



Best Practices for Using Microbiological Sampling

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Table of Contents

I. SCOPE.....	3
II. INTRODUCTION.....	3
III. PROCESS CONTROL VERIFICATION.....	3
A. FOR SLAUGHTER OPERATIONS	4
B. FOR FABRICATION.....	5
IV. GROUND BEEF.....	7
V. HOLDING TESTED PRODUCT:	8
VI. CONCLUSION:	9

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International HACCP Alliance

I. Scope

Due to the wide variety of products and processes employed within the beef industry, BIFSCo has limited the scope of this document to include only the sampling and testing during slaughter, fabrication, and the production of raw ground beef.

II. Introduction

When applied properly, microbiological sampling is a tool that can be used by processing establishments to evaluate process control. If microbiological sampling is not properly used, it can give a false sense of security that the process is in control when it is not. Therefore, this document is designed to provide best practices that can be applied throughout the industry to help develop appropriate procedures for using microbiological testing to verify process control. As with all best practices, each establishment will need to customize the best practices to fit individual operations.

Microbiological testing is not designed to test safety of the product, and end-product testing should not be used to replace verification of process control and validation of microbial interventions. It is important to remember that HACCP was initially developed as a preventive process control system for food safety hazards, because end-product testing was not capable of determining product safety.

Before an establishment develops and implements a microbiological testing program, it should know how the results are going to be used and the impact of the results on the product.

This document will focus on using microbiological testing for:

- process control verification
- trim sampling
- finished product sampling

III. Process Control Verification

Many microorganisms are classified considered as “indicator” microorganisms. Indicators can often be used to determine process control, but are not usually valid for the determination of presence or absence of specific human pathogens. Indicators are beneficial for verifying process control because they are generally present in sufficient numbers allows one to evaluate changes that can be attributed

to the application of the process. Typical indicators include total plate count (TPC), coliforms, *E. coli* species, *enterobacteriaceae*, etc.

There are many different factors that should be considered when evaluating total process control for each segment of the industry. For example, dressing procedure, overall sanitation, and cold chain management should all be considered. Each individual establishment must develop their own process control parameters and take action based on their own evaluation of control.

The basic steps in setting up a system for verifying process control include:

- Identify the locations that will be sampled.
- Establish the frequency of sampling.
- Collect samples.
- Establish a “baseline” for your operation.
- Evaluate the results on an on-going basis and take action when you identify an upward trend from the established baseline.

The following sections provide information on potential sampling locations, as well as factors that may impact the process.

A. FOR SLAUGHTER OPERATIONS

To verify process control within a slaughter operation, it is first necessary to identify the locations that will be sampled and understand what those results indicate about the process.

For example:

- Sampling before and immediately following hide removal may be an indication of sanitary dressing procedures.
- Sampling carcasses that are held on the out-rails or after extended down-time could be used to determine the impact of these practices on process control.
- Sampling at the end of the slaughter process prior to entering the hot-box for chilling, as an indication of the overall harvesting process, including interventions.
- Sampling chilled carcasses could be used to demonstrate cold chain management.
- Sampling locations that relate to by-product production may also be tested.

Taking a sponge sample on carcasses may be the easiest method; however, excision sampling can also be used. An establishment can follow the sampling procedures for taking a generic *E. coli* sample, and have the laboratory conduct APC or other indicator microorganisms on the same sample that is being used for generic *E. coli*. Regardless of the sampling technique, it is important that the sampling locations and the sampling procedures be consistent. In order for the results to be used to create a baseline and to be used to identify changes over time variation due to sampling and testing must be reduced by using the exact same technique at each sample location. A written, detailed standardized procedure is recommended.

Sampling must be designed to detect-find microbial contamination, thus it should be an aggressive sponging action over a large portion of the carcass surface. It is best to sample sites that are more prone to have contamination, i.e., plate, gracillus face, brisket-chuck, etc. Also, a

large surface area of sample sponging is critical to get appropriate process control data. Using the small size sample system is not a good 'best practice' guide to for evaluating process control.

Also, seasonality has been shown to have a major impact on microbial counts. Therefore, if the baseline is developed in winter/cool season, it is invalid for warm season trend analysis or process control purpose – and vice versa. Furthermore, significant changes in the process, equipment, or facility may lead to baseline reassessment and reestablishment.

A "baseline" could be established for the specific locations for each operation through application of statistical process control (SPC) techniques to the data. After the "baseline" is set, then the establishment would use future test results to look for upward trends or changes in the data that indicate the process lacks control. If an upward trend or change is noted, then the establishment must carefully evaluate the process to identify causes in the operation that could have contributed to the change. For example, if a baseline is established for samples taken following hide removal, and then counts indicate an increasing trend, all of the activities that impact the hide removal should be evaluated – condition of cattle coming in, equipment malfunctions, employees (key employees on vacation, out sick, etc.), downtime, and any other factor. Identifying factors that could be contributing to the increase will allow the establishment to make changes to bring the process back into control.

B. FOR FABRICATION

As with slaughter, locations to be sampled and sampling procedures to establish the baselines must be determined. For example:

- Sampling the chilled carcass entering fabrication could provide information on cold chain management.
- Sampling cuts and/or trim could be used as an indication of process control, hygienic conditions through the fabrication lines.

The most commonly used protocol for sampling products exiting the fabrication line, especially trim, is the N=60 sampling protocol. The N=60 sampling protocol is commonly used for sampling beef trimmings for *E. coli* O157:H7 testing, but it can also be used for sampling indicator organisms to establish trends.

The N=60 was initially selected because according to the International Commission on Microbiological Specifications for Food (ICMSF, 1974) Case 15 is used for severe or direct health hazards. It was the highest sampling plan recommended for foods; therefore, it was selected as the optimal sampling scheme for raw beef trimmings intended for ground beef. Additionally, FSIS supports the N=60 sampling program when it is combined with validated interventions.

This level of sampling should be sufficient to identify highly contaminated lots of product; however, it is not 100% effective at identifying contamination. This sampling scheme provides a 95% statistical confidence of detecting a positive in at least one of 60 sub-samples (6.25 g of product) when the expected sub-sample of contamination rate is 5% or greater.

When using this type of sampling plan, it is expected that some level of positives will be detected. Each positive sample requires an evaluation of the process to determine if changes are needed to ensure process control. Some positives may be random, isolated events that cannot be identified to an assignable cause. Detection of multiple positives, especially in a single day, increases the likelihood of an assignable cause. Some items to consider when a positive sample is detected include: employee differences, zero tolerance failures, CCP failures, down-time; higher number and/or extended time on out-rail, construction/deconstruction activities, sewer/drain backups in coolers and processing areas, airflow from dirty-to-clean areas, rendering system failures with the facility, etc. Identifying assignable causes and taking corrective actions will allow the process to be brought back into control. *(Note: On days when there are multiple E. coli O157:H7 positives, it may be wise to consider diverting all trimmings from associated lots, even those that tested negative, to a cook operation. This recommendation is made based on the limitations of testing and an increased likelihood of undetected contamination from product that tested negative.)*

The N=60 sampling protocol uses an excision method to remove the surface of 60 pieces of product from throughout the defined lot. It is best to select samples from pieces of trim taken from the original surface of the beef carcass (i.e., exterior carcass surfaces). This sampling protocol has been accepted and is currently being used by FSIS personnel to conduct sampling of trimmings. The following protocol was modified from FSIS training materials.

Procedure for Collecting Samples N=60 Protocol

When collecting samples, personnel are to:

1. Sanitize the caddy, knife, hook, or tongs before collecting the samples by using the sanitizing solution according to label instructions or by using hot ($\geq 180^{\circ}\text{F}$) water as a sanitizer;
2. Use sterile gloves and handle all sanitized surfaces so that they do not become contaminated;
3. Select samples by using the N60 method of sample collection (as described below) and collect 60 individual pieces from raw beef manufacturing trimmings:
 - a. if a specific production lot is composed of greater than 5 containers of beef manufacturing trimmings, randomly select 5 containers for sampling (i.e., boxed product); and
 - b. if the specific production lot is composed of 5 or less containers (i.e., 5 or fewer combos), use the chart below for sampling;

Number of Sample Pieces to Collect Per Container	
# of containers in each specific production	# of sample pieces to select from each container
5	12 pieces
4	15 pieces
3	20 pieces
2	30 pieces
1	60 pieces

4. Aseptically collect the appropriate number of pieces of beef manufacturing trimmings based on the number of containers that represent one specific production period. Use the sanitized hook or tong to lift a piece of meat off the top of the container. The total number of pieces collected are to be 60 for each sample;
5. Cut off a slice that is approximately a 4 inch length by 1/2 inch width and 1/8 inch thick from each of the 60 pieces. Cut off as much of the beef manufacturing trimmings' outer surface as possible. The priority is to collect samples from pieces of product taken from the original surface of the beef carcass.
6. Collect and bag the sample slices in a sterile bag. Weigh the sample to ensure approximately 375 grams of product are collected in the whirlpak sample bag.

(Note: The FSIS sampling protocol collects approximately 2 pounds of product because it is used fro different laboratory analysis. For normal industry analysis of E. coli O157:H7 the sample must weigh approximately 375 grams.)
7. Check the product temperature of the top pieces of meat from randomly selected containers of beef manufacturing trimmings (do not take the temperature of the actual sample slices). Record the temperature. If the sample pieces came from more than one container, record the temperature of the warmest container. If the product is warmer than 40°F, place the bag containing the sample in a cooler to chill before shipping;
8. Secure samples during preparation, storing, packaging, and submission for testing.

Non-Intact Products:

For information on non-intact products please see the Non-intact Best Practices located at <http://www.bifsc.org/BestPractices.aspx>.

IV. Ground Beef

Some establishments may decide to also sample finished ground beef. The establishment will need to determine if the finished product sampling is being used as a verification of process control by establishing a baseline of indicator microorganisms or if it is being used to sub-lot products. If it is being used to verify process control, then a baseline would be established and when upward trends are

identified the process would need to be reassessed, just as described above for slaughter and fabrication.

If the sampling is being used to sub-lot then the establishment must be able to validate that the sampling scheme and testing protocol is sufficient. One sub-lotting procedure that has been used on finished ground beef and has been demonstrated to be effective was initially designed by Jack-in-the-Box.

For example, some establishments may decide to sample and test finished ground beef along with or in place of sampling of beef trimmings, specifically for *E. coli* O157:H7. The establishment should determine if the finished product sampling and testing for *E. coli* O157:H7 is being used as a verification of process control for raw material supplies or as a method of sub-lotting a day's production. If the sampling and testing is only used as a verification of process control for raw material suppliers then the defined lot for the raw ground beef produced would still be "cleanup to cleanup" as described in USDA Directive 10,010.1, revision 1, 3/31/04.

It is also recommended that establishments producing raw ground beef products establish a baseline of indicator microorganisms as a method of process control verification. This process control microbiological data can then be used to establish a baseline and when upward trends are identified the process would need to be reassessed, just as described above for slaughter and fabrication.

If the sampling and testing for *E. coli* O157:H7 is being used to sub-lot a day's production then the establishment must be able to validate that the sampling scheme and testing protocol is sufficient. One sub-lotting procedure that has been used on finished ground beef and has been demonstrated to be effective was initially designed by Jack-in-the-Box.

This program requires that samples are taken from each batch of product produced (typically 3,000 pounds) and composited with samples from other batches to create a sub-lot representing not more than 12,000 pounds of finished product or approximately 4 batches. These composited samples are then enriched and tested specifically for *E. coli* O157:H7. If a positive result is confirmed then additional testing of backup samples for each batch in the positive composite or composites can be run independently to isolate the contamination and identify affected products. Further backup testing of individual pallets or cases of product may be required to isolate the affected product even further.

(Note: Ground beef testing program designs are quite different depending on whether you are dealing with a fresh ground beef versus a frozen ground beef product. The scheme described above is for frozen ground beef, and may not lend itself as a stand alone best practice for a fresh ground beef sampling scheme.)

V. Holding Tested Product:

Before a microbiological pathogen test is conducted, the establishment must know the impact of a positive result. For example, in ground beef a positive result may impact more than just the lot being sampled because additional lots may have the same raw material sources. To reduce the potential for a recall, all associated products that could be implicated by a positive sample must be held. Also, make

sure you have appropriate hold procedures in place to prevent products from being shipped before the test result is received.

For information on holding tested products, including assistance with proper lot identification, please refer to the “[Industry Best Practices for Holding Tested Products](#).” This document can be found on www.BIFSCo.org or you may request a copy from the International HACCP Alliance (979-862-3643). Additional best practices are located at www.BIFSCo.org.

VI. Conclusion:

When designed and implemented properly, microbiological sampling can effectively demonstrate process control of the total food safety system. Process control is important for all components within a food safety system, including HACCP, SSOP's, sanitary dressing practices, employee practices, and other plant programs.

Key points identified:

1. Food safety cannot be tested into the product, and testing cannot replace proper validation/verification of the process.
2. Consistent sampling methods and appropriate analysis are required for reliable results.
3. If there is an increase or upward trend from a baseline, or a positive result for a *E. coli* O157:H7, then you must evaluate the entire process to determine if it was in control.

Notes:

- 1) If a product test is positive for *E. coli* O157:H7, it can not be re-sampled and re-tested in an effort to obtain a negative result.
- 2) If a microbiological analysis is composed of composites, the individual enrichments may be sampled separately to release sub-lots of a composited sample. You must be able to scientifically validate that the individual enrichment sampling is sensitive enough to detect any possible contamination present in the sample. It is recommended that multiple aliquots be taken from each enrichment to determine true positive or negative results after a positive result is obtained in the original composite.

This document was developed by members of the Beef Industry Food Safety Council. Best Practice documents are ever evolving, and as changes or new information becomes available, these documents will be reviewed and updated. Questions or suggestions are welcome and should be addressed to BIFSCo at bifsc@beef.org.

