

Guidance Document for Lotting and Sampling of Beef Products for Pathogen Analysis

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Introduction

This document provides best industry practices for components (lotting, sampling, laboratory analysis and event window management) of the pathogen-testing program as a part of an overall food safety system. It is important to recognize that these are just components of the system and their success depends on the proper implementation of the best practices leading to and from these steps.

Why do we routinely lot and test beef products for pathogens?

- To contribute to Hazard Analysis and Critical Control Point (HACCP) plan verification
- To satisfy customer specifications/requirements
- To support decisions concerning the size and scope of our response to positive test results

Most consumers unfamiliar with microbiology will tell you that if you want to know if food is contaminated, just test it. Unfortunately, as we all know, microbiological sampling of food to detect presence of pathogens is very difficult. Most bacterial pathogens are not homogeneously distributed in our food and fresh meat is not an exception, so it is difficult to represent the overall level of contamination through the collection of a sample. In addition, the enteric pathogens like *Salmonella* and *Escherichia coli* O157:H7, O26, O103, O45, O111, O121 and O145 (STECs) are most often present in very low numbers in raw foods of animal origin, when they are there at all. To detect them takes examination of a large number of sample units from a lot, and even then the law of probability works against us in ensuring safety.

Assuring the safety of food from production through consumption is a complicated process requiring an organized, deliberate approach to preventing and controlling potential hazards rather than detecting them. The HACCP System is now widely accepted as the most effective and logical way to assure the safety of food. Before a HACCP plan can function with assured control, it must be determined that all hazards reasonably likely to occur have been identified and that the plan to control them is scientifically sound and will be effective. Validation, both of individual Critical Control Points (CCP) as well as the entire HACCP plan, is integral to determining the soundness of a HACCP plan. Pathogen testing is an active and important part of a functioning HACCP plan and may be used in both initial validation and continuing verification of the plan.

Beyond HACCP considerations, pathogen testing for many beef processors has become a requirement for products to enter commerce and a routine practice in their establishment. When individual positive pathogen tests occur, actions that address the hazard found in that product lot are taken to reduce or eliminate the hazard. When multiple positives occur within a shift or across multiple shifts, event period management gives operators the tools and discipline to understand, contain and mitigate the risk.

The objective of this document is to recommend best practices for lotting, sampling, laboratory analysis and event period management for programs conducting pathogen testing in a variety of products produced in a beef processing plant. While an establishment may conduct other microbial testing to monitor the efficacy of their dressing practices, interventions, sanitation programs or other aspects of the food safety program, this document deals only with best practices related to routine pathogen testing. It is imperative that the establishment clearly understand the purpose of all testing, how to interpret the results of such testing and most importantly, know what to do with the results of the testing.

Lotting and Sampling of Beef Products for Pathogen Testing

I. Minimum Lotting and Sampling System Program Components

- Traceability
 - Prior to sampling, all product produced in a sampling system must have 100% reconciliation and have a system to maintain control of the tested lot until actionable results are obtained.
 - Labeling of sample, lot and container must be consistent and performed in a manner to provide complete traceability. It is imperative to ensure that no container or lot ID's can be duplicated in a production day and it is best to not duplicate during a production week.
 - Sequencing of production time and/or line (area) produced should be documented in order to allow for sequencing of finished product in the event of a positive pathogen test result or multiple positive test results.
 - Management of rework has to be performed to maintain identity of time and area of production.
 - Traceability of the original container must be maintained to ensure product is not inadvertently shipped in an event period.
 - Incoming raw material lot management systems must be in place to accurately track raw material, lot and source. It must be documented to ensure all affected product from a production lot of raw material can be easily tracked through the system.
- Frequency
 - Verification sampling should be conducted a minimum of quarterly and increased to once per month during higher prevalence periods (i.e., summer months).
- Microbial Independence
 - Define and support microbiological independence of the lotting scheme. This can vary by product type and/or process.
 - Do lots contain common source materials? If so, in the event of a positive *E. coli* O157:H7 (pathogen) finding, the establishment would have to support why any other product made from those source materials should not be considered adulterated.
 - Is the surge or collection process such that product is not necessarily packaged in time-order? If so, then the most likely lotting scenario would be from clean-up to clean-up. The separation of these lots most likely will include a clean-up step so that cross-contact points are adequately addressed. This is to determine that a true lot separation can be supported as microbiologically independent.
 - There should be a visual verification of this cleaning step. In addition, a microbiological baseline supporting this cleaning step should be conducted and routinely verified.

Separation by production days is supportable due to a full clean-up with an inspection between lots.

- When separating lots into less than a verified cleaning step, the establishment needs to consider how they will support the microbiological independence of those lots. This may be accomplished through:
 - Segregation of collection equipment and packaging.
 - Verify push-through of product through the production system.
 - Non-pathogenic surrogate organism method- inoculate raw material with a validated *E. coli* O157:H7 surrogate organism and process the raw material. Run non-inoculated raw material immediately afterwards and determine if the surrogate can be detected in the finished product in subsequent batches or on common contact processing equipment.
 - DNA detection method – determine the genetic profile of the raw material used then analyze the genetic profile of the finished product associated with the raw material, subsequent finished products, and common contact processing equipment.
- Lotting Effects on Event Periods
 - An “Event Period” is a period of time within a production day where a higher than normal number of presumptive positive tests for *E. coli* O157:H7 or the other top six STECS have been identified.
 - A robust and sensitive sampling plan will allow the establishment to determine a “window” of time and products involved in positive findings.
 - Each facility should have a High Event Period Program detailing specific guidelines for event window decision making.

II. Carcass

When determining lotting practices for individual carcasses, the following should be considered:

- Lotting
 - A minimum of one carcass should comprise the lot. If an establishment chooses more than one carcass for a lot designation, it must provide justification for the lot size.
 - Carcass lots are assigned AFTER the carcass has passed post-mortem inspection and interventions/process aids have been applied.
 - Establishments should have controls and procedures in place to prevent cross-contamination among carcass lots to maintain microbiological independence.
 - Once lots are sampled/tested, the lot should not be broken down into primal(s)/etc. (separated) and should remain intact until negative test results have been received.
 - If a test result shows a positive result, that carcass lot must be kept as an individual lot and no trimming or parts/pieces removed for raw production.

- Positive carcasses should be fabricated at the end of the shift and rendered inedible or sold to a known lethality cooker.
- Verification testing should be sampled from the same carcass lot or using N=60 testing from trim produced by the carcass lot.
- Sampling
 - May utilize the USDA generic *E. coli* sampling method ([9CFR310.25](#)) or the USDA-USMARC carcass sampling method ([www.ars.usda.gov/.../USMARC%20Carcass%20Sampling%20Protocol.pdf](#)).
 - May utilize the excision method by removing surface tissue to equal 375g with a maximum weight determined by the capability of the testing method. (The upper limit must be validated to show that 1-3 CFU of *E. coli* O157:H7 will be detected at >98% sensitivity with the chosen testing protocol.)

III. Trim (Combo)

Definition of Robust Sampling:

Three sampling methodologies have been scientifically verified and validated for use on beef combos. Any of these methods are appropriate for use but should follow these best practices to ensure consistency across the industry. Employee training and verification activities for each method are crucial to provide reliable results.

- Methods must have been validated to be equivalent to or better than N=60 best practice (demonstrate that surface material is targeted during sampling and has equivalent ability to recover bacteria of concern).
- Facilities must support the sampling is representative of the entire lot.
- For combos with large primal pieces (e.g. 2-piece chuck), samples must be taken from different pieces. Therefore, it is advisable to sample as the combo is filled to ensure different random pieces are selected. However, if the lot is less than 5 combos, it is possible there could be fewer primal pieces in the combo than samples required. In these instances, it is acceptable to sample a large primal no more than twice, with at least 8-12 inches between the samples.
- Lotting
 - A minimum of one combo but not more than five combos (~2,000 lbs each) should comprise the lot.
 - Once lots are sampled/tested, the lot cannot be 'split' (separated) and must be sold/shipped intact with adequate traceability records maintained.
 - Sampling for microbial testing should be performed AFTER all quality testing and audits are completed and the combo is a finished combo, ready for shipment.
 - Combo trim should be lotted in one of the following manners: produced by time, lean point, source, or customer requirements, etc. Regardless of the lotting scheme, it is

critical that the tested lot remains intact and clearly identified to the testing laboratory and through to the end users documentation.

- Sampling
 - N60 Sampling
 - Target external surface.
 - Aseptically remove pieces from the external surface that are approximately 1in x 3in in area and 1/8in thick.
 - Target a minimum of 375g of product – The upper weight limit must be validated to show that 1-3 CFU of *E. coli* O157:H7 and/or STEC 6 will be detected at >98% sensitivity with the chosen testing protocol.
 - 60 pieces must be collected across the lot regardless of lot size.
 - Verification Activities
 - Verification of sampling procedure is performed by piece count and sample weight of lots chosen at random using a statistically valid frequency (i.e. Mil Std.).
 - Direct Observation via Camera System – At a defined frequency, trained personnel complete a direct observation of sampling technique (duration, pressure) in an unbiased manner via surveillance cameras.
 - Onsite Verification – An independent team member completes 100% verification at the sampling station by ensuring that the correct time of active sampling is attained with a stopwatch and assuring proper technique is followed.
 - Micro Verification – At a defined frequency, conduct side by side comparison of this sampling procedure to another established single combo sampling method with regard to microbiological indicator organisms (APC and EB).
 - The manual sampling device (MSD) refers to using the food grade sampling cloth manually to sample by hand the exposed trim on the top of a combo after it is filled.
 - Establishments would need to evaluate their process and determine if antimicrobial carryover could affect the results of the laboratory analysis.
 - There are two acceptable sampling techniques:
 1. Aseptically remove the MSD cloth from the sample bag and swab the entire top surface of the combo for 45 seconds. Make sure to use enough pressure to ensure that any bacteria present are dislodged from the product and captured within the cloth. Flip the cloth over and push the cloth material down in between pieces of trim/primal around the entire circumference of the combo for a minimum of 45 seconds. Total sample collection time should be 1.5 minutes.
 2. Using one side of the MSD cloth, swab one half of the meat exposed on the top surface of the combo, flip the cloth and swab the remaining half of the top surface of the combo. Sample collection is conducted for approximately 1.5 minutes total swabbing time for the combo.

- After sample collection, place the used sampling cloth in an appropriately identified sterile bag, in a folded or rolled manner to ensure the cloth remains in contact with the enrichment solution.
- To analyze the samples, enrich the cloths with 200 mL of appropriate diluent and analyze per approved laboratory procedures. Establishments, likely through their laboratory, must have documentation supporting that the laboratory analysis method has been validated for the cloth matrix, as most analysis methods are validated for meat only. Documentation showing a matrix extension of an existing methodology is sufficient in most cases.
- Verification Activities
 - Direct Observation via Camera System – At a defined frequency, trained personnel complete a direct observation of sampling technique (duration, pressure) in an unbiased manner via surveillance cameras.
 - Onsite Verification – An independent team member completes 100% verification at the sampling station by ensuring that the correct time of active sampling is attained with a stopwatch and assuring proper technique is followed.
 - Micro Verification – At a defined frequency, conduct side by side comparison of MSD sampling to another established single combo sampling method with regard to microbiological indicator organisms (APC and EB).
- IEH N60 Plus Sampler™
 - The sample must yield a minimum of 150g, following validated tool sampling methodology.
 - Sanitize the IEH N60 Plus Sampler flute by immersing in hot water sanitizer ($\geq 180^{\circ}\text{F}$). Before collecting a sample, allow the flute to cool in order to prevent injury to microorganisms.
 - A lot sample consists of $\pm 150\text{ cm}^3$ of trim shavings taken from independent locations in one combo. The volume of the sample is set by the sampling head of the IEH N60 Plus Sampler.
 - Collect samples from at least 5 areas of each combo (the four corners and the center) by inserting the sampler up to its maximum depth into the combo bin. DO NOT drill through a single piece.
 - Visually monitor the volume of sample collected by viewing whether the sampling head is full.
 - Open a sterile Whirlpack bag in an aseptic manner around the flute held on a support mount. Allow meat to fall into an open sample bag with the assistance of a sanitized sample removal tool.
- Verification Activities
 - Verification of sampling procedure is performed by a person independent of the operator who collects the sample. This person shall verify that at least 5 insertions have been made and that the sampling head is full by visual inspection during sample collection.

- Direct Observation via Camera System – At a defined frequency, trained personnel complete a direct observation of sampling technique (duration, pressure) in an unbiased manner via surveillance cameras.
 - Onsite Verification – An independent team member completes 100% verification at the sampling station by ensuring that the correct time of active sampling is attained with a stopwatch and assuring proper technique is followed.
- Micro Verification – At a defined frequency, conduct side by side comparison of MSD sampling to another established single combo sampling method with regard to microbiological indicator organisms (APC and EB).
- Lotting Effects on Event Periods
 - An “Event Period” is a period of time within a production day where a higher than normal number of presumptive positive tests for *E. coli* O157:H7 and/or STECS have been identified.
 - A robust and sensitive sampling plan will allow the establishment to determine a “window” of time and products involved in positive findings.
 - Each facility should have a High Event Program detailing specific guidelines for event window decision making.
 - USDA-FSIS issued a compliance guideline (http://www.fsis.usda.gov/wps/wcm/connect/e0f06d97-9026-4e1e-a0c2-1ac60b836fa6/Compliance_Guide_Est_Sampling_STEC_0512.pdf?MOD=AJPERES) for establishments sampling beef trimmings that outlines statistical tables to use when determining high event periods. FSIS determined the possibility of two types of event periods:
 - Localized event – when some specific occurrence or event caused a clustering of STEC contamination in products. FSIS has defined this event as 3 or more STEC positive results out of 10 consecutive samples from production lots containing the same source materials within a defined period. Disposition of combos in this period of time would need to be determined.
 - Systemic event – when a break down or inherent weakness of the food safety system occurs. For example, if 7 or more STEC positive results were found out of 30 consecutive samples from lots containing same source materials, an establishment could define this as a systemic event. In this situation a prudent establishment would consider the combos that were involved as well as subprimals in the systemic event period for disposition.

IV. Trim (Boxed)

- Lotting
 - When determining lotting practices for boxed trim, the following should be considered:

- A minimum box count for a lot can be as small as one box regardless of weight, but the volume of the material in the box should be sufficient to allow for N=60 sampling.
 - The maximum box count should not exceed, by weight, the equivalent of 5 combos (~ 2,000 lbs each) which is approximately 10,000 pounds (ex. 60 lb boxes would be a maximum of 166 boxes per lot).
 - Boxed trim should be lotted in one of the following manners: produced by time, lean point, source, or customer requirements, etc. Regardless of the lotting scheme, it is critical that the tested lot remains intact and clearly identified to the testing laboratory and through to the end users documentation.
 - Product in the same lot is not produced over more than one production day (all boxes in a lot have the same production date).
- Sampling
 - Sampling for microbial testing should be performed AFTER all quality testing and audits are completed and the boxes are finished and ready for shipment.
 - Sampling should be performed as described in the Definition of Robust Sampling section from Trim Sampling (combo) above.
 - Facilities must support the sampling (N=60) is representative of the entire lot.
 - Example for sampling 60 lb boxes that utilizes the [Military Standard MIL STD-105E, dated May 10, 1989, Table 1 \(page 13\), General Inspection Level 1, Table II A Single Sampling Normal Inspection \(page 14\), Acceptable Quality Level of 1.0.](#)

Number of Boxes	Number of Boxes to pull samples from to comprise 60-piece lot
151 – 166	13 (~ 5 pieces/ box)
91 – 150	8 (~ 8 pieces/ box)
26 - 90	5 (~12 pieces/ box)
25 or less	3 (~20 pieces/ box)

- Frozen product sampling is difficult, as it is not easy to target the outside surface of the piece in a frozen state. It is advisable to collect samples for frozen material either prior to freezing or by thawing in a manner to allow for proper surface excision as detailed above. When product is sampled and tested as combos, prior to boxing and freezing, lot integrity must be maintained through the boxing process. If this is not possible, sampling of frozen product should follow USDA Import Procedures (FSIS Directive 10,010.1, Revision 4 - <http://www.fsis.usda.gov/wps/portal/fsis/topics/regulations/directives/10000-series>).

- Boxing Procedure minimum criteria (Program to transfer product from combos into boxes):
 - Provisions to ensure no mixing or co-mingling of product.
 - Segregation parameters to ensure proper handling of all species and/or *E. coli* status. Including but not limited to:
 - Employee hygiene practices.
 - Equipment, tools and conveyance machinery to ensure they do not possess the potential for cross-contamination.
 - A provision to ensure lot integrity is maintained to the finished boxes and appropriate documentation is in place to maintain traceability.
 - Age, labeling, boxing and freezing requirements for the product.
 - Provisions for the handling of less than full boxes at the end of the lot.

V. Ground Beef

- Lotting
 - Each lot should represent a defined volume or timeframe of finished product. This must be logistically feasible, traceable, and maintainable.
 - Rework must be accounted for in the lotting scheme. Rework should be returned to the original lot. If an establishment is not able to re-introduce the ground material into the original lot that the material came from, then that ground material may be placed into a separate “rework” lot. This rework lot may contain ground material from numerous lots of tested product that is produced in that day. This rework lot is kept as a separate individual lot and will be considered a part of any positive lot that may occur during that production day. Since the rework lot is made up of ground material that has already been subjected to sampling and testing, there is no need for any additional testing to be done. No fresh or untested material may be added to the rework lot since the addition of new material will corrupt the lotting integrity. If untested or fresh material is mixed with rework, then product should be diverted to cooking.
 - No finished product (ground material) should be carried over into a new production day due to the fact that this will cause a carryover lotting issue and in the event of a positive test result, would implicate multiple days of production.
- Sampling
 - In order to account for microbiological contamination throughout the process, samples should be collected after a point in the process where no likely additional growth or contamination will occur; thus, the point of sampling depends on the ground beef package variable.
 - Samples **MUST** be collected at a point in the process after the final blender.
 - Every blender or batch must be represented by a sample.
 - All components **MUST** be a part of the blend or batch before samples are taken. (i.e., sample after including, fresh and frozen beef, AMR, seasoning and rework).

- As published by Barkocy-Gallagher et al., 2002, freezing does not significantly affect the persistence or recovery of *E. coli* O157:H7 in ground beef; therefore, sampling can occur before or after freezing.
- The number of samples collected during the processing of the lot should be defined in the sampling program and based on risk assessment.
- The size of the sample should be based on a validated analytical method, with a detection limit of not less than 1-3 CFU/sample unit.
- The combined performance of the sample size, enrichment protocol and screening method should be validated for all sample sizes being analyzed, including individual samples and sample composites.
- Verification Testing or secondary testing (Appendix A):
 - This is performed on finished ground products, produced from raw materials that previously tested negative. Utilized by further processors receiving trim from outside facilities.
 - A minimum of 65g of product (A Set, Diagram 1) produced within the defined lot and representative of each batch within the defined lot.
 - In addition to the initial lot sample, B Set samples (Diagram 2) totaling at least 325g per lot should be taken and held pending initial screen results. There must be a total of 325g per lot of product taken and each blender or batch represented for these library samples. A total of 325g sample per lot is required in order to make disposition when a positive occurs. Both the A and B samples should be representative of each batch within the defined lot. It is inappropriate to collect one 390 g sample for each lot and divide it into a 65g “A sample” and a 325g “B sample,” as the resulting samples may not include product from each batch in the defined lot.
 - B set samples are not analyzed unless there is a positive (detailed in Disposition Testing below).
 - All samples must be documented using blender ID and time.
 - Initial Testing
 - “A” samples for each lot are composited into one 65g sample representing the lot.
 - If the lot tests negative, no additional action needs to be taken.
 - If the lot tests presumptive positive, the B samples must be analyzed to determine disposition of adjacent lots.
 - Disposition Testing
 - Identify the safety zone to be tested based on blender turnover (usually 1-2 hours before and after the presumptive positive lot).
 - Analyze B samples for the safety zone lots based on sampling time.
 - Test 325g of product per lot.
 - If the lots before and after are negative after disposition testing:

- Hold the affected positive lot and the lots before and after. This product should be diverted away from raw ground/non-intact product and could be sent to a USDA inspected, controlled cooking facility, inedible rendering or landfill.
 - A deep understanding of system self-cleaning and supporting evidence is needed if diverting less than a full lot prior to and after an affected lot; See Koohmaraie et al., 2015 (JFP 78(2) 273-80) and AMSA's Role of Microbiological Testing in Beef Food Safety Programs.
- Sublotting of lots before and after the initial positive is only permissible with appropriate data to support these decisions. This cannot be performed without controls for rework, raw material and finished product lot control and system push through (all product processed).
- Remaining raw material lots associated with presumptive lot of finished grind should also be diverted, as noted above, away from raw ground/non-intact product.
- Reassess HACCP plan
- Divert rework lot from end of the production day.
- If the additional lots before or after the positive lot are positive, it will be required to analyze additional lots produced that day.

VI. Box Primal

- Further processors for making non-intact products (i.e., ground, tenderized, injected, vacuum marinated, etc.) should address in their HACCP plans that they intend to use boxed primals as a raw material for making non-intact products, regardless of testing.
- Further processors' specifications should be clearly communicated to the supplier regarding the intent to use boxed primals in a non-intact product to allow for potential testing to be completed by the supplier.
- Whenever practical, raw materials intended for use in non-intact products should be accompanied by a certificate of analysis (COA) from the supplier, to relieve the receiving facility of the need to conduct sampling.
- When a COA is not available, further processors may consider:
 - Application of a validated pre-processing antimicrobial intervention; or,
 - Combining all raw materials that will be used in a day's production and sampling as a lot prior to making non-intact; or,
 - A robust finished product testing program as outlined in the previous section.
- Lotting

- Each individual package of product should be considered microbiologically independent unless the sampling encompasses more than one package. When determining lotting practices for boxed primals, the following should be considered:
 - Product that is collected, bagged and boxed is considered independent of other bagged product from that line or product source. There has been no documented linkage between individual pieces of product and the presence of *E. coli* O157:H7 and therefore, there is no scientific reason to link one primal bag to another. A primal and/or sub-primal lot can be as small as one individual package but must include all product contained within the selected package.
 - In selecting lots, detailed collection of all data available with the primal(s) selected must be maintained in order to clearly identify what was sampled, such as establishment number, pack date, product code, pack time, packaging information, etc.
 - If a sample (n=60) is pulled from one box, then the lot would be that box.
 - If a sample is pulled from 4 boxes consisting of 5 bags of a certain primal, the lot would include all primal products in those 4 boxes.
 - If a sample is pulled from boxes out of five pallets, the lot would include the five pallets and all products in those pallets/boxes.
- Whatever the scheme, it is critical that the tested lot remains intact and can be clearly identified to the end user.
- Sampling
 - Select a representative sample of all products contained in the lot. Each product type must have an equal chance of being selected.
 - Sampling should be performed exactly as described above in the combo section. This would mean a minimum of 60 pieces with maximum surface area. In the event that more than 60 boxes are produced, more than 60 pieces would be selected. In order to maintain the sample weight for lots greater than 60 boxes, it is permissible to reduce the length of the piece of trim to ensure maximum surface area and not exceed the 375g sample standard, unless a higher upper limit has been validated by the manufacturer of the testing platform being used.
 - As with combo sampling, the external surface of the piece must be targeted for sampling (skin surface of the carcass if present).
 - Primal testing can be conducted in one of two ways:
 - Combo Naked Test – This method follows the combo sampling method as detailed above.
 - Product going directly to package – This method would follow the boxed trim method as detailed above, but each primal piece would require a small piece to be incised from individual primal pieces.

- Testing during production has several components that must be assessed:
 - Assess other like-products being produced as the “tested lot” of primal products may implicate other products.
 - Products that were part of the sampled lot and were subsequently rejected for quality or other reasons, must be tracked and controlled. This includes leakers, damaged boxes, held product for specification review, etc.
 - Reconciliation of sampled boxes in inventory must occur. Boxes must be 100% reconciled in inventory before samples are analyzed to ensure all product is under establishment control and no product is shipped prior to receipt of test results.

VII. Bench Trim

- Lotting
 - All source material for bench trim must be tracked.
 - The lot should be linked to a production period.
 - If a sub-primal is made into a non-intact product, the non-intact product should be held until the bench trim results are received.
 - An exception to this rule could be made if the sub-primals are treated with a validated antimicrobial treatment after producing bench trim and prior to making non-intact product.
 - When sampling and testing bench trim from non-intact product, you must take into account the non-intact sub-primals as part of the lot.
 - Bench trim produced after a sub-primal has been processed into non-intact should NOT be used in raw ground product.
- Sampling:
 - Sampling should be performed exactly as described above in combo section. This would mean a minimum of 60 pieces, selected to maximize external surface area. If more than 60 pieces are sampled, it is permissible to reduce the length of the piece of trim to ensure maximum surface area and not exceed the 375g sample standard, unless a higher upper limit has been validated by the manufacturer of the testing platform being used.

VIII. Box Offal

- Lotting
 - Offal products typically used in grinding include hearts, weasands, head meat, cheek meat, oxlips, tongue root trim, etc.

- When determining lotting of offal an establishment must be able to clearly define and support the microbial independence of the lot, if the lot is not clean-up to clean-up. This is due to the fact that harvesting and boxing processes typically used in slaughter operations may include multiple areas of product surge, such as holding bins and totes and commingling of product types on a single conveyor, which makes maintenance of lot independence challenging.
- Depending on the establishment's production and lotting process, the COA's provided for offal product may be a COA letter stating that the product shipped was part of a tested lot but not the entire lot or they may be more traditional COA's if the shipment contained all the product from a tested lot.
- When designing offal lotting systems, the establishment needs to consider if the production of this type of product can support microbial independence.
- When collecting samples for a designated lot, a sampling scheme must account for sampling randomness and represent the entire lot. This may be accomplished by taking a sample from every box or isolating the collection of the tested lot in a method that allows for random sampling such as sampling hearts that do not run down a co-mingled table.
- Sampling
 - Select a representative sample of all products contained in the lot. Each product type must have an equal chance of being selected. Excision sampling or Sponge sampling may be utilized.
 - Excision sampling should be performed exactly as described above in combo section. This would mean a minimum of 60 pieces and a minimum of a 375g sample. The external surface of the piece must be targeted for sampling.
 - Surface sponge sampling should be validated to determine total number of sponges per lot and the compositing schemes that can be used.
 - Validation should determine a specific number of boxes to be sampled per sponge and the total number of sponges allowed per lot.
 - Wet pooling of sponges may be employed to have one test per lot.
 - Target a minimum total surface area of 180 in² per lot.
 - Reconciliation of sampled boxes in inventory must occur. Boxes must be 100% reconciled in inventory before the sample is analyzed to ensure proper controls are in place and no product is shipped prior to receipt of test results. Establishments should consider the risks of shipping partial lots to customers in the event of a downstream, positive finding.

IX. AMR

- Lotting

- All product produced from a clean-up to clean-up must be considered a lot unless an establishment can support alternative lotting and disposition decisions (See Microbial independence section for alternative lotting).
- Sampling
 - Select a small sample from each box as it is being produced ensuring that at least 60 random (6-7g) samples are taken.
 - Composite the 60 or more random samples into one composite of at least 375g for the analytical sample.
- All boxes/containers must be accounted for and retained pending sample analysis.

Sample Analysis

Scope

This document was developed to provide guidelines, recommendations and framework for the development of a program for determining the presence of microbiological targets in samples of beef.

Analysis criteria

Establishments must understand and clearly define the analysis objective(s), potential outcomes and subsequent actions in order to successfully implement and manage a food safety program. Determination of analysis program criteria should be made for each establishment based on complexity of product lines and types, intended use of product, processes, capacity/volume, and contribute added value to the program. The laboratory MUST analyze the entire sample that has been taken – subdividing samples is NOT allowed. **Analysis should only be performed to achieve predetermined objectives of established programs or short term initiatives that involve new analysis technology evaluation or specific process enhancements.**

I. Laboratory Selection

The laboratory should be capable of serving as a qualified guide and information resource in presenting options that best fit the technical requirements, business needs and support analysis objectives. A laboratory partner must provide the expertise and credentials required for unequivocal test results. The laboratory partner must also be capable of interpreting and applying the data in an effective manner.

If the laboratory is utilized in the capacity of guidance on method selection, then the laboratory must be capable of supporting the recommended method by ensuring it is fit for use, performed as validated, and results generated and reported are within the scope of the validation for the target organism(s) or tests. The laboratory must have a strong knowledge and understanding of test systems.

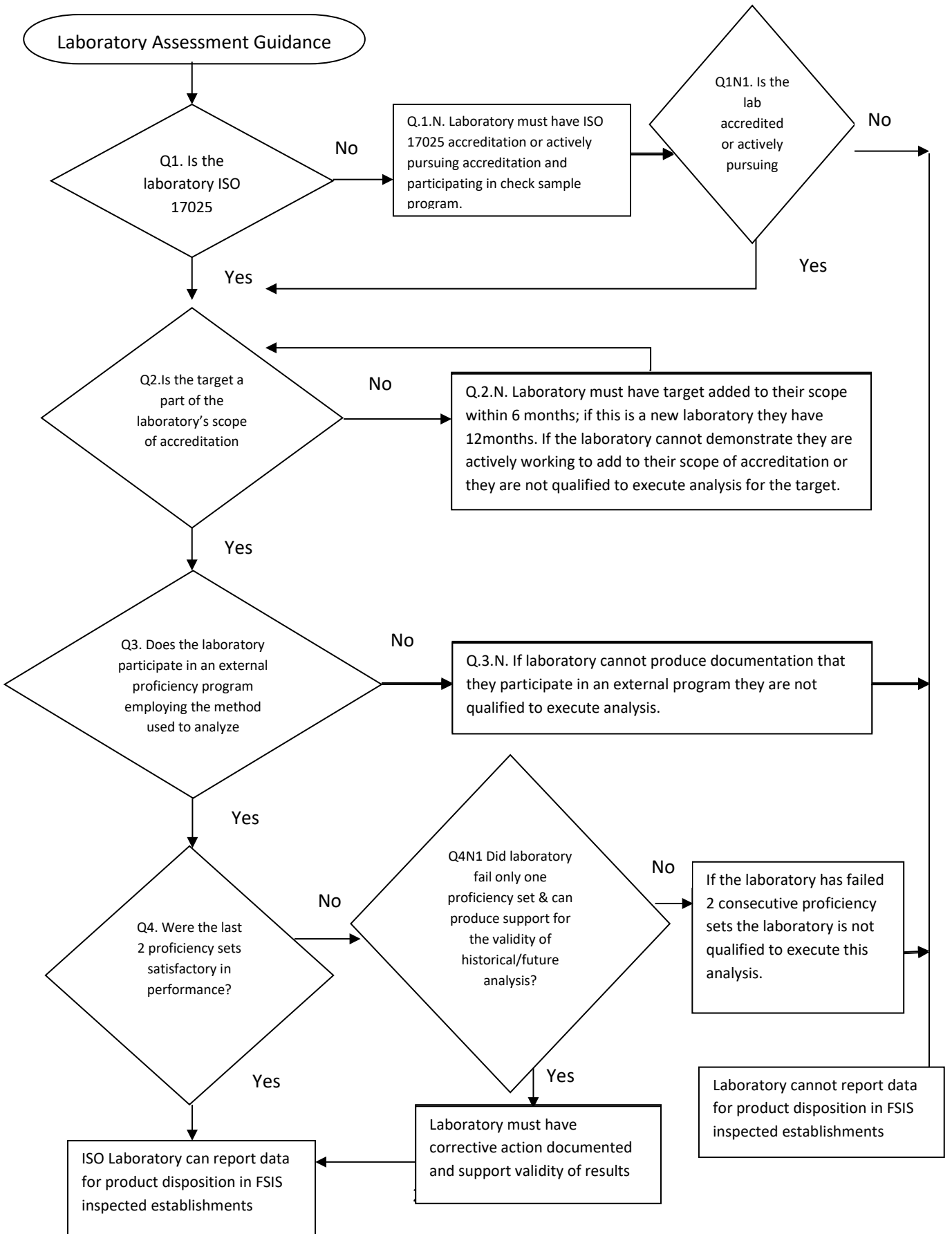
Best practice guidance for accepting a laboratory's qualifications is included below (**FIGURE 1. Laboratory Assessment Guide**).

Key qualifiers include:

- The laboratory must be accredited to the ISO / IEC 17025 standard for testing laboratories
- The test method of choice must be a part of the laboratory's scope of accreditation. The laboratory should provide an official scope of methods which have been included in the accreditation process. The method you choose for testing should specifically be listed on this scope to include the standard method type.
- The laboratory participates successfully in an external proficiency program which includes the pathogen methodology utilized in the establishment's analysis program.
- Final results are reported in a manner consistent and representative of the validated test method and in accordance with any applicable specifications.
- The test method used must be validated for the sample matrix. For example if you wanted to test for *E. coli* O157:H7 in ground beef, the method must have been validated for use in ground product.

For more information, consult USDA-FSIS document on criteria for laboratory selection (Establishment Guideline for the Selection of a Commercial or Private Microbiological Testing Laboratory, June 2013 - <http://www.fsis.usda.gov/wps/portal/fsis/topics/regulatory-compliance/compliance-guides-index#Micro>).

FIGURE 1. Laboratory Assessment Guide



II. Test Method Selection

Method selection should be specific for each analysis program based on factors associated with, but not limited to, product, food safety objectives, time limitations, product intended use, and method performance characteristics relative to specific objectives of established programs. Multiple analysis methods are often warranted due to differences in operational needs.

An establishment must determine methodology for analysis based on factors that include:

1. Test Result Application
 - Method selection should be performed with a complete understanding of the detection target(s) and that the data generated will support food safety objectives.
2. Approvals
 - A validated method should be the method of choice. Validation, approval, or acceptance by an independent body (i.e. AOAC, AFNOR, USDA-FSIS (Letter of No Objection)) is sufficient if all parameters (e.g., sample size, sample type, dilution ratio, incubation time), are validated for the intended application of the test.
3. Fit for Intended Use
 - The method must be validated for the specified product matrix. Assay used must demonstrate effectiveness at detecting or quantifying the target organism (i.e., target analyte) in the same matrix that is being tested in the field (e.g., an *E. coli* O157:H7 test validated for use with spinach should not be used for meat unless validation data demonstrate effective detection of target in both matrices). Key considerations of fit for purpose are:
 - i. Same sample matrix
 - ii. Sample size (e.g., weight) analyzed and analytical units referenced (e.g., result reported per area or unit)
 - iii. Implemented without variation in validated methods
 - Establishment of an appropriate Limit of Detection (LOD) should: (1) be dependent on the target organism; and, (2) detect concentrations of the target analyte consistent with the food safety objectives (i.e., regulatory or establishment requirements: see section on “fractional recovery” in validation section below).
 - i. Method validation should be consistent with the manner in which the method is implemented in the laboratory. A variation in implementation for any reason will require additional validation by the end user or through collaboration with the kit manufacturer. (e.g., sample size, sample type, incubation time)

4. Business Based Considerations

- Does the method provide added value, required by the business, relative to program cost? *This is an organization/business specific question with organization/business specific answers. The answer to this question may vary between establishments. Consider if a method is better suited for business and/or process(es) than what is currently being used.
- Does the method allow an establishment to fulfill the business/customer service requirements (e.g., turn-around time)?

III. Building a Laboratory Relationship

When testing is necessary, it is imperative that the establishment build a working relationship with the laboratory. The establishment must work with the laboratory to assure that the information that is being provided is consistent with the defined program expectations. This relationship extends beyond pricing requirements and data integrity discussions, and encompasses fit for use, qualifications and laboratory practices that may impact the information provided to the establishment.

As a part of building the laboratory relationship, it is recommended that a documented method verification checklist is provided to each laboratory service provider for completion and discussion at least annually.

This form should be completed and reviewed annually

<i>I. Overview</i>		
1) List the test method name as referenced on the laboratory report or COA:		
2) Briefly describe the test as it is performed in the laboratory to achieve reported results:		
3) Does this process require the use of multiple validated methods, not including cultural confirmation? <i>YES</i> <i>NO</i> <i>IF yes is answered, the following sections I – IV must be completed for each method completed, note that the validations must be CONSISTENT as the method(s) are applied</i>		
4) Does this process require the use of cultural confirmations? <i>YES</i> <i>NO</i> <i>IF yes Section V must be completed.</i>		
<i>I. Method Overview - Preliminary or Screening Method</i>		
5) Test Method Name as it appears on package insert:		
6) Test Method Manufacturer:		
7) This method claims to detect:		
<i>E. coli</i> O157:H7	YES	NO
Pathogenic STECs	YES	NO
Other (List): _____		
<i>II. Validations - Preliminary or Screening Method</i>		

<p>8) Has this method been validated by an outside source such as AOAC / AFNOR on a product type consistent to the product(s) that our establishment is submitting for testing?</p> <p>YES NO</p>		
<p>If YES answered in 4) above</p> <p>9) List approval body and the identifying number associated with the method:</p>	<p>If NO answered in 4) above</p> <p>10) Has the method been validated in a manner that meets or exceeds BIFSCO best practices and or current regulatory requirements?</p> <p style="text-align: center;">YES NO</p> <p>11) Validation must be attached to this form.</p>	
<p><i>III. Laboratory Processes - Preliminary or Screening Method</i></p>		
<p>12) Please verify that you are completing this procedure as it has been validated, related to the following elements:</p>		
a) Matrix	YES	NO
b) Amount of product weighed and enriched:	YES	NO
c) Enrichment type and amount of enrichment added	YES	NO
d) Incubation Time	YES	NO
e) Incubation Temperature	YES	NO
f) The method is being performed as validated	YES	NO
<p><i>IV. Laboratory Processes – Cultural Confirmation</i></p>		
<p>13) Is this process completed at the same location as the preliminary result? YES NO</p>		

The following contains expected answers to the questionnaire above and suggested actions if variations occur.

<p><i>I. Overview</i></p>
<p>19) List the test method name as referenced on the laboratory report or COA: <i>This should be the method name as it appears on your report or COA</i></p>
<p>20) Briefly describe the test as it is performed in the laboratory to achieve reported results: <i>Typically the lab will describe a process similar to below:</i> <i>A (list sample size and other pertinent details) is analyzed following (list test manufacturer name and method). If a presumptive positive result is obtained it is (reported or culturally confirmed)</i></p> <p><i>Example: A 375g sample is analyzed following Perfect Test supplied by Perfection Systems. If a presumptive positive result is obtained, according to our customers' requirements, samples are culturally confirmed.</i></p>
<p>21) Does this process require the use of multiple validated methods, not including cultural confirmation? <i>YES</i> <i>NO</i> <i>IF yes is answered, the following sections I – IV must be completed for each method completed, note that the validations must be CONSISTENT as the method(s) are applied</i></p> <p><i>Multiple methods are intended to refer to a series of “screening methods” this could mean a lateral flow type method followed by a PCR based method. If Yes is indicated validations and support must encompass all methods as one system.</i></p>
<p>22) Does this process require the use of cultural confirmations? <i>YES</i> <i>NO</i></p> <p><i>IF yes Section V must be completed. This question is intended only to indicate if section V is necessary or not.</i></p>
<p><i>v. Method Overview - Preliminary or Screening Method</i></p>
<p>23) Test Method Name as it appears on package insert:</p>

<p>This is necessary to assure that it is clear what method is used, often a test is referenced by manufacturer name. Many manufacturers offer multiple tests.</p>										
<p>24) Test Method Manufacturer: Another question for clarity only.</p>										
<p>25) This method claims to detect: Often even sales personnel from manufacturers confuse this – you must understand if the method detects only O157:H7 or O157; typically lateral flows and Enzyme Immunoassays are broader and detect <i>E. coli</i> O157; PCR based methods are typically more specific to <i>E. coli</i> O157:H7</p> <table style="width: 100%; border-collapse: collapse;"> <tr> <td style="width: 40%; text-align: center;"><i>E. coli</i> O157:H7</td> <td style="width: 20%; text-align: center;">YES</td> <td style="width: 40%; text-align: center;">NO</td> </tr> <tr> <td style="text-align: center;">Pathogenic STECs</td> <td style="text-align: center;">YES</td> <td style="text-align: center;">NO</td> </tr> <tr> <td colspan="3"> <p>Other (List): _____</p> </td> </tr> </table>		<i>E. coli</i> O157:H7	YES	NO	Pathogenic STECs	YES	NO	<p>Other (List): _____</p>		
<i>E. coli</i> O157:H7	YES	NO								
Pathogenic STECs	YES	NO								
<p>Other (List): _____</p>										
<p><i>VI. Validations - Preliminary or Screening Method</i></p>										
<p>26) Has this method been validated by an outside source such as AOAC / AFNOR on a product type consistent to the product(s) that our establishment is submitting for testing? Refer to Method Selection guidance in this document for more detail</p> <p>YES NO</p>										
<p>If YES answered in 4) above</p> <p>27) List approval body and the identifying number associated with the method: As an example AOAC Official Method 2016.08</p>	<p>If NO answered in 4) above</p> <p>28) Has the method been validated in a manner that meets or exceeds industry best practices and or current regulatory requirements? YES NO</p> <p>A detailed review of the validation and premise of validation must be completed. If there are questions about the validation refer to BIFSCo Best Practices Guidance Appendix.</p> <p>29) Validation must be attached to this form.</p>									
<p><i>VII. Laboratory Processes - Preliminary or Screening Method</i></p>										

30) Please verify that you are completing this procedure as it has been validated, related to the following elements:		
a) Matrix	YES	NO
b) Amount of product weighed and enriched:	YES	NO
c) Enrichment type and amount of enrichment added	YES	NO
d) Incubation Time	YES	NO
e) Incubation Temperature	YES	NO
f) The method is being performed as validated	YES	NO
<p style="color: red;">If No is the answer to any of these questions there is an issue with the laboratory and or method and it must be addressed immediately.</p>		

VIII. Laboratory Processes – Cultural Confirmation

31) Is this process completed at the same location as the preliminary result?	YES	NO
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32) Are the cultural confirmations completed are consistent with the USDA MLG 5.09 http://www.fsis.usda.gov/PDF/MLG_5_09.pdf	YES	NO
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<p style="text-align: center;">If YES answered in 16 above.</p> <p>33) The method is being performed exactly as prescribed in the USDA MLG 5.09?</p> <p>YES NO</p>	<p style="text-align: center;">If NO answered in 16 above:</p> <p>34) List the differences in method completion</p> <p style="color: red;">Example: A column is defined in the procedure; however, Dynal beads and a bead washer are used.</p> <p>35) Attach validation support for the cultural method being utilized. Dynal support is attached.</p>
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36) We certify that the method is being performed as it has been validated. The laboratory further certifies that the processes and techniques used do not compromise the integrity of results generated

Signed:

Date:

Title:

IV. Supplemental Validation Parameters for *E. coli* O157:H7 Test Methods

Validation data must clearly demonstrate that a method is fit for the intended use by the end user. Sections III and IV above are intended to assist processors in gathering information for determination of a test method's fit for the intended use. Typically test method validation is the responsibility of the test manufacturer, and is achieved through recognized independent body approvals (e.g., AOAC, AFNOR). As with any program operating under commercial conditions, minor deviations from standard procedures may occur. An understanding of procedure robustness may prove valuable when determining the adequacy of existing method validation(s). Scientific rationale should be leveraged to determine if variations to original procedure require supplemental validation. Such variations requiring validation may include, but are not be limited to: 1) Product to enrichment ratio; 2) type of product evaluated (often referred to as matrix); 3) test portion size used in the analysis; 4) product temperature, media temperature and/or enrichment temperature at the time of analysis; 5) enrichment media type; 6) duration of enrichment; and, 7) effect of the initial inoculum dose on sensitivity. In order to achieve a standardized approach to supplemental validation, there are key parameters that must be consistently applied in a validation process in order to consistently demonstrate fit for intended use. Key validation parameters are discussed in detail in Appendix 1.

Questions or suggestions are welcome and should be addressed to:

Beef Industry Food Safety Council (www.bifsc.org or bifsc@beef.org) or Sherri Williams Sherri.Williams@jbssa.com, Pat Mies Pat.Mies@nationalbeef.com, Ted Brown Ted_Brown@cargill.com or Melody Thompson Melody_Thompson@cargill.com, or Noel DCruz Noel.DCruz@tyson.com

Definitions

Association of Analytical Communities (AOAC) - Founded by USDA in 1884 as the Association of Official Agricultural Chemists, AOAC was renamed AOAC International in 1991. AOAC International is a non-profit scientific association whose technical contributions center on the creation, validation and publication of analytical test methods. ([AOAC INTERNATIONAL Homepage](#))

AOAC PT/RI – AOAC Proficiency Tested / Research Institute: Test kit manufacturers seeking *Performance Tested Methodsm* status are required to produce and submit data to support product performance claims. The AOAC-RI recruits independent experts (known as “**Expert Reviewers**”) and selects a **General Referee** to review the performance data of the method. After the data submission for the method have been reviewed and found to support the product performance claims by the Expert Reviewers and General Referee, the method performance is verified by an Independent Testing Laboratory. The evaluation is conducted using protocols developed by the Expert Reviewers and General Referee. The data generated by the Independent Testing Laboratory is sent to the Expert Reviewers and General Referee for evaluation to determine whether the independent laboratory data corroborates the data submitted by the manufacturer. If the two sets of data are found to corroborate each other and support the product performance claims, then the Expert Reviewers and General Referee will recommend *Performance Tested Methodsm* status for the method. (<http://www.aoac.org/testkits/programelements.htm>)

AOAC Official Methods of Analysis (OMA) –Independent method validation, of methods by an inter-laboratory collaborative study, in which experienced, competent analysts work independently in different laboratories under the direction of a study director using a specific method to analyze replicated test samples for a particular analyte. http://www.aoac.org/Official_Methods/Food_Micro_Validation_Guidelines.pdf

AFNOR - Association française de Normalisation (AFNOR) is the French national organization for standardization and it’s International Organization for Standardization member body. The AFNOR Group develops its international standardization activities, information provision, certification and training through a network of 11 key partners in France who are members of the association.

Confirmed *E. coli* O157:H7 Positive**– a biochemically identified *Escherichia coli* isolate that is serologically or genetically determined to be “O157” that meets at least one of the following criteria:

- 1) Positive for Shiga toxin (ST) production
- 2) Positive for Shiga toxin gene(s) (*stx*)
- 3) Genetically determined to be “H7”

**([reference: MLG – 5.09, Detection, Isolation and Identification of Escherichia coli O157:H7 from Meat Products, Revision: 09](#))

Enrichment – process of adding necessary nutrients, typically in a broth form to a sample.

False positive - a true negative that returns a positive result.

Fractional recovery - when 20 – 80% of inoculated samples result in positive detection.

Incubation – process of growing the enriched samples under defined conditions (typically temperature and time).

Inoculation – act of adding a known organism to a sample.

Limit of Detection (LOD) - The lowest concentration of the analyte that can be detected in a sample. This is the level that detection is just feasible.

Lot - The amount of product which is represented by a sample. This can be determined by time, weight, container (combo or boxes) or number of units, that makes it independent of other lots.

Lot Management - The lot should be maintained together and should not expand beyond clean-up to clean-up. All products in the “Lot” should remain under company control until pathogen test results have been received. Lot integrity should be maintained until negative test results are received (no further processing).

Matrix – A substance (gathered or collected) which is the subject of analysis and is considered in terms of specific properties.

Negative - Samples that have been tested with a test method validated as fit for use, and demonstrate an absence of the micro-organism(s) of concern. These results are typically based on an initial screening result.

Negative control - a test portion with known contents to carry through the method to verify performance.

Positive - Any test result that is non-negative. A test result may be suspect, presumptive positive, or confirmed positive.

Potential *E. coli* O157:H7 Positive**- a negative sample that causes a positive reaction with the screen test.

Presumptive *E. coli* O157:H7 Positive**- a sample that has typical colonies, observed on Rainbow Agar, and reacts specifically with O157 antiserum. (See also “Confirmed Positive” above)

Rework - Product that is rejected from the process during a single production run.

Robust Sampling – a process by which a sufficient amount of product is taken to comprehensively represent the entire lot. For trim things to consider: excision sample external

carcass surface area obtaining pieces approximately 1x3 inches and 1/8 inch thick; based on best practices of N=60 (a minimum of 60-individual pieces from DIFFERENT trim pieces per lot); other methods must have been validated to be equivalent to or better than N=60 best practice (demonstrate that surface material is targeted during sampling and has equivalent ability to recover bacteria of concern); facilities must support the sampling (N=60) is representative of the entire lot; for combos with large primal pieces (e.g. 2-piece chuck), samples must be taken from different pieces. Therefore it is advisable to sample as the combo is filled to ensure different random pieces are selected. However, if the lot is less than 5 combos, it is possible there could be fewer primal pieces in the combo than samples required. In these instances, it is acceptable to sample a large primal no more than twice, with at least 8-12 inches between the samples.

Sample - A portion of product that represents the given lot.

STEC - refers here to the 6 specific serotypes (O26, O103, O45, O111, O121 and O145) of shiga toxin-producing *E. coli* that are regulated as adulterants in beef. (See FSIS Microbiological Laboratory Guidebook, Method Number 5B.05 - <http://www.fsis.usda.gov/wps/portal/fsis/topics/science/laboratories-and-procedures/guidebooks-and-methods/microbiology-laboratory-guidebook/microbiology-laboratory-guidebook>).

Unpaired – Inoculated samples randomly assigned to an alternative or a reference method; a proportion of positive (and confirmed) samples using the alternative method compared to the proportion of confirmed positive using the reference method.

Wet Pooling / Compositing -- this occurs when an individual meat sample is enriched in it's own bag and the laboratory removes a liquid aliquot from multiple enrichments to composite into one liquid sample for analysis.

Appendix A- Sample Set Compositing Scheme

Diagram 1- A set testing, 1 hour lots

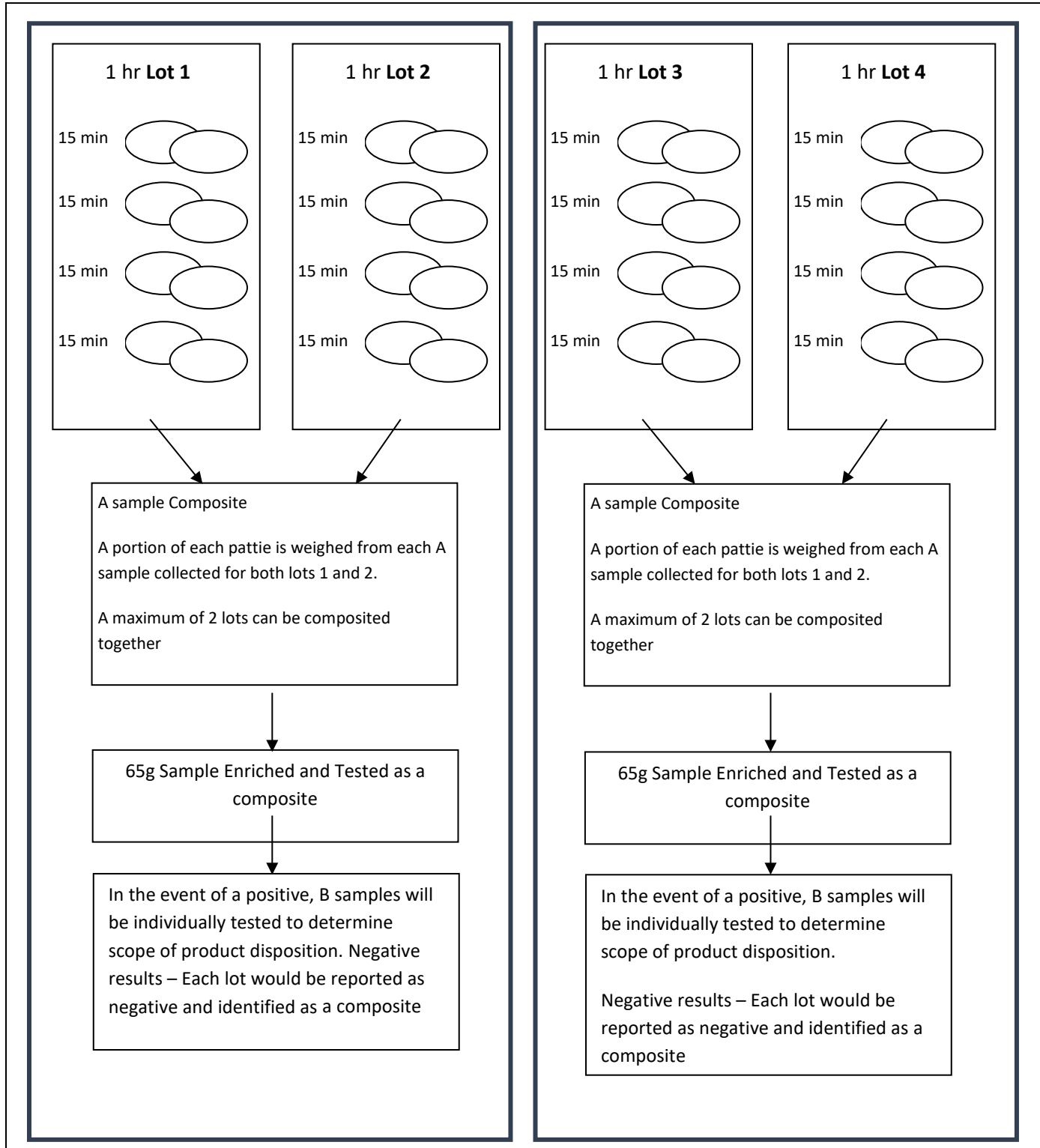


Diagram 2- B Set Testing, 1 hour lots

Each Lot represents 1 hour of production. 2 lots were composited to obtain A sample Results.

ALL PRODUCT REMAINS ON HOLD UNTIL B SET TESTING IS COMPLETED.

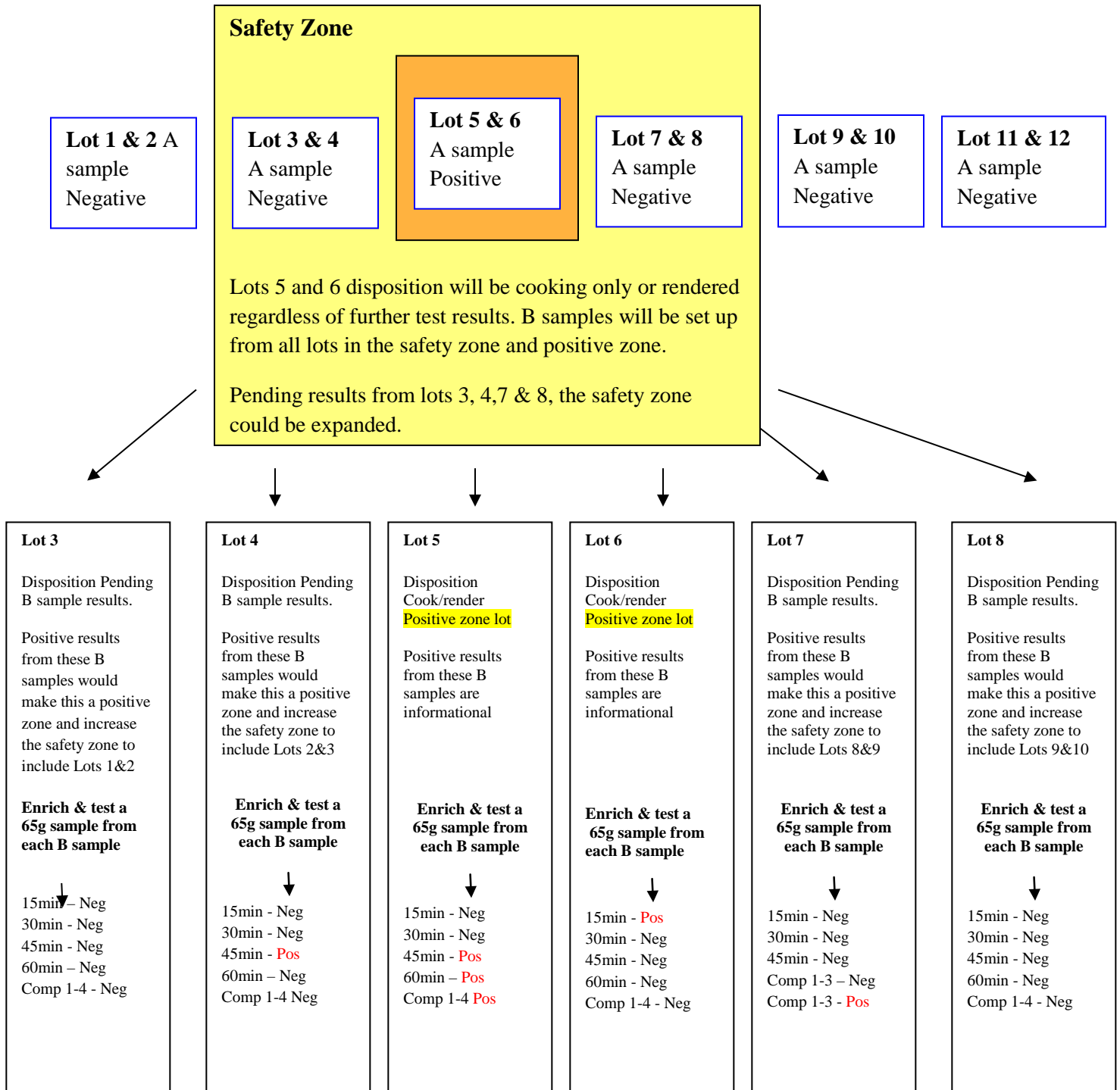
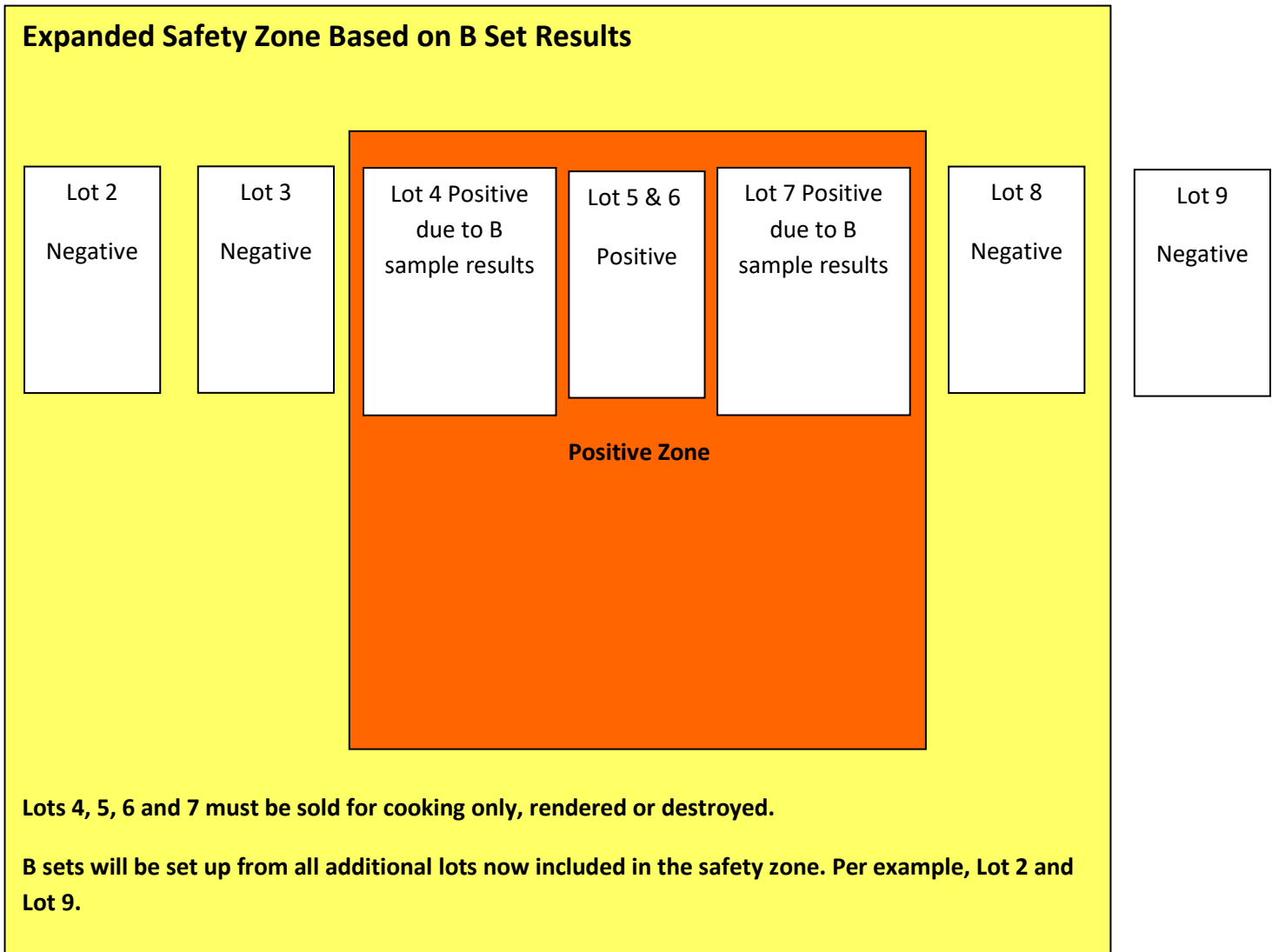


Diagram 3- Additional B set testing, 1 hour lots

ALL PRODUCT REMAINS ON HOLD UNTIL B SET TESTING IS COMPLETED.



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