

The Role of Microbiological Testing in Beef Food Safety Programs

The Scientific Perspective



The American Meat Science Association

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Consensus of the 1999 Symposium

American Meat Science Association

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Contents

Executive Summary	1
Consensus Document.....	2
Introduction, 2	
Sampling, 2	
Microbiological Components and Analytical Methods, 3	
Recommendations for Application of Microbiological Criteria, 3	
Microbiological Sampling for Pathogens, 4	
Food Safety Objective, 5	
Consensus and Conclusion, 5	
References, 8	
Appendix 1 – Sampling & Testing Ground Beef for <i>E. coli</i> O157:H7	9
Appendix 2 – Science Based Applications of Microbiological testing to Fabrication and Trimmings	11
Appendix 3 – Harvest to Carcass	13
Appendix 4 - Role of microbiological testing with regard to sanitation of beef plants	15

Foreword

The American Meat Science Association's mission is to contribute to the betterment of human life through discovery and application of sound scientific and technological principles of the meat sciences in research and education.

On January 20-22, 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 evaluation of sampling programs. Thus, the following objectives were identified:

- 1) To assess current the concept of microbiological testing in a food safety system.
- 2) To describe and define the process to make standardized procedures for microbiological sampling.
- 3) To identify and assess valid statistical approaches to evaluate microbiological sampling plans.
- 4) To assess or examine strategies for *E. coli* O157:H7 sampling for industry applications and economic considerations of these strategies.

Drs. Chris Calkins and Mohammad Koohmaraie co-chaired the symposium and assigned participants to the following working groups: Harvest to Carcass, Fabrication/ Trimmings, Ground Beef, and Sanitation. Individual committee reports were presented during the symposium

followed by discussion of the reports by those in attendance. A consensus conference was held on the last day in which summaries from each group were presented in an effort to reach a consensus on the points presented. Eight consensus points were agreed upon by the participants.

The decision to conduct microbiological testing on beef products by a company will be based on a variety of factors such as: science, humanitarianism, public relations, politics and legal liability. The consensus points agreed upon by the participants of the symposium were, however, based on the availability of scientifically verified data. Microbiological testing is but one component of a food safety assurance program and should not be viewed as a stand-alone approach to food safety. The relative value of microbiological testing for pathogens is always being re-evaluated in light of legislative, legal and public relations concerns and will undoubtedly change with the advent of new detection technologies. Because specific sampling recommendations were made by some working groups within the context of their discussions, these conclusions have been included in the Appendix.

Jimmy T. Keeton
President

Executive Summary

Public concerns over the wholesomeness of the food supply have increased greatly in recent history. These concerns have resulted in increased efforts by the industry to improve the microbiological status of beef products, and by regulatory authorities to implement new requirements and procedures in meat inspection. Another outcome of these concerns and efforts is an increased emphasis on testing products for pathogens (e.g., *Escherichia coli* O157:H7 in raw beef) as a means of assuring consumer safety. The emphasis on product testing has been the subject of debate in the scientific community.

Microbiological testing is an area where a large amount of scientific research has been conducted, yet there is increasing confusion among regulators, industry and the public concerning what can and cannot be accomplished with testing. In January, the American Meat Science Association convened a group of 35 scientists to address the role of microbiological testing in beef food safety programs. The primary achievement of this group was the development of eight consensus points focusing on the effective use of sampling and testing to support a food safety program. The group agreed that:

1. The main purpose of microbiological testing of foods is to validate and verify process control measures in the context of a properly implemented HACCP system.
2. Effective microbiological testing programs are based on sound Food Safety Objectives with definable microbiological performance criteria.
3. Pathogen testing at any stage will not assure food safety.
4. Foodborne pathogens will not be detected consistently when they are not randomly distributed and/or occur at a low incidence.

5. Pathogens or other microorganisms at a low incidence cannot be used to assess process control.
6. Testing for appropriate non-pathogenic organisms will allow validation and verification of process control systems designed to improve food safety.
7. Declaration of a foodborne pathogen as an adulterant in raw products (e.g. *E. coli* O157:H7 in beef). . .
 - discourages testing for that pathogen,
 - leads to a false sense of security among consumers,
 - discourages evaluation of potential control measures, and
 - encourages the inappropriate use of microbiological testing.
8. Microbiological testing of foods in production is important, but is only a part of the overall strategy for controlling food safety. Education concerning proper handling and cooking is essential.

A detailed rationale for these consensus points follows in the next section.

During the course of the meeting, the scientists worked in focus groups to address specific areas of interest. Reports from each of the following groups are included as Appendices to this report.

Sampling and Testing Ground Beef For *E. coli* O157:H7

Science-Based Applications of Microbiological Testing (Sampling and Analyses) to Fabrication and Trimmings

Harvest to Carcass

Role of Microbiological Testing With Regard to Sanitation of Beef Plants

Consensus Paper

Introduction

Successful commercial production of a food product requires control of microbiological contamination and activity to achieve maximum shelf life consistent with safety of the product. Microbiological testing programs may be applied to validate and verify hygiene monitoring or the process of a food, but such programs need to be associated with achievable and verifiable microbiological criteria. Thus, microbiological criteria can provide a tool for evaluating the acceptability of a process or food. However, development and application of microbiological criteria must follow established basic scientific and statistical principles, and success will depend upon a thorough understanding of the raw materials, the food production process, and the significance of various members of the microbial flora.

A microbiological criterion is a standard upon which a judgement or decision regarding acceptability of a food or food product can be made. In most cases, a criterion will specify that a certain microorganism, a group of microorganisms, or a microbial toxin be absent or limited in presence in a specified quantity of food or ingredient. A microbiological criterion should include the following information (NRC, 1985):

1. a statement describing the identity of the food or food ingredient,
2. a statement identifying the contaminant of concern,
3. the analytical method to be used for the detection, enumeration, or quantification of the contaminant of concern,
4. the sampling plan, and
5. the microbiological limits considered appropriate to the food and commensurate with the sampling plan.

Microbiological criteria may be used to assess the safety of a food ready for consumption and, therefore, may involve tests for specific pathogens or toxins of concern. Tests for indicator organisms may be used successfully only when sufficient data have been collected to establish or indicate a relationship between the occurrence or level of the indicator organism and the likely presence or control of a pathogen or toxin. The use of appropriate indicator organisms is especially helpful for validating process implementation and for verifying control at a specific criti-

cal control point (CCP) within a hazard analysis critical control point (HACCP) system. The ultimate purpose of these criteria is to protect the consumer's health.

Microbiological criteria may also be used to make decisions regarding the acceptability of products, or the efficacy of processes, if such criteria are designed to measure adherence to Good Manufacturing Practices (GMPs), HACCP and sanitation standard operating procedures (SSOPs). Criteria can be used to determine the appropriateness of a food or ingredient for a specific purpose. In addition, industry quality assurance programs may use criteria to monitor or predict the potential shelf life of perishable foods.

Sampling

An essential component of a microbiological criterion is an effective sampling plan. To examine a food for the presence of microorganisms, either the entire lot must be assayed or a representative sample should be obtained. A lot is defined as a discrete quantity of product produced, handled, and stored within a limited time period under uniform conditions. The lot is made up of sample units; a sufficient number of units must be selected from the lot for microbiological evaluation in order to determine the acceptability of a lot. Since it is impractical to assay the entire lot, statistical concepts of population probability and sampling must be used to determine the appropriate size of the sample from the lot and permit conclusions to be drawn from the analytical results. The sampling plan must be designed so that it rejects inferior lots with a set level of confidence. 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 a publication by the International Commission on Microbiological Specifications for Foods (ICMSF, 1986).

Two-class plans. A simple method for determining whether to accept or reject a food lot can utilize a microbiological test conducted upon several randomly-selected sample units (n) with a preset maximum number of sample units allowed to yield unsatisfactory results (c). The test will either determine the presence/absence of an organism or it will determine whether microbial levels are above or below a preset concentration (m). Thus, in a two-class

sampling plan designed to make a presence/absence decision on the lot, $n=5$, $c=2$ means that 5 sample units are obtained and examined; if more than 2 of the samples show the presence of the organism of concern, the lot is rejected.

Three-class plans. Three-class plans were designed for situations in which the quality of the product can be divided in three attribute classes based upon the concentration of the organisms within the sample units; 0 to m , m to M , and greater than M . The level of the test organism which is acceptable in the food is denoted by m . M is a hazardous or unacceptable level of contamination. Any count above a concentration M is considered unacceptable; therefore, a count from any of the n sample units exceeding M will result in rejection of the lot. In a three-class plan, c indicates the number of sample units that can contain a concentration above m but only up to and including M . This m to M classification of sample units has been determined to be less than desirable, but some level of microbial contamination of a few sample units (c) will be allowed without rejecting the lot. Thus, in a three-class sampling plan, the food lot will be rejected if the microbial level of any one of the sample units exceeds M or if the number of sample units with contamination levels from m to M exceeds c .

The sampling plan specified in a microbiological criterion should be appropriate for the severity of the hazard expected and its expected incidence in the food. The severity of the expected hazard should reflect not only the type of organism expected to be encountered, but also the handling conditions expected to be applied to the food after sampling. A more stringent sampling plan should be used as the expected degree of hazard increases and the incidence of the hazard decreases. Stringency is affected by both n and c ; the more severe the hazard, the higher the n and the lower the c (NRC, 1985; ICMSE, 1986).

Microbiological Components and Analytical Methods

Microbial components of microbiological criteria of foods include pathogenic bacteria, microbial toxins, and indicator organisms. Adequate, practical and validated methods must be available to detect or enumerate the microbiological component if the criteria are to be effective. Pathogenic bacteria useful as components of microbiological criteria include those that are likely to be found in a ready-to-eat food. Suitable indicator organisms are those whose presence indicates:

1. the likelihood that the pathogens or toxins of concern may be present, or

2. the likelihood that faulty manufacturing practices, or failure of control processes, occurred and may have adversely affected the safety or quality of the product, or
3. that the food or ingredient is not suitable for the intended use.

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 validity of the results.

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). 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 recommended (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. Developments in the U.S. during the last five years have contributed to implementation of most of these recommendations. Through research, industry initiatives, consumer demands, news media scrutiny, and regulatory reform (FSIS, 1996b), adherence to GMP principles is improving, various interventions are being applied to reduce raw meat contamination, HACCP principles are being implemented, and various educational programs have been developed for food handlers and the consumer.

The Codex 'General Principles for the Establishment and Application of Microbiological Criteria for Foods'

(Codex, 1981, 1997) state that a microbiological criterion should be established and applied only where there is a definite need and where application is both practical and likely to be effective. Since current livestock production practices cannot provide pathogen-free live animals, the occurrence of pathogens in raw meat and poultry cannot be entirely prevented by the application of strict sanitary and hygienic principles. Exclusion of pathogens from raw meat and poultry is unlikely without the application of verifiable CCPs which result in pathogen inactivation. The distribution of pathogens in live animals, carcasses and raw meat products such as trimmings and ground beef is extremely variable (non random or unevenly distributed). This variability severely limits the degree of confidence with which a sampling plan can indicate the absence of a particular pathogen in a lot. For example, *Escherichia coli* O157:H7, which has been declared an adulterant in certain raw beef products, occurs sporadically, in low numbers, and is unevenly distributed in those products.

Meat processing controls microorganisms and enhances food safety through the development and use of procedures designed to restrict microbial contamination and growth. Control of processes designed to ensure microbiological safety is managed and monitored by a HACCP system as required by current United States regulations (FSIS, 1996b). The retrospective nature of microbiological testing makes it inappropriate for use in monitoring a CCP if the product is out of control of the producer by the time the results are available. Analysis of the product and the processing environment can be used to validate and verify the effectiveness of a CCP as well as the effectiveness of GMPs and sanitation practices. Aerobic plate counts (APC), or counts of other commonly-accepted indicator microorganisms (e. g., coliforms, *Escherichia coli* biotype I, *Enterobacteriaceae*), can be used to verify proper application of processing procedures, sanitation programs and GMPs. Criteria based upon such examinations are a valuable aid in establishing effective control programs. While these criteria may be effective for evaluating processing conditions (including sanitation, carcass dressing, fabrication and grinding) at the point of production, the perishable nature of the product and the potential for subsequent contamination and microbial growth limit the validity of using microbiological criteria at the retail level or at port of entry. In 1973, the state of Oregon (State of Oregon, 1977) set microbiological standards for fresh and frozen red meat at the retail level and revoked the standards four years later because: (1) the standards were unenforceable and created a general adverse reaction, (2) there was no evidence of reduction of food-borne disease or improvement in quality characteristics of the meat, and (3) the standards may have created erroneous

consumer expectations of improved quality and decreased hazard.

Microbiological Sampling for Pathogens

In what would seem to be the simplest and most direct method for determining the presence of pathogenic bacteria in beef, production lots can be sampled and tested directly for the microorganism using any of several classical or rapid microbiological tests. The principal question is, how many sample units must be collected and analyzed to have a high probability of detecting the presence of the pathogen? Suppose that a large number of sample units (hundreds) have been collected from the lot and analyzed, and that all the samples appear to be negative for the target pathogen. Does this mean that the lot is free of the pathogen? If the producer has data indicating the probable frequency of a pathogen in sample units from a lot, it is possible to determine the probability that all samples collected and analyzed from the lot will be negative for the target pathogen. For example, using data from the FSIS Nationwide Federal Plant Raw Ground Beef Microbiological Survey (FSIS, 1996a) and the ongoing ground beef sampling program (FSIS/OPHS, 1998), one can expect to find *E. coli* O157:H7 in 0.1% of ground beef nationwide. If 100 samples are collected and analyzed from a lot of ground beef, what is the probability (Pr) that all 100 samples will be negative?

$$\text{Pr} = e^{-(100)(0.001)} = 0.90$$

In 9 of every 10 examinations of this lot, all 100 samples are likely to be negative. Conversely, the analyst would expect to detect a positive sample in the lot only 1 out of every 10 occasions that 100 samples from this lot are examined (Dodge and Romig, 1959; Messer et al., 1992). Of course, it is assumed that the pathogens will be detected by the analytical method used (when, in fact, they may not be), and that the pathogen is randomly or evenly distributed within the product (which is highly unlikely). Therefore, the fact that 100 sample units from the lot have been examined without detecting the target pathogen does not eliminate the possibility that the pathogen may still be present in the lot.

Another way to view the problems inherent in sampling plans for the detection of low levels of pathogens within a lot is to determine the number of sample units needed to detect a known or expected level of contamination. Again, accepting the expected incidence of *E. coli* O157:H7 in ground beef as 0.1%, and assuming that at least one sample unit per lot is determined to be contaminated, the following number of samples (n) from a contaminated lot must be examined in order to detect the pathogen with probabilities of 0.90, 0.95 and 0.99:

$n = -\ln(0.10)/(0.001) = 2,303$ samples at a probability of 0.90

$n = -\ln(0.05)/(0.001) = 2,996$ samples at a probability of 0.95

$n = -\ln(0.01)/(0.001) = 4,605$ samples at a probability of 0.99.

Even if it were to be assumed that *E. coli* O157:H7 is present at an incidence ten times higher (1.0%) than that detected by FSIS in their nationwide ground beef survey and ground beef sampling program (FSIS, 1996a; FSIS/OPHS, 1998), the number of samples needed to detect a contaminated lot is still very high:

$n = -\ln(0.10)/(0.01) = 230$ samples at a probability of 0.90

$n = -\ln(0.05)/(0.01) = 300$ samples at a probability of 0.95

$n = -\ln(0.01)/(0.01) = 461$ samples at a probability of 0.99.

Recognizing that the expected incidence of *E. coli* O157:H7 is extremely low, it may be instructive to run these same statistical scenarios using a pathogen with a higher incidence in raw beef. Using FSIS survey data for an expected incidence of *Salmonella* of 7.5% in ground beef (FSIS, 1996a), the following number of samples from a contaminated lot (n) must be examined to detect the pathogen with probabilities of 0.90, 0.95 and 0.99, when at least one positive sample unit is detected:

$n = -\ln(0.10)/(0.075) = 31$ samples at a probability of 0.90

$n = -\ln(0.05)/(0.075) = 40$ samples at a probability of 0.95

$n = -\ln(0.01)/(0.075) = 61$ samples at a probability of 0.99.

Again, even when collecting sufficient samples for a 0.99 probability of detection, nonuniform distribution of pathogens within the lot will still result in a chance that the consumer will be exposed to contaminated product from a lot deemed to be safe by microbiological testing. It is clear that establishment of microbiological criteria for pathogens on carcasses, trimmings or raw ground beef will require extensive microbiological sampling and testing at a significant cost, and still will not guarantee absence of the target pathogen in the lot.

Food Safety Objective

The ICMSF has recommended steps for the management of microbiological hazards in foods in international trade (ICMSF, 1998). The same principles can be applied to food in domestic trade and to beef safety. The steps incorporate existing Codex documents that can be applied in a logical sequence. In Step 1, the Principles and Guidelines for the Conduct of Microbiological Risk Assessment are applied to provide an estimate of the public health

impact associated with the hazards in food. This information provides the scientific basis for subsequent risk management decisions.

In Step 2, options that may be available for managing the identified risk(s) are considered. One outcome of the risk management option assessment process may be to establish a Food Safety Objective (FSO). FSOs are the result of deliberations of risk managers in consultation with risk assessors, affected industry and consumers (ICMSF, 1998). The FSO can be defined as the maximum level of microbiological hazard in a food considered acceptable for consumer protection. Establishment of the food safety objective must consider public health impact and technological feasibility. Whenever possible, FSOs should be quantitative and verifiable. The safety margin of FSOs should reflect the confidence in the risk assessment. Thus, the FSO must be more stringent when the risk assessment is more uncertain.

FSOs must be technically achievable through the application of General Principles of Food Hygiene and the HACCP system. In addition, because good hygiene practices (GHP – a European term similar to the American “GMP”) and HACCP are the only tools available, FSOs must be based upon a realistic assessment of what can be achieved through GHP/GMP and HACCP.

One disadvantage of microbiological criteria is that they do not take into account a scientific assessment of the hazard’s estimated impact on the public’s health. Risk managers are frequently at a loss in developing criteria that are meaningful in addressing key public health issues. It is the intention that FSOs would fill this void. A FSO deals with food safety issues and does not address issues of quality and stability. FSOs are broader in scope than microbiological criteria and are intended to communicate the level of hazard that is considered acceptable to the consumer (ICMSF, 1998).

Consensus and Conclusion

The following consensus points were agreed upon by the participants in the symposium. Consensus points are listed in the order in which they were discussed; listing order is not intended to indicate priority.

1. *The main purpose of microbiological testing of foods is to validate and verify process control measures in the context of a properly implemented HACCP system (Codex, 1997; NACMCF, 1998). Safety of beef products may only be assured through a chain of controls from production through consumption. Microbiological control measures within processing steps in that chain may be validated by microbiological testing. If sufficient data are available to indicate that the behavior or incidence of a particular pathogen or indicator organism is correlated with appropri-*

ate levels of process control, process control points may be developed through research, validated during initial implementation, and verified periodically using microbiological testing. Pathogen testing as a means of HACCP verification is only supportive if incidence is high, distribution is random, and numbers are high enough to reliably permit detection. Expected low numbers and nonrandom distribution of pathogens in meat and poultry products severely impair the usefulness of pathogen testing for verification. Sampling plans used to detect indicator organisms in order to monitor and verify processes may be based, when appropriate, on ICMSF and Codex guidelines (Codex, 1981; ICMSF, 1986).

2. *Effective microbiological testing programs are based on sound Food Safety Objectives (FSOs; ICMSF, 1998) with definable microbiological performance criteria.* FSOs are the result of deliberations of risk managers in consultation with risk assessors, affected industry and consumers. The FSO can be defined as the maximum level of a microbiological hazard in a food considered acceptable for consumer protection. Establishment of the FSO must consider public health impact and technological feasibility and, whenever possible, food safety objectives should be quantitative and verifiable. The safety margin of FSOs should reflect the confidence in the risk assessment; thus, the FSO must be more stringent when the risk assessment is more uncertain.
 3. *Pathogen testing at any stage in food processing will not assure food safety.* Incidence of pathogens, such as *E. coli* O157:H7, on live animals, carcasses, trimmings and ground beef products is non-random and infrequent. Therefore, even when collecting sufficient samples to permit a 0.99 probability of detection, there is still a possibility that pathogens will be present but undetected in a lot of product.
 4. *Foodborne pathogens will not be detected consistently when they are non-randomly distributed and/or occur at a low incidence.* Even when collecting sufficient samples for a 0.99 probability of detection, nonuniform distribution of pathogens within the lot will still result in a chance that the consumer will be exposed to contaminated product from a lot deemed to be safe by microbiological testing. The scientific application of microbiological criteria for pathogens in raw beef will require an extensive amount of microbiological sampling to detect low numbers of pathogens of low incidence at a significant cost, and still will not guarantee absence of the target pathogen. Proper implementation of scientific HACCP principles
- is a better investment for effective pathogen reduction than is product testing. Implementation of the principles of HACCP and product testing for pathogens of infrequent, low and nonrandom occurrence are not comparable in effectiveness for process control. Testing for pathogens in the present context is too unreliable and would be no substitute for the HACCP approach.
5. *Pathogens or other microorganisms which typically occur in the food at a low incidence cannot be used to assess process control.* Effective verification of process control by microbiological sampling and testing requires the analysis of microorganisms that are present or absent with predictable regularity and in numbers that permit reliable detection. Further, the level of presence of the target microorganisms must change in response to the process. Sampling for a pathogen which is normally present infrequently and non-randomly is expected to provide almost no information about process control, since an inability to isolate the pathogen could be due to either the process or simply due to the absence of the microorganism at that particular time in that particular product.
 6. *Testing for appropriate non-pathogenic (indicator) organisms will allow validation and verification of process control systems designed to improve food safety.* For instance, if a processing control point designed to reduce the presence of pathogens is challenged with the pathogen under experimental conditions, a level of possible control can be established. If parallel data are collected using appropriate indicator organisms (e.g., coliforms or *E. coli* biotype I to indicate control of enteric pathogens), a similar level of reduction or control can be established. Control of the indicator organism may then be reliably used to indicate expected pathogen control in commercial application.
 7. *Declaration of a foodborne pathogen as an adulterant in raw products (e.g., *E. coli* O157:H7 in certain raw beef products): discourages testing for that pathogen; leads to a false sense of security among consumers; discourages evaluation of potential control measures; and, encourages the inappropriate use of microbiological testing.* The unavoidable, infrequent, and non-random presence of *E. coli* O157:H7, and the lack of a process to assure the elimination of this organism in raw beef products all argue against its classification as an adulterant. Legal liability issues centered on the adulterant classification severely impede attempts to learn more about *E. coli* O157:H7 in raw beef, and to develop better control procedures.

8. *Microbiological testing of foods in production during processing is important, but such testing is only a part of the overall strategy for controlling food safety. Education concerning proper handling and cooking is essential.* All too often, a discussion of food safety in the news media concentrates on the microbiological testing of food. While microbiological testing is a helpful tool in the overall assurance of food safety, statistical expectations and microbiological realities make testing insufficiently reliable for stand-alone use. With extensive public outcry from various groups to implement more and more testing, the industry is under intense pressure to invest significant time and resources into weaker areas of process control, possibly leading to neglect of other, more effective aspects. Education of the consuming public and the news media is needed – the message must be that the safety of food cannot be assured predictably through testing, but can only be attained through process control. The industry should emphasize the continuous improvement of process control measures instead of extensive pathogen testing programs that are intrinsically unreliable.

The intense coverage of foodborne outbreaks in the news media in recent years has placed responsibility for safety problems mostly on the food industry and regulatory authorities. While blame for a problem is certainly expected to be a major part of any news media coverage, limited understanding of food processing leads the media to make poor assignments of responsibility for food safety. In the process, the food handler and consumer are not always adequately instructed on how to easily protect the food and themselves from exposure to enteric pathogens. It is encouraging to note that recent food safety news stories often include segments on proper food handling, sanitation and hygiene. Also, it should be recognized that news media coverage has increased interest and awareness in food safety issues and has contributed to the support of activities that enhance food safety. Nevertheless, education programs for food handlers, consumers and the news media are needed to better address this problem and contribute to an overall enhancement of the safety of our food supply. Educational efforts must also be aimed at elementary and high-school students in order to ensure that safe food handling practices become a part of our collective conscience.

In conclusion, a number of outbreaks of foodborne illness caused by *Escherichia coli* O157:H7 have been linked with consumption of undercooked ground beef.

Retrospective investigations of these outbreaks have shown that when the organism can be isolated from implicated lots of ground beef, it is present only in a small percentage of samples examined and at low levels (less than 500 cfu/g; usually much less). Also, undercooked product consumed in the vast majority of these outbreaks was not just slightly undercooked, but grossly undercooked. Available evidence indicates that most outbreaks of *E. coli* O157:H7 illness linked to ground beef can be attributed to product contaminated at low, often undetectable, levels which has been grossly undercooked before consumption.

Microbiological testing can be applied within a HACCP system to verify process control or application of a pathogen intervention procedure at a specific CCP. It is important to note, however, that verification activities are more accurate when used to verify the effectiveness of the process which will control hazards at a CCP rather than to verify the safety of the final food product. Implementation of the principles of HACCP and assurance of food safety through product testing for pathogens of infrequent and nonrandom occurrence are two mutually exclusive concepts. The principles of HACCP were developed because end product testing for pathogens was unreliable to assure food safety. With sufficient prior data collection, the reduction of a bacterial indicator at a point in processing can indicate that a specific pathogen also is being controlled effectively. This application of indicator organism testing is especially useful when pathogens are distributed unevenly and at levels too low to allow confirmation of process control through their testing. Although these conditions do apply to pathogen contamination of ground beef, production of raw ground beef currently does not include a processing step capable of consistently reducing the presence of pathogens, so indicator organism testing to ensure process control is a moot point.

Currently, food establishments usually include microbiological testing of end-products as verification activities in their HACCP plans (Hatakka, 1998). However, if microbiological verification activities are limited to end-product testing for pathogens, the ability to isolate the target pathogen will be affected by uneven distribution and infrequent occurrence of the pathogen on the product. Furthermore, testing end-products for the presence of an indicator organism without knowledge of the relative levels of the microorganism throughout the process and within the plant environment provides little information regarding process effectiveness. Since raw ground beef processing does not include a step capable of reducing the presence of enteric pathogens, verification of the effectiveness of a CCP in this process only provides confir-

mation that pathogens, if present, are not becoming a greater problem.

Available "investigative" sampling plans, developed by organizations such as the ICMSF, have been adopted for certain applications; the stringency of these plans is higher when the disease is more severe and the incidence of the agent is low. End-product sampling and testing for enteric pathogens of low and nonrandom incidence, such as *E. coli* O157:H7, may periodically allow detection of extremely contaminated lots of ground beef, but their occurrence is unpredictable. Thus, results of microbiological sampling and testing may mislead the public regarding the safety of raw ground beef, and fail to accomplish the greater goal of protecting the safety of this product.

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Appendix 1

Sampling & Testing Ground Beef for *E. coli* O157:H7

The deliberations of this group were geared towards the larger grinding operations that cater to large food-service establishments. These recommendations assume the existence of control systems to manage microbiological safety and the use of microbiological sampling and testing plans to support those systems. These recommendations may not be suitable for smaller operations, and are not relevant at the retail level.

Because *E. coli* O157:H7 usually occurs sporadically in very low numbers, and is unevenly distributed, it is not possible, by any practical means, to sample ground beef sufficiently comprehensively to determine whether it is free from the organism (Table 1). In rare instances, levels of contamination are higher and these levels may be detected by the use of an appropriate sampling plan. Thus, it may be possible to reduce the number of cases of human illness due to *E. coli* O157:H7 by excluding affected raw product from the human food chain. Whether this reduction in illness will be quantifiable will be dependent upon our ability to demonstrate a significant reduction in illnesses attributed to ground beef (e.g. number of cases/100,000/year).

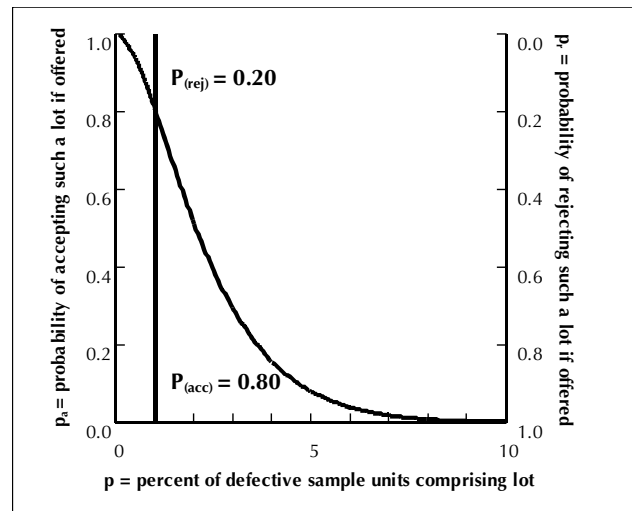
An equally acceptable alternative to finished product testing would be the use of pre-tested raw materials. The use of raw material testing to monitor the efficacy of intervention technologies and process controls at the slaughter level would permit the exclusion of known contaminated raw materials and, thus, reduce the requirement for finished product testing. While no sampling and testing program can assure the complete exclusion of *E. coli* O157:H7, screening of raw materials used to make ground beef can preclude the use of those materials that test positive.

A sampling plan established for ground beef should be based upon the Codex principles. For those establishments that choose to sample ground beef as a management tool, it is recommended that the sampling plan consist of 15 samples per lot (e.g. per half shift or 4 hours of production). This proposed sampling plan will provide a 95% confidence level that acceptable product will contain no more than 1 CFU *E. coli* O157:H7 per 125 g (i.e., no more than 20% of 25-g sample units in the lot will contain *E. coli* O157:H7).

TABLE 1. Probability of accepting a defective lot with indicated proportion of defective sample units.

% Defective	Number of sample units tested			
	15	30	60	100
0.1	0.99	0.97	0.94	0.90
0.5	0.93	0.86	0.74	0.61
1.0	0.86	0.74	0.55	0.37
2.0	0.74	0.55	0.30	0.13
5.0	0.46	0.21	0.05	0.01

FIGURE 1.



The operating characteristic curve for $n = 82$, $c = 1$, i.e. the probability of accepting lots, in relation to the proportion defective, among the sample units comprising the lots.

An appropriate sampling plan and criteria based on this approach would include the following components:

- 1) the nature of the microbiological hazard (e.g. *E. coli* O157:H7);
- 2) lot definition (e.g. half shift);
- 3) the number of sample units to be collected (e.g. number of patties, or quantity of ground beef);
- 4) a description of how the samples are collected;
- 5) a description of the analytical unit(s);
- 6) a description of both the sample preparation method and the analytical method; and

7) lot acceptance criteria ($n = 15, c = 0$).

Different sampling plans should be utilized for different pathogens based upon the risk to consumers and whether the risk will change between the time the product is sampled and the food consumed. The sampling plan will also depend upon the incidence and distribution of the target pathogen in ground beef.

Several voids in our knowledge of *E. coli* O157:H7 were identified during the development of the recommendations presented in this ground beef document. The group believes that answers to these knowledge voids may help clarify certain assumptions used to develop the sampling

and testing plans that are recommended in this document. Knowledge voids include:

- 1) baseline data on the prevalence of food borne illnesses caused by ground beef;
- 2) information on the distribution of *E. coli* O157:H7 throughout the beef production chain;
- 3) verification of the validity of composite sampling for *E. coli* O157:H7 in ground beef; and
- 4) information on the persistence or removal of *E. coli* O157:H7 in a processing system during a production run.

Appendix 2

Science Based Applications of Microbiological Testing (Sampling and Analyses) to Fabrication & Trimmings

The preferred method for microbiological (pathogen) control is the implementation of HACCP. However, in the absence of a step capable of reducing or eliminating microbiological contamination, a sampling/testing plan for trimmings could be integrated with the HACCP plan. This approach would be expected to reduce the chances of contaminated raw beef materials being further processed into ground beef.

The group does not believe that data from microbiological sampling and testing of trimmings can be used for decision making to improve food safety. This is because 1) no sampling and testing regimen (short of 100% testing) can eliminate the risk of pathogen presence; and 2) current preparation protocols for beef trimmings do not have a processing step capable of excluding or destroying pathogens. The group recognizes that data from microbiological sampling and testing can be used to improve the safety of beef trimmings. Specifically, sampling and testing can be used to:

- 1) detect more highly contaminated lots of trimmings which are more likely to yield ground beef which is associated with disease;
- 2) validate or verify process control;
- 3) identify out-of-control processes;
- 4) verify control of the process environment & equipment;
- 5) identify critical stages of the process as sources of contamination;
- 6) identify location, concentration and frequency of contamination;
- 7) establish an individual plant baseline; and
- 8) determine when the process produces trimmings of a microbiological quality which differs from that of the baseline.

Sampling plans

If sampling is done, it should be done according to a statistically valid sampling plan with a known probability of detecting a microbial contaminant, assuming that incidence is statistically random.

Identification of a Lot

A combo holds approximately 2,000 lbs. of trim. A lot is a maximum of 5 combos. A load is 20 combos.

Sampling of the Lot

Sampling plan is based on Case 13 of the ICMSF Plan (corresponding to Category 3 of the FDA Guidelines). Take five random samples (cores) from each combo to make a total of 13 lbs per combo. Grind each of the five samples individually and take three 25 g sub-samples from each sample. Combine the 15 sub-samples (25 g each) into a 375 g composite sample which will be tested after enrichment for the target pathogen (e.g. *E. coli* O157:H7).

If the composite sample tests positive for the pathogen, reject the lot

If the composite sample tests negative for the pathogen, accept the lot

Alternatively, different load sizes can be sampled by using the pallet as a basis for sampling. Three boxes from each of 5 pallets per load can be randomly selected. One (or more) core samples can be taken at random from each box to obtain a 25 g sample. The 15 sub-samples per lot can then be composited to obtain a 375 g sample from the lot. This composite sample can then be enriched and tested.

Assumptions and Limitations

- Pathogens may or may not be randomly distributed in beef trimmings
- If pathogens are randomly distributed, a sampling plan can perform at this confidence level:
<1 organism/125 g with a 95% confidence level for lot (no more than 20% of 125-g sample units in the lot will contain the pathogen)
or
<1 organism/500 g with a 95% confidence level for load (no more than 5% of 500-g sample units in the load will contain the pathogen)

Validation studies on sampling plans

The group believes that validation studies should be performed on any sampling/testing plan that is used for beef trimmings. Core/drilling sampling of the lot has been an accepted procedure in the collection of microbiological samples from a variety of bulk food commodities. The microbiological sub-sample that results from the coring/grinding protocol is indicative of the microbial concentration in the finished product.

The place of sampling

In the absence of a process step capable of reducing or eliminating microbial contamination on beef trimmings, a sampling/testing program is recommended. The sampling plan presented in this report is internationally accepted and used. Based on published risk assessment studies, this sampling plan will minimize the health risks associated with consuming food from the process. A similar sampling testing program has been accepted by USDA-FSIS for fermented sausage that is intended to be further cooked.

Appendix 3

Harvest to Carcass

The goal of this team was to develop 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.

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 for reducing microbial contamination. In the group's opinion, testing for indicator organisms (Aerobic Plate Count & *Escherichia coli* biotype I) is the best approach to the validation and verification of a process control system that is designed to reduce the incidence of microbial contamination. Not only are aerobic organisms and biotype I *E. coli* indicative of environmental and fecal contamination, but the higher expected frequency 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. In the event that problems are encountered or a process is changed, it may be necessary to repeat testing before and after each operational SOP and CCP in order to assess the situation.

The group believes 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. While localized or spot contamination of carcasses with pathogens can possibly occur, it is highly unlikely that such contamination will be detected by routine carcass testing. More likely contamination will only be detected after it spreads to fabrication equipment or is distributed in comminuted products.

The prevalence of *E. coli* O157:H7 on beef carcasses in the FSIS baseline studies (0.2%) and New Zealand baseline survey (0/2840 carcasses) prior to the use of decontamination interventions, and the expected lower prevalence with the use of such interventions, deems carcass

testing statistically unjustified. For instance, statistical analysis [$n = -\ln(0.05)/(0.002)$] indicates that a total of 1,498 samples would be required to provide a 95% confidence of detecting one positive carcass at a prevalence rate of 0.2%. The frequencies and period in which the one *E. coli* O157:H7-positive sample could be expected to be detected on beef carcasses are presented in Table 1.

TABLE 1. Frequency of carcass testing needed to detect *E. coli* O157:H7 when present at pre-intervention incidence levels (FSIS & New Zealand data)

If sample at this frequency	It will take this average time period before a positive sample is detected
1 carcass/month	125 years
1 carcass/week	28 years
1 carcass/day	4 years
1 carcass/300 carcasses	115 days (assuming 3,900 head/day are slaughtered)

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

Frequency of sampling:

For SOP or CCP validation:

Five randomly selected carcasses from each of five consecutive lots. A sample size of 25 provides 95% confidence of detecting a 0.5 log change in the mean count with a standard deviation of 0.6.

For routine process verification:

The same as for validation.

Recommended sampling procedure:

For operational SOP or CCP validation:

One randomly selected 100 cm² carcass site sampled by swabbing (e.g. by following the FSIS *E. coli* carcass-sampling procedure). Randomly selected carcasses should be sampled before and after each operational SOP or CCP. When validating a SOP or CCP for an operation that affects only a limited area of the carcass, targeted sites (likely to be impacted by that process step) must be sampled.

For process verification:

At least one randomly selected 100 cm² anatomical carcass site per carcass in the chiller. Alternative verification procedures may be used if they are demonstrated to be equivalent to random sampling for defining the performance of the process.

Methods of analysis:

Any analytical method accepted by FSIS and/or any method having AOAC approval.

Microbiological criteria:

Total plate counts:

The target total plate count should initially be set below plant baselines. The goal should be to progressively reduce levels to ≤ 2 log CFU/cm².

Escherichia coli biotype I counts:

E. coli biotype I counts should at least meet current FSIS regulatory requirements. The goal should be progressive reductions with process improvements; the ultimate goal is to reach undetectable levels with the stipulated sampling and analytical procedures.

Appendix 4

Role of Microbiological Testing With Regard to Sanitation of Beef Plants

The goal was to examine the role of microbiological testing in assessing the effectiveness of sanitation in beef plants. Essentially, sanitation is a microbial intervention step that impacts the safety of food products by addressing the food production environment and equipment.

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. Environmental sampling and testing programs must consider the exact objective of sampling & testing (e.g. general monitoring or specific trouble-shooting); the organism or organisms being targeted; the stage at which sampling is conducted (pre-operational versus operational); and the use to which the data will be put. Individuals writing the sampling program need to have an extensive knowledge of the equipment and the plant environment in order to identify sampling sites appropriate to a particular objective. The sampling and testing program must provide results which are useful in a retrospective sense since microbiological data are not available for at least 24 hours. Finally, a microbiological sampling and testing program must provide a framework within which results may be interpreted and tied to appropriate actions. In some cases, an environmental test result may have consequences for product manufactured in that facility or on that equipment; this is especially true of plants manufacturing ready-to-eat products.

Most microbiological testing of the plant environment is directed towards pre-operative sanitation since the microbial load in the plant environment and on equipment will increase once product is brought into the area. In addition to gauging whether sanitation procedures have been effective, microbiological testing can also be used to ensure that “niches” of pathogen growth do not exist. Sampling the plant environment and equipment for *Listeria* during production is useful in plant areas where ready-to-eat products are handled but would serve no purpose in slaughter or ground beef plants since animals and raw meats would be expected to carry a variety of *Listeria* species into those environments. Collection of environmental samples at various times during production is a strategy

frequently used when trouble-shooting spoilage or pathogen contamination issues.

Microbiological sampling and testing programs used to evaluate sanitation are much less formal and structured than are sampling plans directed at product. Statistics are seldom (if ever) used when developing environmental sampling plans. Limited statistical analyses (e.g. trending) may be conducted on quantitative data but data are more likely to be informally compared to a historical baseline, yielding a subjective conclusion as to the acceptability of sanitation procedures.

With the exception of standardized laboratory studies of sanitizer activity against known test organisms, quantitative data on the microbiological effectiveness of detergents or sanitizers are rare. Quantitative studies of sanitation efficacy (e.g. the “D-value” type approach) are rarely done, either by food plants or by companies selling sanitation chemicals. In part, this is because such data would be nearly impossible to obtain in the real world where microbial attachment is influenced by a multitude of difficult-to-measure variables and microbial removal or inactivation during sanitation are similarly subject to a variety of unmeasurable influences.

Microbiological sampling protocols — environmental samples

Sample-collection methodologies applicable to environmental samples have been described in the scientific literature. Methodologies include sponge sampling, cotton-gauze sampling, swab sampling, and direct-contact sampling methods. Sponge and swab sampling are probably used most frequently. Standardized environmental sampling plans are uncommon in the meat industry – most plants have their own, internal, sampling plan. Suggestions on sampling for *Listeria* in ready-to-eat processing areas are available (e.g. the AMI *Listeria* guidelines for ready-to-eat products) but typically those guidelines are less detailed than (for example) the ICMSF sampling plans. The number of environmental samples collected varies widely between plants, as does the range of analyses that are conducted on the samples. Typically, the extent of environmental sampling and testing is prescribed by plant or corporate per-

sonnel since there are no regulatory requirements on environmental sampling.

Pre-operational sampling may include both food-contact surfaces and non-food contact surfaces. Sampling hard-to-clean areas is likely to provide the most useful information. Pre-operational sampling may also target such things as air coming from air lines, water, and filters on equipment or air lines. Unless specifically employed as a trouble-shooting tool (e.g. in running down a spoilage issue), in-process environmental sampling is unlikely to be useful in a plant handling raw products.

Microbiological testing protocols — environmental samples

It is important to realize that no official methods (e.g. FSIS) exist for the collection or analysis of environmental samples. AOAC, the organization that serves as a referee for microbiological methods used to analyze food product samples, typically does not address environmental samples. In general, it is more important that methods used in the analysis of environmental samples for indicator organisms give rapid results than precise results since the data are reviewed only for trends. Microbiological data interpretation – environmental samples

Quantitative results indicate either a detectable level of microorganisms or a level below the limit of detection ($\neq 0$). Environmental criteria involving indicator organisms may be based on recommendations (e.g. from a trade group) or on an arbitrarily chosen level or on plant-specific data obtained on environmental samples collected prior to production of microbiologically-acceptable product. No official criteria for environmental samples exist in the U.S. Australian guidelines suggest a maximum APC (at 25°C) of 300 CFU/100 sq. cm. for pre-operational surfaces. In the case of pathogen testing, only qualitative results (presence or absence) are obtained since enrichment procedures are used. The presence of pathogens on pre-operational surfaces is unacceptable and indicative of inadequacies in the sanitation program.

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