dg?view_as=subscriber

6.5 IEH Advanced Pathogen Testing and Carcass Certification Program (APTCCP)

The IEH Advanced Pathogen Testing and Carcass Certification Program (APTCCP) has been granted a Letter of No Objection from FSIS. The APTCCP involves sampling carcasses at the end of the slaughter process after they have entered the chiller. Carcasses are sampled per the procedure briefly described below and if the test result of the N60 sample is negative for the pathogen, the entire carcass lot is certified. The procedure involves the following steps:

- Carcasses grouped into Carcass Certification Lots – a maximum of 10 carcasses = 20 sides
- N=60 excision specimens from carcass surfaces – 3 per side
- Carcass sides staged to prevent cross-contamination until results available
- Lots that test negative released
- Lots that test positive are railed off, along with whole leading and trailing carcasses
- Formed into sublots of one carcass each
- Aggressively reconditioned by trimming followed by application of antimicrobial
- Each carcass sublot is sampled using N=60 and tested
- Sublots that test negative released
- Obtain the LNO issued by USDA-FSIS

7.0 Harvest to Carcass Monitoring

This section outlines a science-based microbiological sampling and testing program to be used in the validation and verification of harvest to carcass HACCP systems. The overall objective is to contribute to a reduced risk of foodborne illness from microbial pathogens. Programs such as the one described here, if used daily or multiple times during a production day (frequency will depend on the production volume), will provide additional information about the microbiological quality of the production process.

The role of microbiological testing during slaughter is to facilitate the implementation, validation, and verification of HACCP programs. Testing may be done before and after each

operational SOP and CCP to determine the effectiveness of a particular process step and/or the entire process for reducing microbial contamination. Testing could also be done at specific steps in the process for routine sampling (hides, carcasses after hide removal, carcasses after the full complement of all interventions and carcasses after chilling). Testing for indicator organisms (Aerobic Plate Count, *Enterobacteriaceae* Count, *Escherichia coli* biotype I Count) is the best approach to the validation and verification of a process control system that is designed to reduce the incidence of microbial contamination. When the objective of sampling and testing is process control, efficacy of interventions or any other process control related objectives, organisms of choice will be the ones that can be readily enumerated (indicator organisms) and not organisms that are not randomly distributed and/or rarely found (pathogens). Not only are aerobic organisms, *Enterobacteriaceae* and biotype I *E. coli* indicative of environmental and fecal contamination, but the higher expected frequency and levels of these organisms makes them much more suitable as process-control indicators than are pathogens. Following validation and routine HACCP implementation, microbiological criteria may be set for end product process verification testing. If problems are encountered, or a process is changed, it may be necessary to repeat testing before and after each operational SOP and CCP to assess the process.

To repeat, the authors believe that sampling carcasses for pathogens serves no valid scientific or statistical purpose, as pathogens are typically present at low levels and at a low incidence on carcasses and are not randomly distributed. These limitations make it impossible to statistically justify the use of pathogens for the validation or verification of a HACCP system in a beef slaughter plant.

Suggested Sampling Plan for APC, *Enterobacteriaceae* Count & *E. coli* Biotype I Count on Beef Carcasses

Frequency of sampling:

<u>For CCP validation, routine process verification and other SOPs:</u>

The number of samples required will depend on the expected difference in the indicator counts before and after the CCP or the step in the process being validated. For a 1.0 log resolution difference, 12 samples before and 12 samples after are needed. For a 0.5 log resolution 42 samples before and 42 samples after are needed. The above criteria use a power of 80% and a standard deviation of 0.80 of log transformed means.

Recommended sampling procedure:

Sponge sampling follows a modification of procedures described in the USDA-FSIS Meat and Poultry Inspection regulations for *E. coli* (biotype I) carcass surface sampling:

- Label the sponge bag prior to sampling and include necessary sample information on the label.
- Wash hands with soap and apply sanitizer prior to sampling.
- Aseptically put on nitrile or similar gloves. Collect sample with a sterile sponge pre-hydrated with a sterile neutralizing buffer. Remove sponges from the bag with the hand

not used to sample with by pushing the sponge up from the outside of the bag. Open the bag and grasp the sponge with the "clean" gloved hand and remove the sponge from the bag.

- Do not let the sponge touch any surface except the designated sampling surface. Discard any sponges that touch surfaces other than sampling surfaces.
- Hold the sponge firmly and sample a surface area by passing the sponge back and forth. Sponging will consist of at least 10 passes vertically (up-and-down being considered as 1 pass) and 10 passes horizontally (side-to-side being considered as 1 pass) with a pressure equivalent of that required to remove dried blood from the surface.
- Carefully place the sponge back into the sterile sample bag without touching the opening of the bag.
- Expel excess air from the bag before it is closed, folded down and secured with wire tabs in preparation for delivery to the laboratory.

Recommended Sampling Area:
Based on literature and/or knowledge of one's own plant, areas of the carcass suspected to be contaminated should be selected for sampling. The likelihood of detecting indicator organisms in anal/hock and brisket areas is far greater that the back side of the carcasses, hence it makes sense that these areas of the carcass should be selected for sampling. As for how much area should be the sampled, the larger the area the better. We recommend 4000 cm^2.

Microbiological Criteria
The target should initially be set below plant baselines for each step in the process with the exception of the hide which cannot be controlled. The goal should be to progressively reduce the levels of APC, EBC and ECC. Target for these indicators on the finished carcass (carcass after the full complement of all interventions) should be:

- APC: less than 2 log CFU/100 cm^2
- EBC: below the detection limit
- ECC: *E. coli* biotype I counts should at least meet current FSIS regulatory requirements.

Reference and additional reading:

1. http://www.fsis.usda.gov/PDF/HACCP_Validation_Ltrs.pdf
2. Arthur, T. M., J. M. Bosilevac, X. Nou, S. D. Shackelford, T. L. Wheeler, M. P. Kent, D. Jaroni, B. Pauling, D. M. Allen, and M. Koohmaraie. 2004. Escherichia coli O157 prevalence and enumeration of aerobic bacteria, Enterobacteriaceae, and Escherichia coli O157 at various steps in commercial beef processing plants. *J Food Prot*. 67:658-65.

8.0 Data Analysis and Utilization

Data collection and analysis are critical to ensuring food safety success in the beef industry, for both process control and pathogen control. There are several considerations when planning

and discussing data collection: question to be addressed, sources of variation, measures to be collected, data aggregation/analysis and recordkeeping.

As a beef industry, millions of data points are collected annually for internal, HACCP / regulatory or for customer auditing purposes. Data collection for any of these reasons should be intentional, meaning there are specific questions to be answered by the data collected. Prior to developing a sampling plan for data collection, efforts should focus on the top 2-3 questions which are to be answered by the data collected. From these questions, specific objectives and hypothesis can be developed. Determining objectives for data collection can prevent unnecessary costs and labor and provide information relevant to specific problems or concerns. The specificity of the objectives is important as well since too broad an objective can prevent in depth conclusions and answers to make improved food safety choices.

Once objectives are determined, outcome measures can be specified along with potential sources of variation. The combination of a testable hypothesis, measurable results, and sources of variation lead to selection of appropriate analytical methods and identification of a sampling plan and sample numbers to meet the data collection objectives. Sample size calculators rely on correct identification of these components to provide sufficient power for testing study objectives. It is important to review the objectives and experimental design to ensure data collected will answer the original questions. There are many options for statistical analysis and it can be complicated so having someone inteam discussions that can provide statistical input is recommended.

8.1 Data Collection Frequency

Data collection frequency is important for monitoring trends and comparison to historical perspectives. The balance of time and knowledge is important to determine during this process and should be based on the identified sources of variation and level of desired data aggregation. A longer history of data collection allows a better picture of process control and can determine trends otherwise missed in a short-term collection. Long term collection also allows a statistician and data scientist to use predictive modeling to identify influences on outcomes related to changes in the process. However, predictive modeling is not effective without the observation of the long term data trends. Building a history within each operation takes time and intent but will allow extremes, seasonality, and trends to be defined. This will allow reactions to any food safety concerns to be early and successful.

8.2 Selecting Appropriate Measures

There are many different methods of sample collection and analysis. Clearly understanding and stating expectations of the sample analysis is critical in understanding trends and data conclusions. Prevalence and load of pathogens allow different perspectives of control in food safety systems. In addition, laboratory methods can have an effect on observed variation. Therefore, understanding the methods used and keeping a record of any changes to

methodology allows improved understanding of the data results.

8.3 Data Aggregation and Analysis

The key to preventing feeling overwhelmed with data is to develop dashboards and implement routine monitoring. Choosing only a few key metrics that align well with the objectives keeps everyone on track to achieving each plant's food safety goals. Metadata can be just as important as the outcome measurement. As an example, time of sample collection, herd data, concentration, source, etc., are all relevant information that potentially influence a sample result. These inputs can be useful when creating a system to then predict and influence outcomes.

There are many useful statistical tools so beware of black box statistical methods and hinging action on p-values less than 0.05. Software will give you an answer but will not tell you whether you have used it correctly. Ensuring that the distributional assumptions of the measurements matches the analysis method selected is vital to correct use of any statistical method and even creation of appropriate data summaries. P-values are influenced by the amount of data you provide, the variation, and the magnitude of the effect; a difference with a practical importance may be worth pursing even if the p-value of 0.08. In fact, many scientists and journals are moving away from the legendary $P<0.05$ means significance. What magnitude of difference is significant in your operation; to answer that you must step back and look at what's important.

8.4 Recordkeeping

Recordkeeping is probably the most important component to having a reliable data source for long term trends, potential problems, and predicting. Maintaining a time log of changes in the system such as equipment or intervention, laboratory methods, feed suppliers, vaccine strategies, and nutritional changes can help determine the "why" behind any pathogen or food safety changes. Change is the only constant, and to understand trends and be able to predict, each processor must be able to document these changes to determine or explain any food safety related changes.

8.5 Final Thoughts

Data collection and analysis can be overwhelming, especially since there is rarely a team designated for this task in plants. Studies can be long term and can involve samples at several collection points which can also become labor or cost prohibitive. However, it is important to keep in mind that any data collection is critical to continuous improvement. Within the beef industry, it is imperative that plants are knowledgeable about their process and process controls as well as their food safety risks. Even if processors begin with a few data points and build upon those over time, the data collected can still be used for determination of history, trends, and areas of improvement. A statistically valid (statistically powered) study or to show a change in the system that is beyond the variability present, is important but if this is not

possible then collecting data for routine monitoring still provides relevant information for system variation and can be indicative of deviations in the system. This monitoring data can still be used in decision making for food safety process improvements.

8.6 Conclusion

The most important aspect of data analysis and utilization is to just get started. Even though it is critical to spend some time determining the question to be addressed, sources of variation, measures to be collected, data aggregation/analysis and recordkeeping, what is most important is that data are collected, and that the data collected is reviewed and discussed frequently so that continuous improvements can be made. Data collection and analysis allows the beef industry to tell the story of continuous improvement. If there are no data, there is no story, and there are no continuous improvements in the system.

9.0 Summary

It is generally understood that companies cannot test their way to safe food, but microbiological testing should be part of the food safety systems for beef processing. The sampling plan is critical for any microbiological testing. You must know why you are testing and how you will react to the results. Depending on the goals of testing, it may involve pathogens, indicator organisms or pathogen index targets, but regardless the method should always be “fit for purpose”. Microbiological criteria can be standards, guidance, or specifications. Non-destructive surface rinse sampling can be used for process control indicator organism data collection. Microbial reduction validation can be for demonstrating that previously established parameters are met or for direct microbial reductions of an in-plant process. Validated processes should be verified periodically to ensure they are functioning as intended. Sampling and testing the plant environment can provide pre-operational sanitation verification and trouble shoot contamination issues. Compliance sampling by FSIS is intended to be regulatory verification of the proper functioning of the food safety system as defined by the HACCP plan. Investigative sampling is intended to identify the root cause of a past or potential contamination problem. Several sampling methods have been validated for various sample types. Data collection, analysis, and utilization can be very complicated, but with proper planning it can be a critically important part of the food safety system that drives continuous improvement in providing safe food to customers and consumers.

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AMSA fosters community and professional development among individuals who create and apply science to efficiently provide safe and high quality meat defined as red meat (beef, pork and lamb), poultry, fish/seafood and meat from other managed species.

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