INTRODUCTION
Foodborne illnesses caused by microorganisms are a food safety concern among consumers and regulatory agencies. Foodborne *Escherichia coli* O157:H7 (*E. coli* O157:H7) and *Salmonella* are common human infectious agents throughout the world and can cause severe debilitating symptoms and in some cases may result in death. Livestock such as cattle, poultry and swine are well known reservoirs of *E. coli* and *Salmonella*. The bacterium often contaminates the animal during rearing and can remain attached to the hide or carcass during processing. If in-plant interventions and sanitary dressing procedures do not effectively reduce pathogen levels, beef products including trim and ground beef can become contaminated. Due to the constant number of *E. coli* and *Salmonella* outbreaks in ground beef over the past decade, some manufacturers want to know how much *Salmonella* is present in order to effectively manage products posing a high public health risk. Therefore, meat processors must implement comprehensive, robust food safety systems to keep meat safe and wholesome for consumers.

OVERVIEW OF HACCP
HACCP (Hazard Analysis and Critical Control Point) is the cornerstone of the meat industry’s preventive food safety approach. Originating in the 1960s, the system has been adopted by many food processors during the past 50+ years and is considered the “gold standard” food safety process management system. Using HACCP, meat companies carefully analyze processes used for each product they make and identify critical control points (CCP) – steps in the production process where potential biological, physical, and/or chemical hazards can be controlled. A CCP could include ensuring the proper cooling of meat to control bacterial growth, or thorough testing of carcasses for contamination. Once these CCPs are identified, companies implement and monitor CCPs to control and document their process – a key component of ensuring their food safety system is working correctly. This type of approach identifies the most high-risk parts of the food manufacturing process and allows companies to focus resources accordingly.

MICROBIOLOGICAL TESTING
Along with process control, a company’s food safety process management system needs to be verified that it is effective by utilizing microbiological testing. Since all raw agricultural products – including meat—may naturally contain bacteria, even pathogenic bacteria, a meat company’s HACCP plans must reflect this fact and address the potential hazards. While a meat plant’s goal is to reduce all bacteria, it is not possible to determine with 100 percent certainty the presence or absence of...
pathogenic bacteria. Why? Because each microbiological test destroys the sample that is tested. And results only apply to the tested sample. It is possible to pull a 300-gram sample of ground beef, test it and receive a negative result despite pathogen’s presence elsewhere in the larger batch. Therefore, pathogen testing of raw meat (and poultry) should not be a measure of plant’s success or failure but used as a tool within a well-designed HACCP plan to verify the food safety system is working correctly. Under HACCP programs, much of the microbial testing is done to track amounts of commonly found bacteria — typically the harmless bacteria that naturally exists in measurable quantities on raw meat. The levels of these generic bacteria on meat are used as an indicator of how well a plant is succeeding in eliminating the much rarer and harder to find pathogenic strains (i.e., how well it is cleaning the facility and equipment). Deviation from established baseline or historical values means the plant needs to implement its action plan to identify potential causes of the increased microbial counts. The increase does not necessarily mean that the product is unsafe, since cooking a raw product destroys bacteria, but it may indicate that perhaps something in the process has changed and should be examined to ensure the food safety process management system is still effective.

In addition, the meat industry is regulated by the U.S. Department of Agriculture (USDA). USDA requires several different tests, some of which are conducted by plants and some of which are collected by USDA inspectors and run in USDA laboratories.

**TESTING PERFORMED IN FEDERALLY INSPECTED PLANTS**

According to the Foundation for Meat & Poultry Research & Education¹, tests and testing programs that may be run in federally inspected meat (and poultry) plants can include:

- **Baseline data collection.** USDA's FSIS has established a series of tests to determine the microbiological profiles of meat for select microorganisms, such as *E. coli O157:H7* and *Salmonella*. These baseline studies are used to develop new prevention programs and develop new pathogen reduction performance standards.

- **Generic *E. coli* for carcasses.** All federally inspected plants that slaughter livestock are required to test for *E. coli* to verify that their process control systems work as intended to prevent contamination.

- **Salmonella for carcasses and raw ground products.** The Pathogen Reduction/HACCP final rule instituted a *Salmonella* performance standard that must be met. Products covered by the standard include carcasses of cattle, swine (and broilers), and ground beef, (ground chicken and ground turkey). This pathogen reduction standard originated due to the wide-spread prevalence of *Salmonella* in each of these products. Plants must conduct a series of tests for the presence of *Salmonella* (the numbers and intervals vary for each product). When a positive sample is found, plants must take corrective actions to prevent *Salmonella* contamination.

- ***E. coli O157:H7* for ground beef.** The USDA collects roughly 10,000 samples of ground beef and raw material used to make ground beef per year in plants, retail stores, import facilities, and tests these samples for the presence of *E. coli O157:H7*. When *E. coli O157:H7* is found in raw ground beef, the product is deemed to be unfit for consumption. In addition to government tests, many meat processors and handlers conduct their own tests for *E. coli O157:H7* - sometimes voluntarily, sometimes to meet customer specifications, or to verify CCPs. The FSIS also collects samples for testing, but these are from cooked, ready-to-eat meat patties and dry fermented sausage in federally inspected plants. When *E. coli O157:H7* is found on these products, they also are considered adulterated and unfit for consumption.
• **Food safety assessments and in-depth verification testing by USDA regulatory personnel.** This is primarily to verify how well HACCP systems are working to produce safe food and to ensure plants' execution of the plans include following up-to-date regulatory requirements.

• **Generic *Listeria* in the environment of a ready-to-eat plant.** The greatest risk for *Listeria* product contamination occurs when a product contact surface is contaminated. This risk is highest between the point where a food is cooked, pasteurized, decontaminated, etc. and the point where the food is packaged. To effectively manage the risk of product contamination, it is necessary to assess where along the product flow the exposed food is most likely to become contaminated.

• ***Listeria monocytogenes* and *Salmonella* testing in ready-to-eat products.** Ready-to-eat products or fully cooked products are intended to be eaten right out of the package. For this reason, in the U.S., there is a “zero tolerance policy” in effect for pathogens on ready-to-eat meat products because they can pose a risk to certain populations, like the elderly, pregnant women and those who are immunocompromised. USDA began testing these products for *Salmonella* in 1983 and for *Listeria monocytogenes* in 1987. Since 2002, FSIS published numerous directives, which have increased the focus on *Listeria* control programs at establishments, including environmental sampling and testing programs. In addition to USDA testing, many companies also voluntarily test their ready-to-eat meat products for the presence of pathogens.

**EXPANDED TESTING REQUIREMENTS**

In addition, the FSIS is expanding its routine verification testing beyond *E. coli* O157:H7 to include six non-O157 Shiga toxin-producing *Escherichia coli* (STEC): O26, O45, O103, O111, O121, and O145 that are also considered adulterants. This testing applies to ground beef, bench trim, and other raw ground beef components (other than raw beef manufacturing trimmings). These pathogens have also been known to cause serious illness, such as the 2019 *E. coli* O103 outbreak linked to raw ground beef that sickened nearly 200 people in 10 states. A 2018 *E. coli* O26 outbreak, also linked to raw ground beef, sickened 18 people in 4 states. Additionally, FSIS also intends to test for non-O157 STEC in ground beef samples it collects at retail stores and in applicable samples it collects of imported raw beef products.²

Slaughter establishments are in the best position to prevent non-O157 STEC contamination because the introduction of the contaminant to the exterior surface of beef products can occur during the slaughter and dressing operation. Processing establishments that receive product for grinding also have an important role in addressing non-O157 STEC. As mentioned, Hazard Analysis and Critical Control Point (HACCP) regulations require establishments to conduct a hazard analysis to determine the food safety hazards that are reasonably likely to occur in their production processes and to identify the preventive measures they can apply to control those hazards in the production of particular products (see 9 CFR 417.2(a)).
TESTING APPROACHES

What is the best way to approach all these testing requirements?

By consolidating testing into the fewest samples and enrichments possible. Identify the best sample collection method(s) and from an enrichment, run multiple assays – for E. coli, non-O157 STEC, and Salmonella species – from that enrichment.

These methods are still labor intensive and result in product loss. Let’s examine some approaches below and identify best practices for testing.

N60 SAMPLING (USDA AND FSIS)

Historically, for beef, the USDA and FSIS collects samples by N60 excision and analyzes for both Shiga toxin-producing Escherichia coli (STEC) and Salmonella. This requires collection of a total of 60 pieces from each lot. These must be aseptically collected by slicing thin strips from the external surface of trim pieces. Each strip is then scored with a knife and placed into a sampling bag. Once all strips are prepared, the bag should weigh approximately 325 g. The bags are then sent to the lab for testing. This process is labor intensive, poses safety issues to the employees, and increases the chances of cross-contamination (as samples may need to be collected from multiple barriers). In addition, it results in product loss. Following the sampling, laboratory testing ensues and if a positive result is obtained, further testing is performed to confirm if the sample is indeed positive. The confirmatory testing usually takes three to four days – again, laborious and time-consuming.

NON-DESTRUCTIVE TESTING

A new approach was developed that is nondestructive. It uses continuous sampling of the trim as the combo is filled. This continuous sampling device (CSD) is positioned at the end of the conveyor so that the trim pieces rub against a sampling cloth as they fall into the combo bin. For situations in which the combo is not filled by a conveyor, a second method was developed that uses the CSD cloth to manually sample all of the trim on the top of the combo by hand (manual sampling device or MSD).

This novel sampling methodology, using a manual sampling device (MSD) swab, eliminate loss of potential final product as it samples the surface rather than taking actual portions of meat to be tested. This swab provides a simplified sampling approach that is nondestructive and efficient. Use of an MSD swab has the potential to produce rapid cost-efficient results when screened with PCR and reduce product waste.3

HOW THE BAX® SYSTEM CAN HELP

To verify this sampling method works for Salmonella and E. coli using the BAX® PCR System, the MSD swab method was compared to the USDA FSIS reference culture method for multiple beef sample types. Beef trim samples were analyzed for the presence of E. coli O157:H7, STEC, and Salmonella using MSD swabs, specifically, MicroTally™ manual sampling swabs. In addition, a limits-based testing approach was utilized to evaluate high levels of Salmonella. Last, ground beef and beef trim were analyzed for contamination using both Salmonella limits testing and quantification methods (SalQuant™) using MicroTally™ swabs and the BAX system for analysis.
**CURRENT INDUSTRY WORKFLOWS – GROUND BEEF**

**SALMONELLA DETECTION IN BEEF**

For the beef trim, 60 pounds was sourced from a local butcher for samples. MicroTally™ swabs were removed from the sample bag, unfolded and firmly used to swab the beef trim. Once both sides of the cloth were used, swabs were folded back to the original dimensions with an additional horizontal fold per the manufacturer’s instructions and placed into the original sample bag. The MSD swabs were then spiked with various amounts of *Salmonella* and homogenized and incubated in the proper medium. After 3-6 hours, samples were analyzed using the BAX System Real-Time PCR Assay for *Salmonella*. All inoculated samples were detected after 4, 5, and 6 hours of enrichment and all 30 were confirmed with culture.

**SALMONELLA QUANTITATION**

When repeated for ground beef and beef trim (and MicroTally™ MSD swabs) using contamination levels of 10 CFU/g, the BAX System Real-Time PCR Assay for *Salmonella* detected the presence of organisms at 4-5 hours of enrichment. In addition, after a 6-hour enrichment, *Salmonella* could be quantified from 1-10,000 CFU/g when following the SalQuant™ approach. This allows beef processors to not only identify the presence of *Salmonella*, but also to identify which lots of ground beef or beef trim contain higher levels of *Salmonella*, allowing for rapid action to reduce risk of exposure to consumers and to improve food safety processes internally. (Similar results were obtained for poultry too.)
SALMONELLA AND E. COLI DETECTION FROM A SINGLE ENRICHMENT

For E. coli O157:H7 detection, a number of PCR approaches can work such as the BAX System Real-Time PCR Assay for E. coli O157:H7, the BAX System Real-Time PCR STEC Assay, or the BAX System Real-Time PCR Assay for E. coli O157:H7 EXACT. However, when it comes to time savings, the best option is to use the same sample enrichment for both Salmonella and E. coli O157:H7 testing. Using organism spiked MicroTally™ MSD swabs, we confirmed that the BAX System Real-Time PCR assays for Salmonella and E. coli O157:H7 accurately detected the presence of both organisms from beef trim in 8 hours from a single enrichment from the same MSD swab. Results were again equivalent to those obtained by the reference culture methods. The bottom line is this: choose the method that provides you the best results, in the shortest amount of time, without wasting product for testing. To meet all these requirements, the BAX System Real-Time PCR assays are an excellent choice.

References:
1. https://www.meatpoultryfoundation.org/fact-sheets/microbiological-testing
escherichia-coli-stec-testing-to-additional-raw-beef

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