ORIGINAL SUBMISSION

Advanced Food Technologies, LLC

11230 Magnolia Glen
Shreveport, LA 71106

October 12, 2011

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Division of
Biotechnology and
GRAS Notice Review

Via Federal Express

Antonia Mattia, Ph.D.
Office of Food Additive Safety
Division of Biotechnology and GRAS Notice
Review (HFS-255)
Center for Food Safety and Applied Nutrition
Food and Drug Administration
5100 Paint Branch Parkway
College Park, MD 20740

Re: GRAS Notification for Sulfuric Acid and Sodium Sulfate Blend Used as an Anti-Microbial in Meat and Poultry Processing

Dear Dr. Mattia:

By this letter, Advanced Food Technologies, LLC ("AFT") is submitting four copies of a GRAS Notification for its AFTEC 3000 product which is a blended combination product containing sulfuric acid and sodium sulfate. AFT has determined that AFTEC 3000 is generally recognized as safe ("GRAS") for use as an anti-microbial agent for the treatment of meat and poultry to reduce levels of micro-organisms (bactericidal) and to prevent microbial growth (bacteria static). The product is intended for use on the surface of meat or poultry and can be delivered via spray, wash or dip.

AFTEC 3000 and its proposed uses are exempt from the premarket approval requirements of the Federal Food, Drug, and Cosmetic Act because AFT has determined through scientific procedures that such use is GRAS. AFT's GRAS determination is likewise supported by the fact that the Food and Drug Administration ("FDA") has previously affirmed each of the constituent components (i.e., sulfuric acid and sodium sulfate); various blends containing at least one of AFTEC 3000's included constituent components (i.e., sulfuric acid / ammonium sulfate / copper sulfate blends); and a variety of other acids (i.e., acetic acid, phosphoric acid) as GRAS.

AFT is including a fourth copy of the submission for FDA to share with the U.S. Department of Agriculture ("USDA") since the proposed use occurs within USDA regulated facilities.

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Dear Dr. Mattia:

By this letter, Advanced Food Technologies, LLC ("AFT") is submitting four copies of a GRAS Notification for its AFTEC 3000 product which is a blended combination product containing sulfuric acid and sodium sulfate. AFT has determined that AFTEC 3000 is generally recognized as safe ("GRAS") for use as an anti-microbial agent for the treatment of meat and poultry to reduce levels of micro-organisms (bactericidal) and to prevent microbial growth (bacteria static). The product is intended for use on the surface of meat or poultry and can be delivered via spray, wash or dip.

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AFT is including a fourth copy of the submission for FDA to share with the U.S. Department of Agriculture ("USDA") since the proposed use occurs within USDA regulated facilities.

If for any reason the agency has any questions or requires any additional information to aid its review of AFT's conclusion, please contact us at the address listed above or our counsel, Robert G. Hibbert (202-778-9315) at your earliest convenience.

Sincerely,

(b) (6)

Stephen Mixon /Rett
Director of Operations

Enclosures

cc: Judith L. Kidwell, FDA
Susan Carlson, FDA
John Hicks, FSIS
Robert G. Hibbert, K&L Gates

GRAS NOTIFICATION

FOR SULFURIC ACID

AND

SODIUM SULFATE BLEND

SUBMITTED BY

ADVANCED FOOD TECHNOLOGIES, LLC

GRAS NOTIFICATION FOR A SULFURIC ACID AND SODIUM SULFATE BLEND FOR USE AS AN ACIDIFIER OR ANTI-MICROBIAL ON MEAT AND POULTRY

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1 GRAS NOTIFICATION FOR A SULFURIC ACID AND SODIUM SULFATE BLEND FOR USE AS AN ACIDIFIER OR ANTI-MICROBIAL AGENT FOR MEAT AND POULTRY

Advanced Food Technologies, LLC ("AFT") manufactures a blended product containing sulfuric acid and sodium sulfate for use as an acidifier or anti-microbial agent for meat or poultry and is intended to be delivered via spray, wash or dip. The trade name of the product is AFTEC 3000 but it will also be known as AFT Clear 3000 (hereafter, referred to as "AFTEC 3000").

AFTEC 3000 is intended for use as an acidifier or anti-microbial agent for meat and poultry to reduce levels of micro-organisms (bactericidal) and to prevent microbial growth (bacteria static). Chemically equivalent to Sodium Bisulfate in Solution, the product contains both sulfuric acid and sodium sulfate in blend. The included sodium sulfate, which is a natural conjugate salt of sulfuric acid, serves as a buffering agent to the sulfuric acid. Diluted to a targeted pH of 1.0-2.2 for use in the form of a spray, wash or dip; AFTEC 3000 kills microbes via the low pH effect.

AFT's product is manufactured using food grade raw materials recognized under the Food Chemical Code ("FCC") in accordance with the U.S. Food and Drug Administration's ("FDA") current Good Manufacturing Practices ("cGMPs") by Harcros Chemicals Inc. in Vicksburg, Mississippi.

Pursuant to the regulatory and scientific procedures set forth in the Proposed Rule "Substances Generally Recognized as Safe," 62 Fed. Reg. 18937 (April 17, 1997) (proposed 21 C.F.R. § 170.36) ("GRAS Proposed Rule"), AFT has determined, through scientific procedures, that AFTEC 3000 is GRAS for use as an antimicrobial agent in levels not to exceed cGMPs and is therefore exempt from the requirement for premarket approval. General and specific information identifying and characterizing AFTEC 3000, its applicable conditions for use, and other supporting information provide the basis for AFT's GRAS determination.

There are several sulfuric acid blends in use today in meat and poultry plants in the U.S. They are used for their anti-microbial properties. Examples of such blends include 1) sodium acid sulfate or sodium bisulfate (used as an acidifier for meat and poultry, and used in bread making); 2) sulfuric acid + ammonium sulfate + copper sulfate (used as an acidifier for poultry scalders, pickers, NY rinse and post-chill treatment); and 3) sulfuric acid + citric acid + phosphoric acid (used as a poultry application including on-line reprocessing, chill treatment and post chill applications). In all of these products, the sulfuric acid is the main active anti-microbial component. The other components serve simply as buffering agents to allow for the safe handling of the sulfuric acid.

Likewise, several additional acids are currently used individually or in combination as blends with meat and poultry in the U.S including among others, acetic acid, phosphoric acid, sulfuric acid, hydrochloric acid, citric acid, sodium bisulfate (sodium acid sulfate) and hypochlorous acid (from chlorine + water). These acids are designated as acidifiers or anti-microbial agents, and are identified in the U.S. Department of Agriculture ("USDA") Food Safety and Inspection Service's ("FSIS") Directive 7120.1. *See* "Safe and Suitable Ingredients Used in the Production of Meat, Poultry, and Egg Products," FSIS Directive 7120.1, Revision 7 (July 1, 2011).

The material and data included in this submission clearly shows the safety of AFTEC 3000 for use as an acidifier or anti-microbial agent with meat or poultry. As outlined in the prior Table of Contents, Section 1 provides general information identifying and characterizing AFTEC 3000, its applicable conditions for use, and the basis for AFT's GRAS determination. Section 2 describes the AFTEC 3000 product and its chemical solution. Section 3 discusses the manufacturing process to make AFTEC 3000. Section 4 discusses the product's formulation. Section 5 includes a discussion of application and Section 6 is the Safety Evaluation. Lists of attachments and references also accompany the notification.

1.1 Name and Address of Notifier

NOTIFIER

Advanced Food Technologies, LLC
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Shreveport, LA 71106
Email:dsmithyman@advfoodtech.com
Tel: 908-385-7216
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PERSON RESPONSIBLE FOR THE DOSSIER

Stephen Mixon
Director of Operations
Advanced Food Technologies, LLC
PO Box 1208
Fairhope, AL 36533
Email: smixon@advfoodtech.com
Tel: 713-261-0674
Fax: 936-622-6826

1.2 Common or Usual Name of Substance

AFT produces a sulfuric acid and sodium sulfate blend for use as an acidifier or anti-microbial on meat or poultry. The trade name of the product is AFTEC 3000. The product is also known as AFT Clear 3000.

1.3 Applicable Conditions of Use

AFTEC 3000 is intended for use as an acidifier or anti-microbial to reduce the level of micro-organisms (bactericidal) and to prevent microbial growth (bacteria static) on meat and poultry. It is delivered in the form of a spray, wash, or dip. Equivalent to Sodium Bisulfate in Solution, AFTEC 3000 contains both sulfuric acid and sodium sulfate, and kills microbes by the low pH effect. *See* Growth Factors for Selected Bacteria, Pathogen Modeling Program Online, USDA Agricultural Research Service (Last accessed on October 11, 2011) (Attachment 1).[1]

When used as a spray, wash, or dip, the microbial reduction has a temporary effect and the chemical solution quickly drips off, evaporates, or otherwise leaves no significant chemical residue and has no lasting technical effect.

1.3.1 Substances Used In

AFTEC 3000 is intended for use directly on meat and poultry surfaces. This includes whole carcasses, parts, trimmings, organs and cut meats.

1.3.2 Levels of Use

AFTEC 3000 is diluted with water to a pH level that is suitable for the intended purposes stated above. For proper anti-microbial efficacy this is usually a pH range of 1.0 – 2.0 which amounts to a 1/25 – 1/500 volumetric dilution of the product with water respectively. The most common working target is pH 1.5 or a 1/100 dilution. A table comparing pH levels by volumetric dilution rate and product weight is included below.

Dilution (v/v)	pH	Wt% AFTEC	Wt% H_2SO_4	Wt% Na_2SO_4
1/25	1.0	5.44	2.12	0.27
1/100	1.5	1.38	0.54	0.07
1/500	2.0	0.28	0.11	0.01

1.3.3 Purposes

AFTEC 3000 is intended for use as an acidifier or anti-microbial to reduce the level of micro-organisms (bactericidal) and to prevent microbial growth (bacteria static) on meat and poultry. Examples of proposed applications are described below:

Beef Processing - In the multi-hurdle approach in beef processing, AFTEC 3000 can be used in several processing steps to reduce microbial contamination, including hide wash, eviscerated carcass wash, parts wash, on primals and cut meats, and on trimmings prior to grinding.

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[1] Available at http://pmp.arserrc.gov/PMPOnline/References/GrowthFactors.aspx

Poultry Processing - In the multi-hurdle approach in poultry processing, AFTEC 3000 can be used in several processing steps to reduce microbial contamination, including with scalders, pickers, New York rinse, on-line reprocessing, chillers, post-chill dips, on parts such as organs and feet (paws), and trimmings prior to grinding.

1.4 Basis for AFT's GRAS Determination

Pursuant to the GRAS Proposed Rule, AFT has determined, through scientific procedures that the combination of sulfuric acid and sodium sulfate is GRAS for use as an antimicrobial agent in levels not to exceed current good manufacturing practices. The safety of AFTEC 3000 is supported by the fact that the blended product readily dissolves in aqueous media into the same constituent ions as the components that are used for its manufacture. Individually, each of the constituent components has been affirmed as GRAS by the FDA. For the reasons explained below, AFTEC 3000 is affirmed to be GRAS for use as an acidifier and by extension, a processing aid.

FDA states in its 2009 and 2011 letters to AFT with respect to sulfuric acid:

> "Sulfuric acid (CAS Reg. No. 7664-93-9) is listed under 21 C.F.R. § 184.1095 for use as a pH control agent and as a processing aid."

FDA states with respect to sodium sulfate:

> "Sodium sulfate (CAS Reg. No 7727-73-3) is listed for use as a direct food additive under §172.615 where it is permitted for use as a masticatory substance, stabilizer, thickener or gelling agent. It is also listed for use as a secondary direct additive under §173.310 as permitted for use as a boiler water additive or processing aid."

FDA also noted:

> "Sodium sulfate is the soluble salt formed by sulfuric acid and sodium hydroxide. Both sulfuric acid (21 CFR § 184.1095) and sodium hydroxide (21 CFR § 184.1763) are generally recognized as safe (GRAS) substances that completely ionize in water to form sodium sulfate ions and more water. Therefore, sodium sulfate is also GRAS and can be used as a processing aid in accordance with 21 CFR § 174.5(d)(1), which authorizes GRAS substances for use as indirect additives. The only limitation on the use levels of either of these compounds would be based on good manufacturing practice (GMP) in accordance with 21 CFR §148.1(b)(1), which means using the minimum amount to accomplish the intended technical effect."

See FDA Letters to Stephen Mixon, Director of Operations, Advanced Food Technologies, LLC (July 1, 2009; August 30, 2011) (Attachment 2). *See also* 21 C.F.R. § 172.5 (In the context of direct food additives, the term "good manufacturing practice" means using the minimum amount to accomplish the intended technical effect.)

Furthermore, FSIS has previously recognized the use of AFTEC 3000 in meats and poultry in different capacities.[2] Equivalent to sodium bisulfate, FSIS Directive 7120.1 allows for the product's use 1) as a pH control agent and processing aid in water used in meat and poultry processing at levels "sufficient for purpose;" 2) as a pH control agent in meat and poultry soups at levels not to exceed 0.8 % of the product formulation; and 3) for addition to sauces used as separable components in the formulation of various meat products at levels sufficient for such purposes (citations to GRAS Notice GRN No. 3 included). *See* FSIS Directive 7120.1. Since FSIS requires assurances of the GRAS status of an ingredient before accepting the suitability of the use, the above-listed uses can be considered GRAS.

Based on FDA's various GRAS determinations with respect to sulfuric acid, sodium sulfate and the various blends referenced above; it is appropriate to assume by extension that the FDA also recognizes the GRAS status of AFTEC 3000 in all foods, including meat and poultry, as a pH adjuster and processing aid. Furthermore, FSIS has already accepted the GRAS status of these ingredients in combination as a pH adjuster, and considering that but for notation of the impact on microbes, there is no substantive difference in the use and ingredient levels of such products and AFTEC 3000, it is appropriate to conclude that AFTEC 3000 is equally safe for use as a pH adjuster and as an antimicrobial agent.

Since the use range, method of application, and targeted products for AFTEC 3000 when used as an antimicrobial will be the same as the use range, method of application, and targeted products for AFTEC 3000 when used as a pH adjuster, there are no novel safety issues presented that have not previously been addressed. Accordingly, AFTEC 3000 is GRAS when used as an antimicrobial agent in meat and poultry.

When used as an anti-microbial agent, AFTEC also satisfies the regulatory requirements for being a processing aid under 21 C.F.R. § 101.100(a)(3)(ii)(c). Processing aids are substances that are added to a food for their technical or functional effect in the processing but are present in the finished food at insignificant levels and do not have any technical or functional effect in that food. AFTEC 3000 clearly meets this definition.

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[2] FSIS confirmed by letter that AFTEC 3000 is the same as sodium bisulfate, and thus, would be considered to have the same regulatory status as sodium bisulfate. *See* FSIS Letter to Advanced Food Technologies, LLC (November 4, 2008) (Attachment 3).

1.5 Availability of Information for FDA Review

The data and information that are the basis for AFT's GRAS determination are available for the FDA's review, and copies will be sent to FDA upon request. Requests for copies and arrangements for review of materials cited herein may be directed to:

Robert G. Hibbert, Esq.
Gary L. Yingling, Esq.
K&L Gates LLP
1601 K Street, NW
Washington, DC 20006-1600

2.0 DETAILED INFORMATION ABOUT THE IDENTITY OF THE NOTIFIED AFTEC 3000 PRODUCT

2.1 Identity

AFTEC 3000 is a blend of sulfuric acid and sodium sulfate in purified water.

Sulfuric Acid (CAS #7664-93-9) is commonly used as an acidifier. It is a strong mineral acid that fully dissociates in water ($H_2SO_4 \Rightarrow 2H^+$ and $SO_4^=$). While it therefore could be an ideal anti-microbial solution, its corrosive nature makes sulfuric acid difficult to handle in its concentrated form. Even when diluted, sulfuric acid can cause organoleptic damage to treated meats. However, when blended with its conjugated base salt (any sulfate) or even a weaker organic acid (like citric acid) the equilibrium solution is significantly less corrosive to skin and meat tissue.

Sulfuric acid is GRAS and is included by FDA in the regulations. *See* 21 C.F.R. § 184.1095. As per the regulations, it meets the specifications for the FCC. It is intended for use as a pH control agent and as a processing aid. The regulations set maximum use levels for the chemical.

The USDA also recognizes sulfuric acid as an approved single ingredient acidifier and processing aid at levels sufficient for certain defined purposes when used in accordance with cGMPs. *See* FSIS Directive 7120.1. FSIS also allows for sulfuric acid to be blended with other acids or sulfates to create a safer acidifier. Such combinations include:

- Sulfuric acid, ammonium sulfate, copper sulfate, and water <u>for use in poultry processing</u>;

- An aqueous solution of sulfuric acid, citric acid, and phosphoric acid to adjust the pH <u>in poultry chill water and processing water in meat and poultry plants</u>; and

- An aqueous solution of sodium bisulfate and sulfuric acid as a pH control agent in <u>poultry processing water</u> to a pH of 1.0-6.0.

See FSIS Directive 7120.1.

Sodium Sulfate (CAS #7757-82-6) serves strictly as a buffering salt for the sulfuric acid in this case. It has no anti-microbial properties of its own. Sodium sulfate is a salt formed from the reaction of two GRAS substances (sulfuric acid and sodium hydroxide) and it fully dissociates in water ($Na_2SO_4 \Rightarrow 2Na^+$ and $SO_4^=$). It is GRAS and permitted for use as a direct food additive under 21 C.F.R. § 172.615 as a masticatory substance, stabilizer, thickener or gelling agent. It is also listed for use as a secondary direct additive under 21 C.F.R. § 173.310 as a boiler water additive or processing aid. The desired buffering capability can be achieved at 1:5 – 1:10 ratios with sulfuric acid (that is 10-20% of the amount of sulfuric acid by weight in the finished blend).

2.2 Characteristic Properties

AFTEC 3000 Characteristic Properties	
Appearance	It is a clear, colorless to light amber liquid.
Odor	None to slightly acidic.
Solubility	Very soluble in water.
pH (1:100 v/v dilution with neutral water)	1.4 – 1.6
Specific Gravity @25°C	1.38

2.3 Quantitative Composition

AFTEC 3000 is the product of blending the following materials to achieve the targeted levels.

Material	**Target Wt%**	**Spec Range Wt%**
Sulfuric Acid (H_2SO_4)	39.0	38.5 – 39.5
Sodium Sulfate (Na_2SO_4)	5.0	4.5 – 5.5
Water (H_2O)	56.0	55.0 – 57.0
Total	100.0%	

3 MANUFACTURING PROCESS

3.1 Overview

The sulfuric acid and sodium sulfate blend described in this dossier is produced by Harcros Chemicals Inc. at its Vicksburg, Mississippi facility for AFT in accordance with industry recognized cGMPs.

Batch sheets are used for the recording of raw material and production information for every batch produced. Every batch is assigned a unique lot number for identification / traceability purposes.

The production process uses 98% sulfuric acid that is diluted with water to make a 41% sulfuric acid solution in a dedicated blend / storage tank. Sodium sulfate (anhydrous) crystals are added to the tank containing the diluted acid solution while the tank is circulated (pumps are used to draw the solution from the bottom and re-circulated through the top). The tank in which the blend is produced is thoroughly mixed and samples are taken for Quality Control ("QC") purposes to ensure that product specifications are met. The product is then filtered and transferred through dedicated pipelines to designated packaging areas for packaging into bulk (tank trailer), IBC (totes) and drums.

Typical batch amounts for 1,000gals of AFTEC 3000 are below. Note the final water content comes from the 41% sulfuric acid.

Material	Amount in lbs	Wt%	Wt% in Final Product
41% Sulfuric Acid	10,925	95.0	39.0% sulfuric acid
Sodium Sulfate	575	5.0	5.0% sodium sulfate
Water			56.0% water

3.2 Raw Materials

The raw materials used for the blending of the product are suited for the intended use thus supporting the safety of the finished product. The raw materials meet predefined quality standards that are controlled by the Quality Assurance Department of AFT ("QA Dept.") and the contracted Chemical Blender. The raw materials used for the formulation meet food grade standards (i.e., FCC standards) and are sourced from NorFalco (98% sulfuric acid) and Saltex (sodium sulfate, anhydrous). Water used is from the local municipal/city water source.

Raw material lot numbers are recorded for every batch produced and every lot is inspected / received based on purchase order specifications and certificates of analysis.

3.3 Quality Control of Finished Product

The specifications for the AFTEC 3000 product are outlined below:

Material	Target Wt%	Spec Range Wt%
Sulfuric Acid (H_2SO_4)	39.0	38.5 – 39.5
Sodium Sulfate (Na_2SO_4)	5.0	4.5 – 5.5
Water (H_2O)	56.0	55.0 – 57.0
Total	100.0%	

The wt% target and specification ranges are based on the material amounts (weights) recorded on the batch sheet. Samples are collected from every batch produced and are tested for appearance, specific gravity and pH to meet the following specifications.

Specifications for Sample Testing	
Appearance	Clear, colorless to slight amber liquid. Free of particulates.
Specific Gravity @ 25°C	1.37 – 1.39
pH (1/100 v/v dilution)	1.4 – 1.6

Final product batch samples are retained for 1 year.

4 COMPOSITION AND SPECIFICATIONS

4.1 Formulation

The common starting materials for the blended product are sulfuric acid, sodium sulfate and water. All of the raw materials used are of food grade quality and satisfy FCC standards.

Apart from these materials, the blended product may also contain substances commonly found and tested for in food grade raw materials. Such substances generally consist of sulfate impurities of iron and magnesium, and are recognized under FCC specifications and testing requirements.

4.2 General Production Controls and Specifications (Good Manufacturing Practices)

4.2.1. Technical Measures

AFTEC 3000 is prepared, preserved and stored in such a way that contamination is avoided. This is achieved by dedicated production equipment, piping and tanks; trained production personnel, documented standard operating procedures; and the preparation and maintenance of batch records. The product is clearly labeled and stored in dedicated tanks and shipping containers. Only new drums and totes are used for packaging. Bulk trailer shipments are made in rubber-lined trailers that undergo food grade washouts, washout certificates are issued before loading, and such certificates are maintained by the manufacturer.

The manufacturing of product and the cleaning of the equipment are laid down in Quality Assurance documents and strictly followed by trained personnel.

4.2.2. Control Measures

The raw materials used to produce the product are appropriate for the intended use thus supporting the safety of the finished product. The raw materials meet predefined quality standards that are controlled by the AFT's QA Dept. and the chemical blender. The raw materials are of food grade quality and comply with FCC specifications.

The finished product is subjected to extensive product and quality controls as outlined in Section 3 above.

5 APPLICATION

5.1 Mode of Action

As is well recognized by scientific experts in the industry, most microorganisms cannot tolerate a high or low pH environment.[3] Any pH environment below a 4.0 pH begins to kill the organisms. The efficacy of any antimicrobial kill step is a function of concentration and time (K = C x T). Since pH is a negative log scale of the concentration of the Hydrogen ion (H+), the lower the pH, the higher the concentration and the faster the kill step.

The function of the AFTEC 3000 product is to provide significant microbial reductions on the surfaces of poultry and meat without imparting any lasting technical effects or chemical residues, or causing any organoleptic damage to the meats. AFTEC 3000 performs this function by utilizing a strong inorganic acid, sulfuric acid, to provide the microbe killing Hydrogen ions (H+) and a buffering agent, sodium sulfate, to minimize any damage to the treated meats, plant workers, and USDA inspection personnel.

5.2 Application

AFTEC 3000 is intended for use directly on meat and poultry surfaces as a spray, wash, or dip in order to temporarily reduce the level of microbes on the food surface. This would include whole carcasses, parts, trim, organs and cut meats. As a spray, wash, or dip the microbial reduction is a temporary effect and the chemical solution will drip off, evaporate, or otherwise leave no significant chemical residue and have no lasting technical effect. Examples of such application are included below.

AFT submitted a request to FSIS for waiver of the regulatory requirements under 9 C.F.R. § 381.91(b) (1) for permission to conduct an on-line reprocessing ("OLR") trial to determine whether AFTEC 3000 is an effective antimicrobial agent in an OLR application which FSIS granted. In support of its request, AFT provided FSIS with data from seven studies conducted at the University of Georgia showing that AFTEC 3000 is an effective antimicrobial agent when used as a dip or spray for the reduction of microbes on broiler chickens. FSIS granted AFT's waiver request. *See* Summary and Report from Background Studies with Poultry to Support Waiver Request (Log #10-OLR-0514-N-A (March 15, 2010) (Attachment 4).

5.2.1. FSIS Approved Poultry On-Line Reprocessing

The approved poultry on-line reprocessing study was conducted at Tyson Foods, Inc.'s Forest, Mississippi facility ("Tyson's Study"). Over a 10 day period, 400 carcasses were identified as being either visually uncontaminated or visually contaminated. 100 carcasses (10 per day per condition) were rinsed before and after the OLR. The pH of the AFTEC solution applied to the carcasses was measured before and after each trial. The protocol provided for an operating range

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[3] *See* Growth Factors for Selected Bacteria. Attachment 1.

from 1.4-2.2 pH. Samples pulled during the tests ranged from 1.5-1.9 pH. *See* Analysis of On-Line Reprocessing Results with AFTEC 3000 as an Antimicrobial Agent on Poultry (2010 - 2011) (Attachment 5).

The APC and *E. coli* count numbers showed 90 and 85 percent statistically significant reductions from pre-OLR to post-OLR, respectively. *Salmonella* spp. showed a low positive incidence pre and post-OLR, so even though there was a 50 percent reduction in positive incidence post-OLR compared to pre-OLR, the reduction could not be shown to be statistically significant. The study authors concluded that AFTEC 3000 is an effective antimicrobial agent in the OLR.

Upon completion, the results of the Tyson's Study were submitted to FSIS in support of a request to expand the waiver to two additional facilities, which was approved by FSIS. *See* FSIS Letter Granting AFT Permission to Conduct Additional In-Plant Trials (March 8, 2011) (Attachment 6). Based upon its review of the results of the Tyson's Study, FSIS granted the waiver request concluding that the data:

> ... showed that the number of aerobic plate count (APC) bacterial, *Escherichia coli*, and *Salmonella* positive samples was statistically reduced after passage through the AFTECT 3000 (AFT Clear 3000) OLR system. The data showed that there was no statistical microbiological difference between carcasses marked visibly clean and those marked visibly contaminated after decontamination with the AFTECT 3000 (AFT Clear 3000) OLR treatment.

Under the FSIS granted waiver, AFT was given permission to conduct testing at two additional facilities. This included a second in-plant trial at Pilgrim's Pride (establishment # P6638) in Enterprise, Alabama and a third in-plant trial at Gold'n Plump (establishment # P322) in Cold Springs, Minnesota. Testing at the two facilities is ongoing and data will be provided to FSIS upon completion of the studies. While AFT continues to conduct studies to validate the application methods of AFTEC 3000, initial data indicates that the solution is clearly effective as an antimicrobial agent in poultry processing.

5.2.2. Secondary Beef Processing Study at Oklahoma State University

The Oklahoma State University ("OSU") study was conducted in two phases to address blade tenderized beef contamination issues for the potential to carry surface contamination (i.e., *E. coli* O157:H7) into the interior of steak cuts, and whether this presents a potential health risk to consumers. *See* Summary and Technical Report of Integral Antimicrobial Solution Application on the Ross Blender Tenderizer, Phase 1 and Phase 2 Results Report (April 2, 2011) (Attachment 7).

Phase 1 determined the effectiveness of AFTEC 3000 at a pH of 1.0 as an antimicrobial on beef surfaces inoculated with *E. coli* O157:H7 through quantification of survivors to determine reduction of initial population on the surface. *E. coli* O157:H7 colonies were measured at 1 hour, 1 day, 7 days, and 14 days, and the data was plotted on a chart depicting the comparative difference in amount of pathogens (log reduction (cfu/cm^2)) in the treated sample as compared to the control (rinsed with water). In phase 1, lean beef wafers sprayed with AFTEC were observed

to have a 1.19 log reduction (cfu/cm^2) of *E. coli* O157:H7 after 1 hour. Data collected after 1 day, 7 days, and 14 days revealed a slight increase in comparative difference of amount of *E. coli* between the control and samples treated with AFTEC 3000, consistent with expectations. The comparative difference in amounts of pathogens between the control and treated samples after the initial kill step persisted over time. AFT believes this is because a reduced amount of pathogens likely remained after the treatment of the sample with AFTEC 3000 generating a lower amount of pathogens as compared to the control whose colonies flourished and multiplied. As with all pH treated meats however, the pH quickly increased back to a neutral pH providing an environment more suitable for all microorganisms, including spoilage bacteria and pathogens, to grow. This also occurred with samples treated with AFTEC 3000. The data showed only a slight increase in the comparative difference of *E. coli* colonies after 1 day, 7 days, and 14 days demonstrating that AFTEC 3000 had an immediate, non-continuous impact on *E. coli* growth.

Phase 2 used surface-inoculated beef whole muscle cuts followed by spray treatment of AFTEC 3000 at a pH of 1.0. Blade tenderization was performed immediately to determine the level of microbial entry after interventions are applied compared to an untreated control. Researchers looked for recovery of internalized *E. coli*, which was expected to be proportional to the reduction of *E. coli* O157:H7 on the surface (i.e., the greater the reduction on the surface, the less likely one will recover it from internalized sections after blade tenderization). That is, a sufficiently high population on the surface was needed to observe translocation to internal sections. For the samples inoculated with *E. coli* O157:H7, spray-treated with AFTEC 3000 and then blade-tenderized during Phase 2, only 8 core sections tested positive for *E. coli* O157:H7 as compared to 15 core control sections.

The study report concludes that the use of antimicrobial spray interventions prior to blade tenderization (i.e., integral solution intervention) has an immediate, non-continuous impact on reducing the population of *E. coli* O157:H7 thus lowering levels than those that exist prior to spray treatment, and therefore, reduces the likelihood of translocation to beef internal sections concomitant with lower surface populations.

There were a total of 14 different anti-microbial chemicals or brands tested in this OSU study. All were applied in the same equipment at constant flows and pressures at manufacturer's recommended dosage rates. AFTEC 3000 consistently performed in the top quartile of microbial performance.

5.3 Use Levels

When using a low pH spray or dip in a meat or poultry plant,, the generally accepted operating range of the dilute solution is 1.0-2.2 pH, with a target of 1.3-1.8 pH for the best combination of efficacy and cost.

As supported by the results for each of the product categories, AFT concludes that it is suitable to use AFTEC 3000 in beef and poultry products as an antimicrobial agent at levels necessary to achieve targeted pH ranges. Typical pH ranges will vary between 1.0-2.2 pH and will vary according to instrumentation and application as validated by individual processors for specific product.

5.4 Residues in the Final Food

AFTEC 3000 has been tested as a spray and as a dip in various meat and poultry plants and in controlled plant-equivalent laboratory settings at several major universities.

5.4.1. Antimicrobial Beef Study

In a study conducted at Kansas State University ("KSU"), researchers examined whether AFTEC 3000 has a continuing technical effect after the product is spray-treated and then stored. *See* Summary and KSU Study Report on Anti-Microbial Treatment of Beef Trimmings (October 13, 2009) (Attachment 8). The study conducted a sensory evaluation comparing a control to ground beef manufactured using beef trimmings treated with AFTEC at 10, 20, and 30 second time intervals in triangle tests using a trained sensory panel. No differences between the treated samples and the control were reported.

The study also examined the shelf life of treated products. Samples were evaluated daily for visual color and microbiological testing for total aerobic plate count. No differences in color stability were observed between treated samples versus the control. No significant differences in aerobic plate counts were reported between treated and control ground beef samples.

The study report states:

> There was no long term residual effect on the color, shelf life, or microbiological quality of ground beef manufactured from the treated trimmings versus the control. In addition, no statistical differences in residual levels of sodium sulfate or sulfuric acid were reported in ground beef treated with the *AFTEC* solution versus control samples.

Therefore, any AFTEC 3000 that remains on the product is insignificant, and after the initial antimicrobial impact during the spray treatment, AFTEC 3000 has no technical or functional effect in the beef product. Accordingly, this study demonstrated that AFTEC 3000 may properly be qualified as a processing aid based on the KSU data indicating that only de minimis residues are found on the final product and that AFTEC 3000 does not have a long-term residual effect on the color, shelf life, or microbiological quality of ground beef.

5.4.2. Poultry Studies

In support of its initial waiver request referenced above, AFT provided FSIS with a University of Georgia study that examined the potential chemical residue of AFTEC 3000 as an antimicrobial spray solution. *See* Summary of Studies Conducted to Support FSIS Waiver Request, Pages 5-7 (Attachment 4). In that study, 5 control carcasses were sprayed with tap water, and 5 test samples were sprayed with AFTEC 3000 for 5 seconds and allowed to drip for 2 minutes. The meat from each part (i.e., skin, fat) was compared (control versus test) for each chemical component (sulfur or sodium) using statistical analysis of variation or ANOVA. From a review of the data, it was determined that the study demonstrated no statistical difference between samples treated with tap water (controls) and samples treated with AFTEC 3000 in a spray

solution. Therefore, the study concluded that no chemical residue exists on the skin, fat, or meat of chicken carcasses treated using AFTEC 3000.

In the same FSIS waiver request, AFT included another University of Georgia study that evaluated the ability of AFTEC 3000 to impact aerobic plate counts or psychrotrophic plate counts (spoilage bacteria) after treatment and storage of poultry. *See* Summary of Studies Conducted to Support FSIS Waiver Request, Pages 7-9 (Attachment 4). In this study, eighty carcasses were collected before entering the on-line reprocessing system in a commercial poultry operation. The carcasses were then transported to the Poultry Research Center at the University of Georgia where they were separated into groups of 10 each and subjected to the following:

1) 10 carcasses were tested prior to any treatment as controls

2) 10 carcasses were sprayed with tap water using a commercial sprayer and allowed to hang on the line for 1 minute, then placed for 1 hour in tap water with ice and aeration, then sampled as chill controls

3) 10 carcasses were sprayed with tap water using a commercial sprayer and allowed to hang on the line for 1 minute, then sampled as spray controls

4) 10 carcasses were sprayed with tap water using a commercial sprayer and allowed to hang on the line for 1 minute, then placed for 1 hour in tap water with ice and aeration, then held for 24 hours at 4°C, and sampled as chill 24 h controls

5) 10 carcasses were sprayed with tap water using a commercial sprayer and allowed to hang on the line for 1 minute, then placed for 1 hour in tap water with ice and aeration, then held for 48 hours at 4°C, and sampled as chill 48 h controls

6) 10 carcasses were sprayed with AFTEC 3000 at a pH of 1.5 and allowed to sit for no longer than 1 minute, then sampled

7) 10 carcasses were sprayed with AFTEC 3000 at a pH of 1.5 and allowed to sit for no longer than 1 minute, then placed for 1 hour in tap water with ice and aeration, then held for 24 hours at 4°C, and sampled (AFTEC Chill 24 hr)

8) 10 carcasses were sprayed with AFTEC 3000 at a pH of 1.5 and allowed to sit for no longer than 1 minute, then placed for 1 hour in tap water with ice and aeration, then held for 48 hours at 4°C, and sampled (AFTEC Chill 48 hr)

9) Carcasses were sampled using 400 mL of 0.1% buffered peptone water with neutralizer

10) Rinses were diluted and plated onto 2 sets of Petrifilm Aerobic Count Plates

11) 1 set of plates was incubated at 37°C for 48 hours and counted (APC Counts)

12) 1 set of plates was incubated at 7°C for 10 days and counted (psychrotrophic plate counts)

13) Means for each group were graphed and compared using the ANOVA procedure of SAS

The study data indicates that even though AFTEC 3000 was sprayed onto the carcasses, no additional reductions in aerobic plate counts ("APC") or psychrotrophic plate counts (spoilage bacteria-PPC) were observed when compared to the other groups (except the original control / untreated group). Moreover, and most importantly, APC and PPC bacteria counts on carcasses sprayed with AFTEC 3000 and tested immediately, after 24 hours of storage or after 48 hours of storage, were not significantly different. This means that no residual effect on spoilage bacteria occurred for carcasses treated with AFTEC 3000 during storage.

Based on the test data obtained, AFTEC 3000 clearly satisfies the regulatory requirements for being considered a processing aid under 21 C.F.R. § 101.100(a)(3)(ii)(c). Despite being sprayed on beef and poultry for its antimicrobial effects during processing, it is present in the finished product only at insignificant levels and does not have any technical or functional effect on that food.

AFT intends to label the AFTEC 3000 product with the following ingredient declaration: "purified water, sulfuric acid, sodium sulfate." Since it will be used as a processing aid, manufacturers using AFTEC 3000 will not be required to include AFTEC 3000 on their labels. Despite their usage of AFTEC 3000, the finished meat and poultry products will comply with all applicable FSIS labeling requirements.

6. SAFETY EVALUATION

6.1 Safe Acid Chemistry

AFTEC 3000 is an example of 'Safe Acid' technology in which a strong acid is buffered by a weaker acid or conjugate base salt. AFTEC 3000 is made by blending a solution of water, sodium sulfate (salt) and sulfuric acid. The sodium sulfate acts as a conjugate base when mixed in a water solution with sulfuric acid. Sodium sulfate is the salt formed from the reaction of a strong acid (sulfuric acid) and strong base (sodium hydroxide). Salts from the reaction of a strong acid and strong base generally are neutral in pH (7), and can be used as a buffering compound for the strong acid. There are many other examples of 'Safe Acids' that work according to this same chemical principal that are used daily in various food and water applications.

The AFTEC 3000 product is made by blending a solution of sulfuric acid (H2SO4), water (H2O), and sodium sulfate (Na2SO4). The resulting solution contains sulfuric acid, sodium sulfate, sodium bisulfate, water, sodium ions (Na^+), hydrogen ions (H^+), hydrogen sulfate ions (HSO_4^-) and sulfate ions ($SO_4^=$). These are the exact same ionic species that are formed when Sodium bisulfate (NaHSO4) is dissolved in water (H2O).

6.2 FSIS & FDA Recognition of Safety

There are three listings in FSIS Directive 7120.1 for sodium bisulfate. These include 1) for use as a pH control agent and processing aid in water used in meat and poultry processing at levels "sufficient for purpose"; 2) pH control agent in meat and poultry soups at levels not to exceed <u>0.8 % of product formulation</u>; and 3) for <u>addition to sauces</u> used as separable components in the formulation of various meat products at levels <u>sufficient for purpose</u>.

In addition, FDA GRAS Notice GRN No. 0003 discusses the use of sodium bisulfate as a leavening agent in cake mixes at a level of 1 to 10 grams sodium bisulfate per 1000 grams of total mix (0.1% to 1.0% by weight). *See* Sodium Bisulfate GRAS Notice, GRN No. 3 (June 5, 1998).

Sulfuric acid as a single ingredient product is affirmed as GRAS in 21C.F.R. § 184.1095. The ingredient is used as a pH control agent and processing aid. "Current good manufacturing practice results in a maximum level, as served, of 0.014 percent for alcoholic beverages...and 0.003 percent for cheeses..."

Sodium sulfate is listed for use as a <u>direct food additive</u> under 21 C.F.R. §172.615 where it is permitted for use as a masticatory substance, stabilizer, thickener or gelling agent. It is also permitted for use as a secondary direct additive under 21 C.F.R. §173.310 as permitted for use as a boiler water additive used in the preparation of steam that will contact food.

6.3 Residual Studies

As an anti-microbial, AFTEC 3000 is intended for use directly on meat and poultry as a spray, wash, or dip in order to temporarily reduce the level of microbes on a food's surface. This includes whole carcasses, parts, trimmings, organs and cut meats. Whether delivered as a spray, wash, or dip; the microbial reduction is a temporary effect and the chemical solution drips off, evaporates, or otherwise leaves no significant chemical residue and has no lasting technical effect.

During the Kansas State University study referenced above, AFTEC 3000 was applied as an anti-microbial wash to beef trimmings prior to grinding. The study demonstrated significant microbial reduction without any chemical residues or lasting technical effects. *See* Attachment 7. Specifically, the report states:

> There was no long term residual effect on the color, shelf life, or microbiological quality of ground beef manufactured from the treated trimmings versus the control. In addition, no statistical differences in residual levels of sodium sulfate or sulfuric acid were reported in ground beef treated with the *AFTEC* solution versus control samples.

Likewise, the numerous studies on poultry conducted by Dr. Scott Russell at the University of Georgia produced similar results. (Attachment 4). In those studies, significant microbial reductions were achieved with no chemical residue remaining on the skin, fat, or meat of the chicken carcasses treated with AFT's AFTEC 3000 product. Also, no lasting technical effects were measured or observed.

When used as an anti-microbial agent, AFTEC 3000 satisfies the regulatory requirements for a processing aid as defined under 21 C.F.R. § 101.100(a)(3)(ii)(c). The regulations define processing aids as substances that are added to a food for their technical or functional effect in the processing but are present in the finished food at insignificant levels and do not have any technical or functional effect in that food.

Therefore, when used as an anti-microbial agent, foods treated with an AFTEC 3000 spray, wash, or dip would have insignificant levels of the product or its components in the final food. These levels, if any, would be far less than those levels already generally recognized as safe in foods for the components sulfuric acid, sodium sulfate, and sodium bisulfate. Thus, it can be concluded that there are no novel safety issues presented and no risk to the population consuming food that has been treated with AFTEC 3000. Accordingly, AFTEC 3000 should be recognized as GRAS when used as an antimicrobial agent in meat and poultry processing.

7. LIST OF ATTACHMENTS

Attachment 1: Growth Factors for Selected Bacteria, Pathogen Modeling Program Online, USDA Agricultural Research Service (Last accessed on October 11, 2011)

Attachment 2: FDA letters to AFT from 2009 and 2011 regarding the GRAS status of AFTEC 3000 and its components for use as a pH control agent and processing aid on meat and poultry.

Attachment 3: USDA FSIS letter to AFT dated Nov 4, 2008 confirming that AFT Clear 3000 (AFTEC 3000) is equivalent to sodium bisulfate and permitted by FSIS to be used as a pH control agent in water used in meat and poultry processing sufficient for the purpose.

Attachment 4: Summary and Report from Background Studies with Poultry to Support Waiver Request (Log #10-OLR-0514-N-A) (March 15, 2010)

Attachment 5: Analysis of On-Line Reprocessing Results with AFTEC 3000 as an Antimicrobial Agent on Poultry (2010 - 2011)

Attachment 6: USDA FSIS letter to AFT dated March 8, 2011 granting permission to conduct the second and third in-plant trials of AFTEC 3000 in the On-Line Reprocessing application in poultry slaughter plants.

Attachment 7: Summary and Technical Report of Integral Antimicrobial Solution Application on the Ross Blender Tenderizer, Phase 1 and Phase 2 Results Report (April 2, 2011)

Attachment 8: Summary and KSU Study Report on Anti-Microbial Treatment of Beef Trimmings (October 13, 2009)

8. LIST OF REFERENCES

1. Safe and Suitable Ingredients Used in the Production of Meat, Poultry, and Egg Products, USDA FSIS Directive 7120.1, Revision 7 (July 1, 2011).

2. 21 C.F.R. § 184.1095 (Lists sulfuric acid as GRAS for use as a pH control agent and processing aid.)

3. 21 C.F.R. § 172.615 (Supports the use of sodium sulfate as a direct food additive for use as a masticatory substance, stabilizer, thickener or gelling agent.)

4. 21 C.F.R. § 173.310 (Lists Sodium Sulfate as a secondary direct additive permitted for use as a boiler water additive and processing aid for steam generation for direct contact with food.)

5. 21 C.F.R. § 174.5(d)(1) (Authorizes GRAS substances for use as indirect additives.)

6. 21 C.F.R. § 172.5(a)(1) (In the context of direct food additives, the term "good manufacturing practice" means using the minimum amount to accomplish the intended technical effect.)

7. Sodium Bisulfate GRAS Notice, GRN No. 3 (June 5, 1998) (The GRAS notice allowing for the use of sodium bisulfate as a pH control agent and use as a leavening agent for cake mixes.)

8. 21 C.F.R. § 101.100(a)(3)(ii)(c) (Defines a processing aid as substances that are added to a food for their technical or functional effect in the processing, but are present in the finished food at insignificant levels and do not have any technical or functional effect in that food.)

Attachment 1

United States Department Of Agriculture
Agricultural Research Service

ERRC
EASTERN REGIONAL RESEARCH CENTER

Pathogen Modeling Program (PMP) Online

- PMP Home
- PMP Online
  - About PMP
  - Tutorial
  - Frequently Asked Questions
  - ***References***
    - pH of Selected Foods
    - Water Activity in Food
    - ***Growth Factors***
    - Model Development
    - Publications List

You are here: PMP Home / PMP Online / References / Growth Factors

Growth Factors For Selected Bacteria

ORGANISM	TEMP °C[a]	pH[a]	a_W[a]
Salmonella spp.	5.2 / 35-43 / 46.2	3.8 / 7.0-7.5 / 9.5	0.94 / 0.99 / >0.99
Clostridium botulinum			
A & B	10 - 50	4.7 - 9	>0.93
nonproteolytic B	5 - ?	-[b]	NR[c]
E	3.3 - 15-30	-[b]	>0.965
F	4 - ?	-[b]	NR[c]
Staphylococcus aureus	7 / 37 / 48	4.0 / 6.0-7.0 / 10	0.83(0.9) / 0.98 / >0.99
Campylobacter jejuni	32 / 42-43 / 45	4.9 / 6.5-7.5 / ca9	>0.987 / 0.997 / -
Yersinia enterocolitica	-1.3 / 25-37 / 42	4.2 / 7.2 / 9.6	- / - / 5% NaCl
Listeria monocytogenes	-0.4 / 37 / 45	4.39 / 7.0 / 9.4	0.92 / - / -
Vibrio cholerae O1	10 / 37 / 43	5.0 / 7.6 / 9.6	0.970 / 0.984 / 0.998
V. cholerae non-O1	-[b]	-[b]	-[b]
Vibrio parahaemolyticus	5 / 37 / 43	4.8 / 7.8-8.6 / 11	0.940 / 0.981 / 0.996
Clostridium perfringens	4 / 43-47 / 50	5.5-5.8 / 7.2 / 8.0-9.0	0.97 / 0.95-0.96 / 0.93
Bacillus cereus	4 / 30-40 / 55	5.0 / 6.0-7.0 / 8.8	0.93 / - / -
Escherichia coli	ca7-8 / 35-40 / ca44-46	4.4 / 6-7 / 9.0	0.95 / 0.995 / -
Shigella sonnei	6.1 / - / 47.1	4.9 / - / 9.34	- / - / 5.18% NaCl
Shigella flexneri	7.9 / - / 45.2	5.0 / - / 9.19	- / - / 3.78% NaCl

a. minimum / optimum / maximum values.
b. The value, though unreported, is probably close to other species of the genus.
c. NR denotes that no reported value could be found, but for most vegetative cells, an a_W of >0.95 would be expected.

Values taken from:

ICMSF(1996) Microorganisms in Foods 5: Characteristics of Microbial Pathogens, Roberts, T. A., Baird-Parker, A. C. and Tompkin, R. B. (eds.), Blackie Academic & Professional, London [ISBN 0 412 47350 X]

Microbial Survival in the Environment, E. Mitscherlich and E.H. Marth (eds.), Springer-Verlag, Berlin and Heidelberg, 1984. [ISBN 3-540-13726-2 Springer-Verlag, Berlin, New York, Tokyo] [ISBN 0-387-13726-2 Springer-Verlag, Heidelberg, Berlin, Tokyo].

ARS.USDA.gov

Attachment 2

DEPARTMENT OF HEALTH & HUMAN SERVICES

Public Health Service

Food and Drug Administration
College Park, MD 20740

July 1, 2009

Stephen Mixon
Director of Operations
Advanced Food Technologies, LLC
3614 Windhill Ln.
Montgomery, TX 77356

Dear Mr. Mixon:

This responds to your inquiry dated May12, 2009, requesting information on the regulatory status of your product (Trade name: AFTEC 3000) for use as a pH control agent or processing aid in water used on poultry, red meat and seafood processing. Specifically, you provided the information on the chemical composition, proposed applications, and use levels for your product.

In general, FDA does not "certify" products or packaging for use in contact with food. Instead the agency authorizes the use of specific chemicals in the production of such food-contact articles or products. FDA's primary method for authorizing such uses is the food contact notification (**FCN**) process. Please note, however, that only the listed manufacturer/supplier and their customers are authorized to market the product of an effective FCN. Previously, FDA published regulations for such uses in Parts 174 to 189 of Title 21 of the U.S. Code of Federal Regulations (CFR). These regulations prescribe safe conditions of use for components of food contact materials. Therefore, in order to market your product(s) in the U.S., all the chemical components would have to be authorized for their intended use or we would suggest that you should submit a food contact notification following FDA's current guidelines for the preparation and submission of a food contact notification which may be accessed on the internet at: http://www.cfsan.fda.gov/~lrd/foodadd.html.

FDA recognizes that opinion letters from the agency can serve as a valuable tool of assurance for consumers and for this reason we are available to assist the manufacturer in producing compliant products by providing interpretations of food additive regulations or policy. Moreover, it is the manufacturer's responsibility to ensure that their products comply with all appropriate regulations whenever the products enter into interstate commerce in the U.S.

By way of background, when reviewing a product to determine compliance in the 21CFR, you should consider each regulation to be composed of three parts: the *identity* of the substance,

specifications including purity or physical properties, and *limitations* on the conditions of intended use. In order for your products to be suitable for use in contact with food, each chemical component must comply with all three criteria.

For your product you have correctly identified the components of your product and their corresponding applicable regulations. Sulfuric acid (CAS Reg. No. 7664-93-9) as listed under 21 CFR §184.1095 is permitted for use as a pH control agent and as a processing aid.

Sodium sulfate (CAS Reg. No 7727-73-3) is listed for use as a direct food additive under §172.615 where it is permitted for use as a masticatory substance, stabilizer, thickener or gelling agent. It is also listed for use as a secondary direct additive under §173.310 as permitted for use as a boiler water additive or processing aid. Sodium sulfate (Anhydrous) (CAS Reg. No 7757-82-6) is listed under §186.1797 as permitted for use as an antimicrobial agent. Also note, sodium sulfate is the soluble salt formed by sulfuric acid and sodium hydroxide. Both sulfuric acid (21 CFR §184.1095) and sodium hydroxide (21 CFR §184.1763) are generally recognized as safe (GRAS) substances that completely ionize in water to form sodium sulfate ions and more water. Therefore, sodium sulfate is also GRAS and can be used as a processing aid in accordance with 21 CFR 174.5(d)(1), which authorizes GRAS substances for use as indirect additives. The only limitation on the use levels of either of these compounds would be based good manufacturing practice (GMP) in accordance with 21 CFR §148.1(b)(1), which means using the minimum amount to accomplish the intended technical effect.

I hope that this information has been responsive to your questions regarding your product. If you have any further questions concerning this matter, please do not hesitate to contact us.

Sincerely,

Vivian Gilliam
Division of Food Contact Notifications, HFS-275
Office of Food Additive Safety
Center for Food Safety
And Applied Nutrition

cc: HFA-224 HFS-200 HFS-275
Letter No. 93332
R/D:HFS-275:VGilliam:06/20/09
INIT:EMachuga:HFS-275:07/01/09
F/T:HFS-275:VGilliam:

DEPARTMENT OF HEALTH & HUMAN SERVICES

Public Health Service

Food and Drug Administration
College Park, MD 20740

August 30th, 2011

Stephen Mixon
Director of Operations
Advanced Food Technologies, LLC
3614 Windhill Ln.
Montgomery, TX 77356

Dear Mr. Mixon:

This is to clarify our response of June 24, 2009 to your inquiry dated May12, 2009, requesting information on the regulatory status of your product (Trade name: AFTEC 3000) for use as a pH control agent or processing aid in water used on poultry, red meat and seafood processing. Specifically, you provided the information on the chemical composition, proposed applications, and use levels for your product.

In general, FDA does not "certify" products or packaging for use in contact with food. Instead the agency authorizes the use of specific chemicals in the production of such food-contact articles or products. FDA's primary method for authorizing such uses is the food contact notification (**FCN**) process. Please note, however, that only the listed manufacturer/supplier and their customers are authorized to market the product of an effective FCN. Previously, FDA published regulations for such uses in Parts 174 to 189 of Title 21 of the U.S. Code of Federal Regulations (CFR). These regulations prescribe safe conditions of use for components of food contact materials. Therefore, in order to market your product(s) in the U.S., all the chemical components would have to be authorized for their intended use or we would suggest that you should submit a food contact notification following FDA's current guidelines for the preparation and submission of a food contact notification which may be accessed on the internet at: http://www.cfsan.fda.gov/~lrd/foodadd.html.

FDA recognizes that opinion letters from the agency can serve as a valuable tool of assurance for consumers and for this reason we are available to assist the manufacturer in producing compliant products by providing interpretations of food additive regulations or policy. Moreover, it is the manufacturer's responsibility to ensure that their products comply with all appropriate regulations whenever the products enter into interstate commerce in the U.S.

By way of background, when reviewing a product to determine compliance in the 21CFR, you should consider each regulation to be composed of three parts: the *identity* of the substance, *specifications* including purity or physical properties, and *limitations* on the conditions of intended use. In order for your products to be suitable for use in contact with food, each chemical component must comply with all three criteria.

Sodium sulfate is the soluble salt formed by sulfuric acid and sodium hydroxide. Both sulfuric acid (21 CFR §184.1095) and sodium hydroxide (21 CFR §184.1763) are generally recognized as safe (GRAS) substances that completely ionize in water to form sodium sulfate ions and more water. Therefore, sodium sulfate is also GRAS and can be used as a processing aid in accordance with 21 CFR 174.5(d)(1), which authorizes GRAS substances for use as indirect additives. The only limitation on the use levels of either of these compounds would be based good manufacturing practice (GMP) in accordance with 21 CFR §148.1(b)(1), which means using the minimum amount to accomplish the intended technical effect. Sodium sulfate would also be considered GRAS for use as a secondary direst additive when used as a pH control agent or processing aid in water used on poultry, red meat and seafood processing.

I hope that this information has been responsive to your questions regarding your product. If you have any further questions concerning this matter, please do not hesitate to contact us.

Sincerely,

(b) (6)

Vivian Gilliam
Division of Food Contact Notifications, HFS-275
Office of Food Additive Safety
Center for Food Safety
And Applied Nutrition

DEPARTMENT OF HEALTH & HUMAN SERVICES

Public Health Service

Food and Drug Administration
College Park, MD 20740

August 30, 2011

Correction Letter

Stephen Mixon
Director of Operations
Advanced Food Technologies, LLC
3614 Windhill Ln.
Montgomery, TX 77356

Dear Mr. Mixon:

This letter is to correct a statement provided in our previous opinion letter. The response letter dated June 24, 2009 provided information that was in error. Specifically, the letter provided incorrect information when it cited the following statement: "Sodium sulfate (Anhydrous) (CAS Reg. No 7757-82-6) is listed under §186.1797 as permitted for use as an antimicrobial agent."

Please note that sodium sulfate (Anhydrous), listed under 21 CFR §186.1797, as permitted for use as a constituent of paper and paperboard and cotton and cotton fabric, only and **not** for use as an antimicrobial agent. I apologize for any inconvenience that this incorrect advice may have caused. I will also send you a revised response letter for your files.

If you have any further questions concerning this matter, please do not hesitate to contact us.

Sincerely,

(b) (6)

Vivian Gilliam
Division of Food Contact Notifications, HFS-275
Office of Food Additive Safety
Center for Food Safety
And Applied Nutrition

Attachment 3

United States Department of Agriculture

Food Safety and Inspection Service

Washington, D.C. 20250

Mr. Dennis Smithyman
President
Advanced Food Technologies, LLC
11230 Magnolia Glen
Shreveport, LA 71106

NOV 0 4 2008

Dear Mr. Smithyman:

This is in response to your October 30, 2008 email (Log number 08-NT-0387-N-A) requesting a letter from the Food Safety and Inspection Service (FSIS) stating that AFT Clear 3000 is the same as sodium bisulfate and under FSIS Directive 7120.1, "Safe and Suitable Ingredients Used in the Production of Meat and Poultry Products" can be used as an acidifier in meat and poultry plants.

After reviewing your submitted information, FSIS has determined that AFT Clear 3000 is considered the same as sodium acid sulfate (SAS) or sodium bisulfate and, thus, would not be considered new technology. SAS or sodium bisulfate is already permitted by FSIS to be used as a pH control agent (acidifier) in water used in meat and poultry processing sufficient for the purpose.

This letter should not be considered as validation that your process will be effective in any particular FSIS establishment. Your technology, as described in your notification, will need to be factored into an establishment's hazard analysis and, if appropriate, incorporated into its HACCP Plan or SSOP, or other prerequisite program, validated for its application, and verified on an ongoing basis for its effectiveness. If the establishment does not address the effects of using your technology in its hazard analysis, FSIS would be unable to determine that the product processed using your technology is safe, including microbiologically, not adulterated; and therefore, the product would not be eligible to bear the mark of inspection.

If you have any questions, please contact Dr. David Zeitz at (202)690-3556 or david.zeitz@fsis.usda.gov.

Sincerely,

(b) (6)

Dr. John Hicks, Director
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Office of Policy and Program Development

FSIS FORM 2630-9 (6/86)

EQUAL OPPORTUNITY IN EMPLOYMENT AND SERVICES

Attachment 4

Study title: Background Studies to support request for an in-plant trial of AFTEC 3000 for use in on-line reprocessing of poultry (Log#10-ING-0498-N-A)

Conducted by: Scott M Russell, Ph.D., Professor, Dept. of Poultry Science, University of Georgia

Study dates: March 15, 2010

Objective: to evaluate the efficacy of application of AFTEC 3000 as a means of reducing pathogenic and indicator populations of bacteria on ready-to-cook carcasses. Also, evaluate effect on shelf life, color, and residues.

Methodology: A total of 7 anti-microbial studies (Section IV) were conducted over a 1 year time frame on chicken carcasses. Studies 1 & 3 were in-plant post-chill spray cabinets; study 4 was a lab study for a post-chill dip, while study 2 was an in-plant post-chill dip; Studies 5, 6, and 7 were in-plant pre-chill spray cabinets or dips. All of the tests were conducted with AFTEC 3000 diluted to a pH range between 1.4 and 2.0.

The residual studies (Section III) addressed both chemical residues and evaluation of the ability of AFTEC 3000 to have a residual impact on aerobic plate counts or psychrotrophic plate counts (spoilage bacteria) after treatment and storage of poultry. The chemical residual study compared 5 treated and 5 control carcasses. The skin, meat, and fat of of each carcass was aseptically excised and analyzed for component levels of sulfuric acid and sodium sulfate. A total of 80 carcasses were used for various treatments and controls to determine the effect on psychrotrophic plate counts (PPC) immediately after treatment and after storage for 24 or 48 hours.

Results and discussion: Across the 7 anti-microbial studies, AFTEC 3000 consistently validated statistically significant reductions in overall microbial levels and *Salmonella* spp. Whether as a spray or a dip, AFTEC 3000 usually achieved a greater than 1 log reduction in generic E.coli CFU/ml and a measurable percent reduction in Salmonella incidence if sufficient Salmonella were present pre-treatment in the plant.

In the chemical residue study, 5 carcasses were sprayed with AFTEC 3000 for 5 seconds and allowed to drip for 2 minutes. 5 untreated carcasses were used for control. The means for each part (skin, fat, or meat) were compared (control versus test) for each chemical component (sulfur or sodium) using ANOVA. The study demonstrated no statistical difference between samples treated with tap water (controls) or samples treated with AFTEC 3000 in a spray solution.

In the study on the impact of AFTEC 3000 on psychrotrophic plate counts (spoilage bacteria), there was no residual effect on spoilage bacteria compared with normal water sprays or dips used in the plant after treatment and storage.

Background Studies to support request for an in-plant trial of AFTEC 3000 for use in on-line reprocessing of poultry

(Log#10-OLR-0514-N-A)

March 15, 2010

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I. Purpose of the research

The purpose of this research was to evaluate the efficacy of application of AFTEC 3000 (AFT Clear 3000) as a means of reducing pathogenic and indicator populations of bacteria on ready-to-cook carcasses. The components of the AFTEC 3000 concentrate are as follows: sulfuric acid (25-35%), sodium sulfate (5-15%), water (50-70%). This concentrate is diluted by mixing with tap water to reach the target levels of pH for each of the applications.

II. Literature Review

Studies have been conducted for the last three decades in an effort to discover means of reducing pathogenic bacterial populations on raw poultry products; however, contamination of raw poultry continues to be a concern for consumers, industry, and regulatory agencies [1]. Addition of chlorine, hydrogen peroxide [1], and antimicrobials such as halogenic compounds, organic acids, and salts [2] to the chiller have been used effectively to reduce microorganisms in the water, but no significant reduction in *Salmonella* has been demonstrated on carcasses. This may be explained because there are numerous areas on the carcass that afford protection for bacteria, such as feather follicles, and cuts or folds in the skin or fat [3,4]. Using electron microscopy, it has been shown that bacteria appear to be entrapped in ridges and crevices, which become more pronounced in the skin and muscle following water immersion [5]. This may make bacteria on carcasses inaccessible to antimicrobial agents.

Because the chiller is a common bath, opportunities exist for *Salmonella* to wash off of one contaminated carcass and to be transported to other uncontaminated carcasses. Lillard [6] reported that populations of aerobic bacteria and Enterobacteriaceae on broiler carcasses were significantly reduced by commercial processing steps, but cross-contamination still occurred. The author found that there was no increase in *Salmonella* prevalence on carcasses at five sampling points from the kill line through the final washer, but a significant increase in *Salmonella* prevalence occurred on carcasses exiting the immersion chiller, indicating that this may be the point of most significant cross-contamination in broiler processing plants [6].

The most commonly used chiller disinfectant in the broiler industry is chlorine. Lillard [7] observed that *Salmonella* Typhimurium, inoculated onto broiler breast skin and treated using 0.5 ppm free residual Cl_2, were not readily accessible to the chlorine and were only reduced by $< 1 \log_{10}$. It is suspected that greater reductions do not occur using chlorine because of the abundance of organic material and nitrogenous compounds associated with chicken carcasses that deactivate

forms of chlorine [8]. In addition, chlorine is coming under increasing scrutiny by European countries and Canada because of the formation of carcinogenic trihalomethane compounds. Hydrogen peroxide has been shown to be effective for eliminating total aerobic bacteria (95-99.5% reduction using 6,600 ppm or higher) and *E. coli* (97 – 99.9% reduction using 5,300 ppm or higher); however, the concentrations required to successfully eliminate these bacterial populations caused the carcasses to become bleached and bloated as the catalase in the blood of the chicken reacted with the H_2O_2 [4]. Ozone has been used successfully to eliminate 99% of the bacteria washed off of carcasses into chiller water as a means of controlling cross-contamination [9].

Numerous trials have indicated that organic acids, such as lactic and acetic acid, can be used in a variety of ways to either decrease or eliminate salmonellae from the carcass and extend shelf-life of processed broilers [10]. However, some chemicals at high concentrations may produce undesirable organoleptic characteristics. Dickens and Whittemore [11] reported that Enterobacteriaceae counts on broiler carcasses were reduced by 0.50, 0.71, and 1.4 $\log_{10}$ when using 0.6% acetic acid (AA), air agitation and 0.6% acetic acid (AGAA), or a paddle chiller with 0.6% acetic acid (PAA), respectively. *Salmonella* prevalence on inoculated carcasses after treatment were 87% for control carcasses, 80% (AA), 53% (AGAA), and 6.7% for the (PAA) treatments [11].

Trisodium phosphate has become popular as a solution approved by USDA-FSIS for disinfecting carcasses as an automated reprocessing method. Lillard [12] evaluated trisodium phosphate for eliminating *Salmonella* from broiler carcasses. The author reported that salmonellae levels were reduced by 2 $\log_{10}$ colony forming units (CFU)/mL rinse. However, use of high levels of phosphates (10%) to wash chickens during processing creates an enormous amount of phosphorous in the waste-stream that must be eliminated prior to release to the environment.

The scalder is the first common bath during poultry processing. As such, it represents the first location where pathogenic bacteria from one carcass may wash off and contaminate many other carcasses. This is of concern because of the USDA HACCP/Pathogen Reduction Final Rule [13] which uses *Salmonella* prevalence as a regulatory criterion, as opposed to the number of *Salmonella* cells per carcass. Therefore, any operation that presents an opportunity for one *Salmonella* positive carcass to cause many other *Salmonella* negative carcasses to become positive is of great concern to the industry. A major problem exists in that the chemicals listed above are not used in the scalder because they are inappropriate for use in high temperature, high organic load situations.

AFTEC 3000 (AFT Clear 3000) has been a USDA-FSIS approved acidifier since November 2008. This product has been tested and used in numerous poultry plants over the past year in scalders, pre- and post-evis sprays, pre- and post-chill dips and sprays and is used to acidify chillers.

AFTEC 3000 has been shown that it can be used in scalder water to eliminate bacterial populations. It is not affected by organic material as are the oxidants (Cl, ClO_2, ozone, acidulated sodium chlorite, and H_2O_2), it is not affected by scalder temperatures (130 – 135ºF), and does not cause organoleptic defects.

AFTEC 3000 has been used and tested in poultry plants in pre- and post-evis spray cabinets at a pH range of 1.5 to 2.0. Carcass dips are also conducted in this 1.5-2.0 pH range. Significant microbial reductions are achieved with no organoleptic damage to carcasses and no off-gassing of the chemical. This is a stable low-pH safe acid with less risk of off-gassing than the commonly used hydrochloric acid blends used in OLR's, chillers, and finishing chillers.

III. Residual Studies

Study I: Evaluation of potential chemical residuals

A study was conducted in which two groups of ready-to-cook chicken carcasses (5 each for a total of 10 carcasses) were collected from a commercial processing plant. The 5 control carcasses were sprayed with tap water and the 5 treated carcasses were sprayed with AFTEC 3000 at a pH of 2.0 (use dilution strength) for 5 seconds and allowed to drip for 2 minutes (to simulate actual poultry processing line conditions prior to the carcasses entering the chill system). Then the 5 control carcasses and the 5 treated carcasses were placed into separate containers with 30 gallons of ice water that was agitated using compressed air for 1 hour, to simulate commercial poultry chilling.

After chilling, the skin, fat, and meat of each carcass was aseptically excised, placed into sterile plastic bags on ice, and transported to the University of Georgia Water and Soil Test Lab for evaluation of the components in AFTEC 3000 (sulfur from sulfuric acid and sodium from sodium sulfate) and compared to controls to determine if any chemical residuals were present in the skin, fat, or meat of the carcasses.

Sodium: Samples were prepared using EPA Method 3052 "Microwave assisted digestion of siliceous and organically based matrices" and then evaluated using EPA Method 6010B "Inductively-coupled plasma atomic emission spectrometry."

Sulfuric acid: Samples were prepared using EPA Method 3052 "Microwave assisted digestion of siliceous and organically based matrices" and then evaluated using EPA Method 6010B "Inductively-coupled plasma atomic emission spectrometry."

The raw data results in parts per million (ppm) of the residual studies are presented in Table 1 below.

AFT 3000 Residual Data

Item	Compound	Number	Control	Treated	P Value
Skin	Sulfur	1	12.4	7.58	
Skin	Sulfur	2	4.3	8.29	
Skin	Sulfur	3	6.42	11.2	
Skin	Sulfur	4	11.3	14.2	
Skin	Sulfur	5	6.95	10.4	
			8.274	10.334	0.3173
Fat	Sulfur	1	6.41	11.1	
Fat	Sulfur	2	11.8	12.5	
Fat	Sulfur	3	10.5	11	
Fat	Sulfur	4	9.57	14.2	
Fat	Sulfur	5	9.27	11	
			9.51	11.96	0.0549
Meat	Sulfur	1	24.9	28.9	
Meat	Sulfur	2	30.7	20.6	
Meat	Sulfur	3	16.3	24.8	
Meat	Sulfur	4	19.3	26.7	
Meat	Sulfur	5	29.9	21.8	
			24.22	24.56	0.9187

Item	Compound	Number	Control	Treated	
Skin	Sodium	1	155	207	
Skin	Sodium	2	98.6	94.3	
Skin	Sodium	3	82.4	202	
Skin	Sodium	4	115	207	
Skin	Sodium	5	102	137	
			110.6	169.46	0.0538
Fat	Sodium	1	112.4	112.1	
Fat	Sodium	2	109	123.4	
Fat	Sodium	3	80.1	115	
Fat	Sodium	4	88.9	91.1	
Fat	Sodium	5	84.7	121.8	
			95.02	112.68	0.0785
Meat	Sodium	1	306	242	
Meat	Sodium	2	378	254	
Meat	Sodium	3	360	397	
Meat	Sodium	4	341	361	
Meat	Sodium	5	303	260	
			337.6	302.8	0.3493

The means for each part (skin, fat, or meat) were compared (control vs. treated) for each chemical component (sulfur or sodium) using ANOVA. These data demonstrate that no significant differences ($P \leq 0.05$) were observed between post-chill chicken skin, fat, or meat samples that were treated with tap water (controls) or treated using AFTEC 3000 in a spray solution prior to chilling. Thus, it may be concluded that no chemical residual exists on the skin, fat, or meat of chicken carcasses treated using AFTEC 3000 in a spray solution prior to chilling.

Because of these results, Advanced Food Technologies, LLC (AFT) feels that use of AFTEC 3000 as an antimicrobial at various locations throughout processing would not pose any risk of leaving any chemical residual on chicken skin, fat, or meat.

Study II: Evaluation of the ability of AFTEC 3000 to have a residual impact on aerobic plate counts or psychrotrophic plate counts (spoilage bacteria) after treatment and storage of poultry.

A study was conducted to determine if treating carcasses with AFTEC 3000 had any residual impact on bacteria during storage.

The procedure used was as follows:

Eighty carcasses were collected before the online reprocessing system in a commercial poultry operation. These carcasses were transported to the Poultry Research Center at the University of Georgia. The carcasses were separated into groups of 10 each and subjected to the following:

1) 10 carcasses were tested prior to any treatment as controls

2) 10 carcasses were sprayed with tap water using a commercial sprayer and allowed to hang on the line for 1 minute, then placed for 1 hour in tap water with ice and aeration, then sampled as chill controls

3) 10 carcasses were sprayed with tap water using a commercial sprayer and allowed to hang on the line for 1 minute, then sampled as spray controls

4) 10 carcasses were sprayed with tap water using a commercial sprayer and allowed to hang on the line for 1 minute, then placed for 1 hour in tap water with ice and aeration, then held for 24 hours at 4°C, and sampled as chill 24 h controls

5) 10 carcasses were sprayed with tap water using a commercial sprayer and allowed to hang on the line for 1 minute, then placed for 1 hour in tap water with ice and aeration, then held for 48 hours at 4°C, and sampled as chill 48 h controls

6) 10 carcasses were sprayed with AFTEC 3000 at a pH of 1.5 and allowed to sit for no longer than 1 minute, then sampled

7) 10 carcasses were sprayed with AFTEC 3000 at a pH of 1.5 and allowed to sit for no longer than 1 minute, then placed for 1 hour in tap water with ice and aeration, then held for 24 hours at 4°C, and sampled (AFTEC Chill 24 hr)

8) 10 carcasses were sprayed with AFTEC 3000 at a pH of 1.5 and allowed to sit for no longer than 1 minute, then placed for 1 hour in tap water with ice and aeration, then held for 48 hours at 4°C, and sampled (AFTEC Chill 48 hr)

9) Carcasses were sampled using 400 mL of 0.1% buffered peptone water with neutralizer

10) Rinses were diluted and plated onto 2 sets of Petrifilm Aerobic Count Plates

11) 1 set of plates was incubated at 37°C for 48 hours and counted (APC Counts)

12) 1 set of plates was incubated at 7°C for 10 days and counted (psychrotrophic plate counts)

13) Means for each group were graphed and compared using the ANOVA procedure of SAS

Results:

The results obtained in this study are presented in Figures 1 and 2 below:

Figure 1

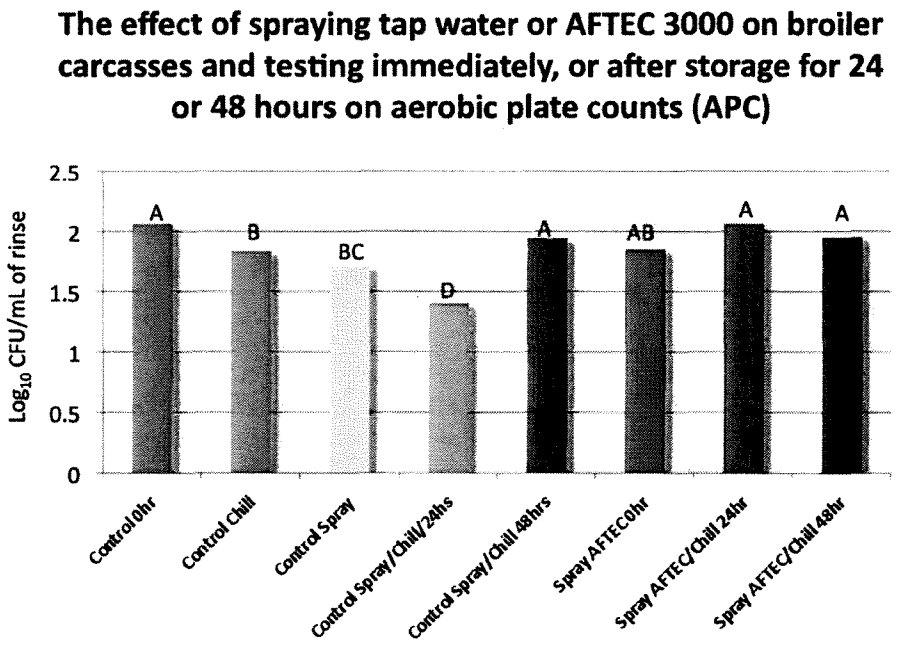
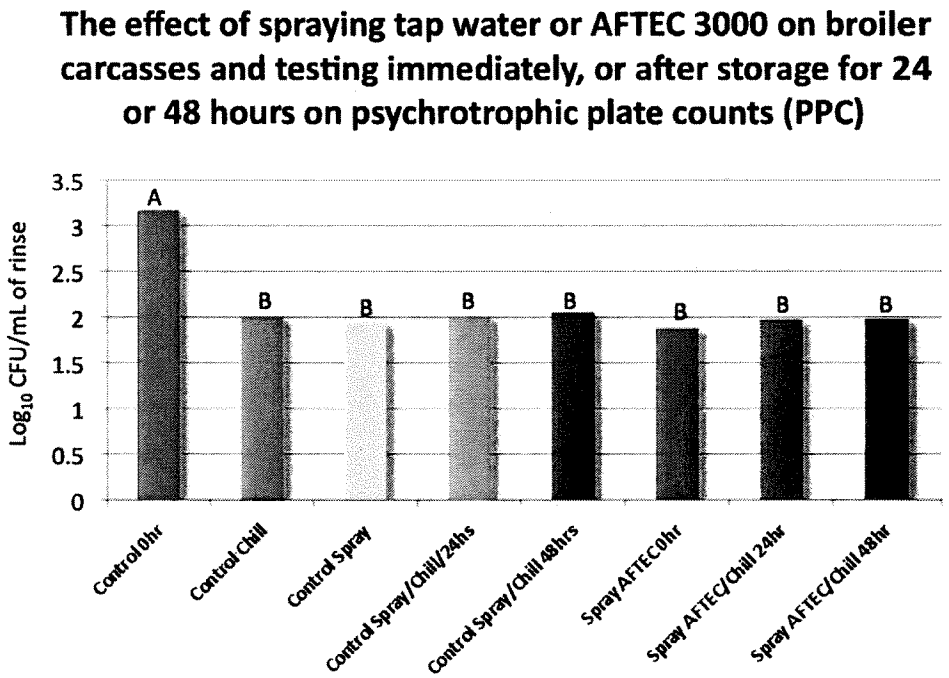


Figure 2



These data indicate that even though AFTEC 3000 was sprayed onto carcasses, no additional reductions in aerobic plate counts (APC) or psychrotrohic plate counts (Spoilage bacteria-PPC) were observed for carcasses when compared any other group except the original control (untreated group). Moreover, and most importantly, APC and PPC bacteria on carcasses sprayed with AFTEC 3000 and tested immediately, after 24 hours of storage, or after 48 hours of storage were not significantly different. This means that no residual effect on spoilage bacteria would occur if carcasses treated with AFTEC 3000 were stored.

IV. Antimicrobial Studies

Numerous studies have been conducted at several different poultry processing plants at different times during the past year:

- Study 1 is an in-plant post chill spray system comparing 40 treated and 40 control carcasses.
- Study 2 is an in-plant commercial finishing chiller comparing 10 treated and 10 control carcasses.
- Study 3 was another post-chill spray cabinet test using 10 control and 10 treated carcasses.

- Study 4 was a lab study simulating a post-chill dip using inoculated *Salmonella* carcasses. A total of 30 carcasses were used in this study.
- Study 5 was an in-plant study of a commercial installation of AFTEC 3000 as an intervention placed between the OLR and the chiller in a poultry plant that was struggling in a USDA set. The intervention was a spray/dip combination.
- Study 6 was the same Study 5 plant after the USDA set. A new post-chiller finishing chiller was installed with AFTEC as the anti-microbial dip. The post-OLR/pre-chill dip tank was shortened and the spray cabinets removed.
- Study 7 was an in-plant comparison of AFTEC 3000 in spray cabinets at the New York Rinse (NYR) and pre-OLR.

Studies 1-3 are analyzed and summarized as a group (early studies). Studies 4-7 are each analyzed and discussed separately.

Study 1: Evaluation of AFTEC 3000

Approach:

1) In Plant 1, ten carcasses within a given flock, were removed from the line just after chilling using sterile gloves, sprayed with tap water using a hand-held sprayer, and these carcasses were termed Post-Chill Controls (PCC). Ten carcasses were allowed to traverse through a post-chill spray system, and collected from the line using sterile gloves. These carcasses were termed Post-chill AFTEC 3000 spray (PTC).

2) After allowing the carcasses to drip thoroughly, all carcasses were individually bagged in sterile polyethylene bags and rinsed using 400 ml of sterile Butterfield's phosphate buffer containing neutralizing buffer (Neutralizing Buffer - Formula per one liter of distilled water 0.0425g KH_2PO_4, 0.16g $Na_2S_2O_3$, 5.0g aryl sulfonate complex pH adjusted to 7.2 ± 0.2 at 25°C). This rinsate neutralized any residual AFTEC 3000 that may be rinsed from the carcass. The rinsates were encoded using a 4 digit number (to prevent identification by Silliker Laboratory employees and the introduction of bias) and sent on blue ice in a cooler using FedEx to Silliker Laboratories for evaluation for APC, *E. coli* counts, and *Salmonella* prevalence.

3) This procedure was conducted 4 times on 4 separate days such that 40 carcasses were collected before and after post-chill spraying for a total of 80 carcasses.

4) The post-chill spray consisted of tap water dosed with AFTEC 3000 to a target pH level of 1.5 ± 0.2.

5) Microbiological tests conducted included aerobic plate counts (APC), *Escherichia coli* (*E. coli*) counts, and *Salmonella* prevalence tests (% positive).

6) Encoded microbiological results were received from Silliker labs and submitted to the Department of Statistics at The University of Georgia for analysis.

Study 2: Evaluation of AFTEC 3000

Approach:

1) In Plant 2, ten carcasses within a given flock, were removed from the line just after chilling using sterile gloves, dipped into tap water, and these carcasses were termed Post-Chill Controls (PCC). Ten carcasses were allowed to traverse through a post-chill dip system for 30 seconds, and collected from the line using sterile gloves. These carcasses were termed Post-chill AFTEC 3000 Dip (PTD).

2) After allowing the carcasses to drip thoroughly, all carcasses were individually bagged in sterile polyethylene bags and rinsed using 400 ml of sterile Butterfield's phosphate buffer containing neutralizing buffer (Neutralizing Buffer - Formula per one liter of distilled water 0.0425g KH_2PO_4, 0.16g $Na_2S_2O_3$, 5.0g aryl sulfonate complex pH adjusted to 7.2 ± 0.2 at 25°C). This rinsate neutralized any residual AFTEC 3000 that may be rinsed from the carcass. The rinsates were encoded using a 4 digit number (to prevent identification by Silliker Laboratory employees and the introduction of bias) and sent on blue ice in a cooler using FedEx to Silliker Laboratories for evaluation for APC, *E. coli* counts, and *Salmonella* prevalence.

3) This procedure was conducted 1 time such that 10 carcasses were be collected before and after post-chill dipping for a total of 20 carcasses.

4) The post-chill spray consisted of tap water dosed with AFTEC 3000 to a target pH level of 1.5 ± 0.2.

5) Microbiological tests conducted were total aerobic plate counts (APC).

6) Encoded microbiological results were received from Silliker labs and submitted to the Department of Statistics at The University of Georgia for analysis.

Study 3: Evaluation of AFTEC 3000

Approach:

1) In Plant 3, ten carcasses within a given flock, were removed from the line just after chilling using sterile gloves, sprayed with tap water using a hand-held sprayer, and these carcasses were termed Post-Chill Controls (PCC). Ten carcasses were allowed to traverse through a post-chill spray system, and collected from the line using sterile gloves. These carcasses were termed Post-chill AFTEC 3000 spray (PTC).

2) After allowing the carcasses to drip thoroughly, all carcasses were individually bagged in sterile polyethylene bags and rinsed using 400 ml of sterile Butterfield's phosphate buffer containing neutralizing buffer (Neutralizing Buffer - Formula per one liter of distilled water 0.0425g KH_2PO_4, 0.16g $Na_2S_2O_3$, 5.0g aryl sulfonate

complex pH adjusted to 7.2 ± 0.2 at 25°C). This rinsate neutralized any residual AFTEC 3000 that may be rinsed from the carcass. The rinsates were encoded using a 4 digit number (to prevent identification by Silliker Laboratory employees and the introduction of bias) and sent on blue ice in a cooler using FedEx to Silliker Laboratories for evaluation for APC, *E. coli* counts, and *Salmonella* prevalence.

3) This procedure was conducted 1 time such that 10 carcasses were be collected before and after post-chill spraying for a total of 20 carcasses.

4) The post-chill spray consisted of tap water dosed with AFTEC 3000 to a target pH level of 1.5 ± 0.2.

5) Microbiological tests conducted included aerobic plate counts (APC), *Escherichia coli* (*E. coli*) counts, and *Salmonella* prevalence tests (% positive).

6) Encoded microbiological results were received from Silliker labs and submitted to the Department of Statistics at The University of Georgia for analysis.

Description of the experimental design, including the methods for control of bias:

All carcasses were selected from the line or after the post-chill dip using a pick one, count five and select the sixth carcass method to avoid bias. All carcass rinses were encoded using 4 digit number so that the laboratory technicians and statistician were not aware of which treatment corresponded to each sample.

Test method references:

Aerobic Plate Counts (APC) were determined using The Official Methods of Analysis of the AOAC, Method 990.12, and reported in colony forming units (CFU).
E. coli – *E. coli* were conducted using The Official Methods of Analysis of the AOAC, Method No. 998.08, and reported in colony forming units (CFU).
Salmonella – *Salmonella* were tested using The Official Methods of Analysis of the AOAC, Method No. 2000.07, and reported as either positive or negative.

Statistical methods:

Statistical evaluation was conducted by the Statistical Consulting Group in the Department of Statistics at the University of Georgia. Treatment effects were determined using t-tests and the Statistical Analytical Software (SAS) program for APC and *E. coli* counts. For *Salmonella* prevalence, logistical regression or Fisher's exact test was conducted using SAS.

Results and Discusson:

Results for Studies 1, 2, and 3 are presented in Tables 2, 3, and 4, respectively.

Table 2: Study 1 Effect of AFTEC 3000 used in a post-chill spray application on aerobic plate counts (APC), *E. coli* counts (*E. coli*), and *Salmonella* prevalence on broiler chicken carcasses in a commercial processing facility.

Treatment	Rep	APC	P value	*E. coli*	P value	*Salmonella percent incidence*	P value
Post-Chill Control	1	2.45		0.68		30	
Post-Chill Spray	1	1.05	0.0000006	0.03	0.0000007	0	<0.05
Post-Chill Control	2	2.39		0.56		0	
Post-Chill Spray	2	0.21	<0.000001	0.00	0.0038	0	NS
Post-Chill Control	3	2.20		0.58		10	
Post-Chill Spray	3	0.00	<0.000001	0.00	0.0051	0	<0.05
Post-Chill Control	4	1.23		0.03		0	
Post-Chill Spray	4	0.08	0.0005	0.00	0.3306	0	NS
Overall			<0.00001		0.0000007		<0.05
N		10		10		10	

Table 3: Study 2 Effect of AFTEC 3000 used in a post-chill dip application on aerobic plate counts (APC) on broiler chicken carcasses in a commercial processing facility.

Treatment	Rep	APC	P value
Post-Chill Control	1	1.89	
Post-Chill Dip	1	0.05	<0.0000001
N		10	

Table 4: Study 3 Effect of AFTEC 3000 used in a post-chill spray application on aerobic plate counts (APC), *E. coli* counts (*E. coli*), and *Salmonella* prevalence on broiler chicken carcasses in a commercial processing facility.

Treatment	Rep	APC	P value	*E. coli*	P value	*Salmonella*	P value
Post-Chill	1	2.11		0.74		20	
Post-Chill Spray	1	0.71	0.0004	0.03	0.0185	10	<0.05
N		10		10		10	

The results in Study 1 (Table 2) demonstrate that AFTEC 3000 was effective for significantly reducing APC, *E. coli*, and *Salmonella* prevalence in all 4 repetitions except for *E. coli* in Rep 4. This is because the levels of *E. coli* on Post-Chill control samples were extremely low at 0.03 $\log_{10}$ cfu/ml. With controls being this low, it is impossible to show a reduction in *E. coli*. The *Salmonella* prevalence in Reps 2 and 4 was also not reduced by AFTEC 3000 because there were no *Salmonella* detected on the controls in these Reps. The results for Study 2 (Table 3) indicate that APC was significantly reduced on poultry carcasses dipped in AFTEC 3000 for 20-30 seconds. Study 3 (Table 4) clearly demonstrated that AFTEC 3000 was effective for significantly reducing APC, *E. coli*, and *Salmonella* prevalence on chicken carcasses sprayed in a post-chill spray application on-line in a commercial processing plant. These data from Studies 1-3 demonstrate that AFTEC 3000 is an effective antimicrobial for use in poultry processing, whether used in a post-chill spray or dip application.

Study 4: Evaluation of AFTEC 3000 for reducing *Salmonella* on broiler chicken carcasses

A research study was conducted to determine the effect of Sulfuric Acid and Sodium Sulfate (AFTEC 3000) on *Salmonella* firmly attached to the surface of broiler chicken carcasses from a commercial processing facility.

Procedure:

1) Thirty eviscerated ready-to-cook chicken carcasses were collected just prior to the online reprocessing system in a large poultry processing facility.

2) The carcasses were transported to the Poultry Research Center at The University of Georgia for analysis.

3) To preclude counting native *Salmonella* that may already be attached to carcasses and vary considerably from carcass to carcass, each of the 30 carcasses were inverted and suspended from a shackle in the pilot poultry processing facility, inoculated with 0.1mL of an actively growing culture of nalidixic acid-resistant *Salmonella* Typhimurium (10^5 concentration) obtained from the USDA-Agricultural Research Service in Athens, GA.

4) After inoculation, the *Salmonella* were allowed to attach for a period of 1 hour to ensure that they were firmly attached.

5) Ten carcasses were tested immediately without dipping in water as untreated controls.

6) Ten carcasses were dipped in potable water for 25 seconds as a water control.

7) Ten carcasses were dipped in a solution of Sulfuric Acid and Sodium Sulfate (pH 1.54) for 25 seconds.

8) All carcasses were dipped and agitated during the treatment to imitate the agitation action in a post-chill dip system.

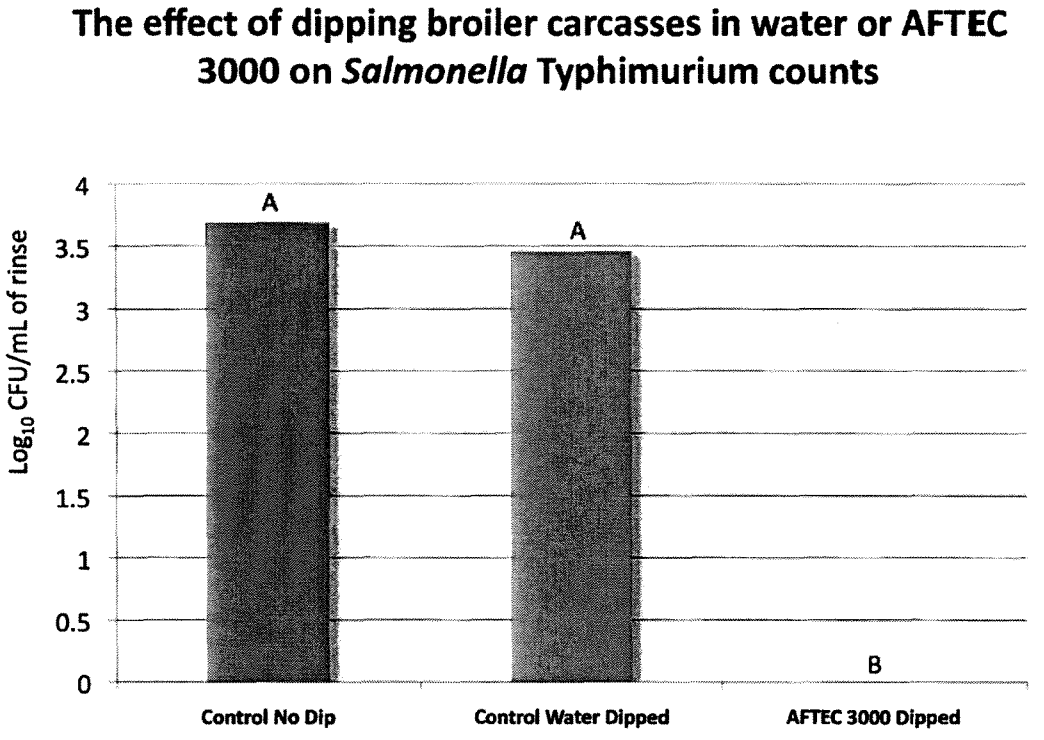
9) Each carcass was re-suspended on shackles for two minutes prior to bagging and rinsing with 400 mL Butterfield's Phosphate Buffer.

10) Samples were plated on BGS agar supplemented with nalidixic acid.

Results:

The data demonstrating the effect of Sulfuric Acid and Sodium Sulfate (AFTEC 3000) on *Salmonella* Typhimurium firmly attached to the surface of broiler chicken carcasses are presented in Figure 3:

Figure 3



Study 5: Evaluation of AFTEC 3000 as a Pre-Chill dip followed by a Pre-Chill spray

A research study was conducted to determine if a sulfuric acid, sodium sulfate blended product (AFTEC 3000) was effective as an antimicrobial treatment when used prior to the chiller system at a pH of 2, when used as a pre-chill dip followed by a pre-chill flood. Total contact time of the treatment on the carcasses before entering the chiller was 45 seconds to one minute. This Facility was in a USDA "A" set with 38 samples of 51 total and 8 positive results. For the first 38 samples the facility was using an approved anti-microbial as its OLR, Pre-Chill dip, Pre-Chill spray and in the chiller. It was felt that something should be done to assure that 13 positive of the 51 were not reached. The plant replaced the previous anti-microbial in the Pre-Chill dip system and the subsequent Pre-chill cabinet that flooded carcasses with AFTEC 3000.

Experimental Design: Carcasses were sampled at three locations, Post-OLR but prior to the Pre-Chill dip, before the chiller after the Pre-Chill spray, and after the chiller, (Post-Chill). Carcasses were rinsed using the whole carcass rinse technique (400 mL buffered peptone water) as per USDA-FSIS protocol. Ten carcasses were sampled daily at each location for six days, (3 locations x 10 carcasses x 6 days = 180 total samples). Rinses were evaluated for APC counts and *Salmonella* spp. Presence

or absence. Samples were pulled simultaneously at the Post-OLR and Pre-Chill sites, however the post chill carcasses were taken after a 100 minute time lapse from the time the Pre-Chill sampling began so as to allow the sampling of the relatively same lot of carcasses that were sampled Pre-Chill.

Statistical Analyses: APC count data were $\log_{10}$ transformed prior to analyses so that the data would more closely meet the underlying assumptions of the Analysis of Variance, (ANOVA). Prior to transformation values of zero or <1 were changed to 0.1 since the $\log_{10}$ transformation of zero is undefined. APC count data was analyzed by an ANOVA with the aid of SAS, (Statistical software) and *Salmonella* spp. prevalence was analyzed using a 2 by 3 Chi Square test using the Tables option of the PROC FREQ procedure of SAS.

Results: APC levels were significantly highest before entering the Post-OLR dip and significantly the lowest exiting the chiller, (Table 5). For Salmonella spp. incidence, the carcasses prior to the Post-OLR dip were significantly higher than the other two sites. Four positive carcasses were found Pre-Chill after the dip and flood, and 2 positives were found after the chiller, (Table 6), there was not a large enough difference between the Pre-Chill and Post-Chill values to detect a significant difference.

Table 5. Average Aerobic Plate Counts at three locations in the plant.

Plant Location	Aerobic Plate Counts	Log_{10} Aerobic Plate Counts
Post-OLR (Base level)	5012	3.70[a]
Pre-Chill (AFTEC treated)	288	2.46[b]
Post-Chill	40	1.60[c]

Table 6. *Salmonella* prevalence at three locations in the plant.

	Locations		
Salmonella	Post-OLR (control)	Pre-Chill (treated)	Post-Chill
Number Positive	15	4	2
Number Negative	45	56	58
Percent Positive	25%	7%	3%

Discussion: The AFTEC Post-OLR dip followed by an AFTEC Pre-Chill spray was effective at reducing microbiological counts and *Salmonella* spp. frequency. Since its placement on the evisceration lines at this facility, zero carcasses sampled by the

USDA were found to be positive for *Salmonella* spp. Another observation was that no product defects occurred.

Study 6: Effect of AFTEC 3000 on Aerobic Plate Counts and *Salmonella* spp. prevalence on chicken carcasses treated during Pre-Chill, Chilling, and in the Finishing Chiller.

A study was conducted to evaluate the effect of AFTEC 3000 on Aerobic Plate Counts at 5 locations and *Salmonella* spp. percentages at 2 locations after evisceration in a poultry processing facility where the Pre-Chill Dip, the Chiller and the Finishing Chiller were acidified.

This Study VI was conducted in the same plant as Study V after the USDA-FSIS had taken their sample set for *Salmonella*. A new post-chiller finishing chiller (Morris) was installed with AFTEC as the anti-microbial dip. The post-OLR/pre-chill dip tank was shortened and the spray cabinets removed. A commercial chiller management system (Hope Technical) was installed to manage chlorine dosage and the use of AFTEC acid to targeted pH and ORP settings.

Experimental Design: Carcasses were sampled at five locations:1) Pre-OLR; 2) Post-OLR but prior to the AFTEC Pre-Chill dip; 3) after the AFTEC dip at pH 2.0 and before the chiller; 4) after the chiller, but before the finishing chiller (labelled Post-Chill); and 5) after the AFTEC treated finishing chiller (pH of 1.7-1.8) labelled as Post Finish. Carcasses were rinsed using the whole carcass rinse technique (400 mL buffered peptone water) as per USDA-FSIS protocol. Ten carcasses were sampled at each location for one production day, (5 locations x 10 carcasses x 1 day = 50 total samples). Rinses were evaluated for APC counts and *Salmonella* spp. presence or absence. Samples were pulled simultaneously at thePre-OLR, Post-OLR and Pre-Chill sites; however the Post-Chill and Post-Finish carcasses were taken after a 100 minute time lapse from the time the Pre-Chill sampling began so as to allow the sampling of the relatively same lot of carcasses that were sampled pre-chill.

Statistical Analysis: Count data were analyzed by analysis of variance, (ANOVA) using the proc GLM procedure of SAS statistical software. Prior to analyses counts were $\log_{10}$ transformed so that the data met the underlying assumption of the ANOVA. When differences among means were detected, means were separated using Duncan's multiple range test. Since a zero dilution was used for some locations, when zero colony forming units were found a value of 0.1 replaced >1 for purposes of analyses. Since only one sample was detected positive for *Salmonella* spp. at the Pre-OLR location, no statistical analysis was performed on *Salmonella* spp. results (Figure 4).

Results: Aerobic Plate Counts (APC) were steadily decreased along the process from Pre-OLR to Post-Finishing Chiller (Figure 5). The OLR itself did not show a significant decrease, but with a larger sample size the decrease seen across the OLR would have most likely been significant. Pre-Chill carcasses had significantly lower counts than Pre-OLR carcasses though not different from Pre-Dip counts, again

sample size was probably the reason. The final two locations, Chiller and Finishing Chiller reduced counts by a full log, (90% reductions), and these were significant reductions.

Figure 4. Percent *Salmonella spp.* Positive by Location

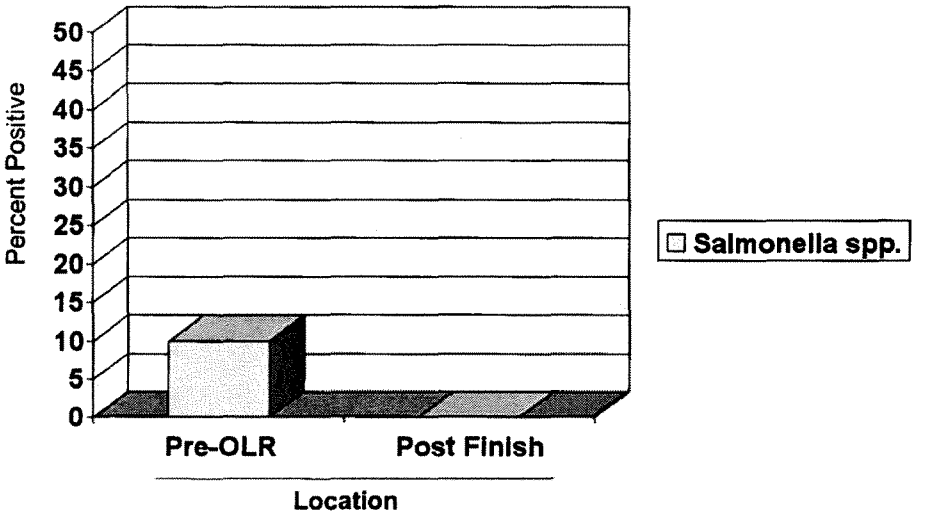
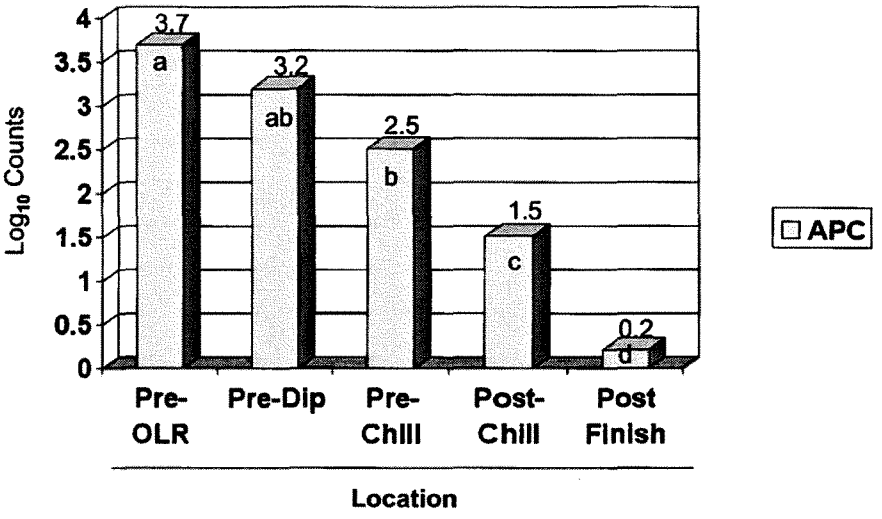


Figure 5. Mean Log_{10} Aerobic Plate Counts by Location



Study 7: An evaluation of the effect of AFTEC 3000 on Aerobic Plate Counts and generic *E. coli* on broiler carcasses in a large poultry processing facility when used in a New York Rinse cabinet and as a Pre-OLR cabinet.

A study was conducted to evaluate the impact of AFTEC 3000 on Aerobic Plate Counts and generic *E. coli* on chicken carcasses that were treated using an on-line cabinet at the New York Rinse and as a pre-OLR cabinet in a large poultry processing facility.

Approach:

1) In Plant 7, ten carcasses within a given flock were removed from the line just prior to the NY Rinse cabinet using sterile gloves and these carcasses were termed NYR Controls. Ten carcasses were allowed to traverse through the NYR cabinet and were sprayed with an AFTEC solution at a pH of 1.6 and collected from the line using sterile gloves. These carcasses were termed NYR Treated (AFTEC 3000 spray).

A second cabinet was installed just prior to the existing OLR cabinet. This was used to mimic AFTEC in a OLR cabinet due to the placement on the line while still permitting the carcasses to subsequently pass through the existing OLR system. Ten carcasses were removed from the line just prior to the pre-OLR cabinet using sterile gloves and these carcasses were termed Pre-OLR Controls. Ten carcasses were allowed to traverse through the Pre-OLR cabinet and were sprayed with an AFTEC solution at a pH of 1.6 and collected from the line using sterile gloves. These

carcasses were termed Pre-OLR Treated(AFTEC 3000 spray). All carcasses were selected by the count 5 pick 6th method to eliminate bias.

2) After allowing the carcasses to drip thoroughly, all carcasses were individually bagged in sterile polyethylene bags and rinsed using 400 ml of sterile Butterfield's phosphate buffer containing neutralizing buffer (Neutralizing Buffer - Formula per one liter of distilled water 0.0425g KH_2PO_4, 0.16g $Na_2S_2O_3$, 5.0g aryl sulfonate complex pH adjusted to 7.2 ± 0.2 at 25°C). This rinsate neutralized any residual AFTEC 3000 that may be rinsed from the carcass. The rinsates were encoded using a 4 digit number (to prevent identification by Silliker Laboratory employees and the introduction of bias) and sent on blue ice in a cooler using FedEx to Silliker Laboratories for evaluation for APC and *E. coli* counts.

3) This procedure was conducted 1 time such that 10 carcasses collected before and after NY Rinse spraying and before and after Pre-OLR spraying for a total of 40 carcasses.

4) The spray consisted of tap water dosed with AFTEC 3000 to a target pH level of 1.6 ± 0.2.

5) Microbiological tests conducted included aerobic plate counts (APC) and *Escherichia coli* (*E. coli*) counts.

6) Encoded microbiological results were received from Silliker labs and submitted to The University of Georgia for analysis. The results are summarized in Table 7.

Table 7. Average Aerobic Plate Counts and E. coli

	Aerobic Plate Counts (Log_{10})		***E. coli* (Log_{10})**	
	Control	**Treated**	**Control**	**Treated**
NY Rinse	**4.62**	**3.41**	**1.49**	**0.86**
Pre-OLR (NYR off)	**4.34**	**2.82**	**1.57**	**0.26**

Results and Discussion: When carcasses were treated using AFTEC 3000 in spray cabinets in commercial poultry operations, APC's were reduced by over 1.0 log_{10}. *E. coli* were also reduced by a significant amount. These results would indicate that AFTEC could be a good candidate for an OLR study.

Overall Summary:

The collective data in this report demonstrate that AFTEC 3000 is an effective antimicrobial when used as a dip or spray in a variety of applications for reducing total Aerobic Plate Counts, *E. coli* counts and *Salmonella* spp. prevalence on broiler chickens. Moreover, the data also show that treatment of carcasses with AFTEC 3000 does not result in chemical residuals being present on the carcasses and there was no residual impact on Aerobic Plate Counts or Psychrotrophic Plate Counts during storage. Therefore, Advanced Food Technologies, LLC (AFT) respectfully requests that the USDA-FSIS grant them a letter of no objection to allow the use of sulfuric acid and sodium sulfate blend (AFTEC 3000) as an antimicrobial agent for poultry processing.

Appendix A:

Raw Data for Efficacy of AFTEC 3000 in 3 separate studies.

Study I: Efficacy of AFTEC 3000 as a post-chill spray antimicrobial on APC and *E. coli* counts, and *Salmonella* prevalence on chicken carcasses.

	Total Aerobic Count		E.coli Count		Salmonella
Sample ID	CFU/ml	LOG	CFU/ml	LOG	1 = +, 0 = -
Post-Chill Control (Butterfield's Phosphate Buffer)					
1101	310	2.49	6	0.78	0
1102	360	2.56	4	0.60	0
1103	450	2.65	45	1.65	0
1104	260	2.41	2	0.30	1
1105	440	2.64	4	0.60	0
1106	200	2.30	8	0.90	0
1107	310	2.49	3	0.48	0
1108	90	1.95	1	0.00	0
1109	290	2.46	31	1.49	1
1110	310	2.49	1	0.00	1
Average		2.45		0.68	30.00%
Post-Chill Control (Butterfield's Phosphate Buffer)					
1201	270	2.43	18	1.26	0
1202	430	2.63	3	0.48	0
1203	280	2.45	1	0.00	0
1204	430	2.63	5	0.70	0
1205	290	2.46	1	0.00	0
1206	170	2.23	1	0.00	0

1207	110	2.04	37	1.57	0
1208	310	2.49	2	0.30	0
1209	460	2.66	5	0.70	0
1210	70	1.85	4	0.60	0
Average		2.39		0.56	0.00%
Post-Chill Control (Butterfield's Phosphate Buffer)					
1211	110	2.04	9	0.95	0
1212	110	2.04	1	0.00	0
1213	49	1.69	21	1.32	1
1214	220	2.34	2	0.30	0
1215	170	2.23	4	0.60	0
1216	73	1.86	1	0.00	0
1217	3100	3.49	44	1.64	0
1218	98	1.99	5	0.70	0
1219	170	2.23	1	0.00	0
1220	110	2.04	2	0.30	0
Average		2.20		0.58	10.00%
Post-Chill Control (Butterfield's Phosphate Buffer)					
1221	17	1.23	1	0.00	0
1222	4	0.60	1	0.00	0
1223	1	0.00	1	0.00	0
1224	1	0.00	1	0.00	0
1225	56	1.75	1	0.00	0
1226	250	2.40	2	0.30	0
1227	19	1.28	1	0.00	0
1228	170	2.23	1	0.00	0
1229	12	1.08	1	0.00	0
1230	49	1.69	1	0.00	0
Average		1.23		0.03	0.00%

	Total Aerobic Count		**E.coli Count**		**Salmonella**
Sample ID	CFU/ml	LOG	CFU/ml	LOG	1 = +, 0 = -
AFTEC 3000 Post-Chill Spray (Butterfield's Buffer; pH 1.51)					
1121	21	1.32	1	0.00	0
1122	20	1.30	1	0.00	0
1123	26	1.41	2	0.30	0
1124	9	0.95	1	0.00	0
1125	3	0.48	1	0.00	0

1126	5	0.70	1	0.00	0
1127	2	0.30	1	0.00	0
1128	15	1.18	1	0.00	0
1129	5	0.70	1	0.00	0
1130	150	2.18	1	0.00	0
Average		1.05		0.03	0.00%
AFTEC 3000 Post-Chill Spray + Hand Spray(pH 1.39)					
1231	1	0.00	1	0.00	0
1232	1	0.00	1	0.00	0
1233	3	0.48	1	0.00	0
1234	1	0.00	1	0.00	0
1235	19	1.28	1	0.00	0
1236	1	0.00	1	0.00	0
1237	1	0.00	1	0.00	0
1238	1	0.00	1	0.00	0
1239	1	0.00	1	0.00	0
1240	2	0.30	1	0.00	0
Average		0.21		0.00	0.00%
AFTEC 3000 Post-Chill Spray + Hand Spray(pH 1.39)					
1241	1	0.00	1	0.00	0
1242	1	0.00	1	0.00	0
1243	1	0.00	1	0.00	0
1244	1	0.00	1	0.00	0
1245	1	0.00	1	0.00	0
1246	1	0.00	1	0.00	0
1247	1	0.00	1	0.00	0
1248	1	0.00	1	0.00	0
1249	1	0.00	1	0.00	0
1250	1	0.00	1	0.00	0
Average		0.00		0.00	0.00%
AFTEC 3000 Post-Chill Spray + Hand Spray(pH 1.39)					
1251	2	0.30	1	0.00	0
1252	1	0.00	1	0.00	0
1253	3	0.48	1	0.00	0
1254	1	0.00	1	0.00	0
1255	1	0.00	1	0.00	0

1256	1	0.00	1	0.00	0
1257	1	0.00	1	0.00	0
1258	1	0.00	1	0.00	0
1259	1	0.00	1	0.00	0
1260	1	0.00	1	0.00	0
Average		0.08		0.00	0.00%

Study II: Evaluation of AFTEC 3000 as an antimicrobial in a post-chill dip application, Plant 2.

	Total Aerobic Count	
	CFU/ml	LOG
Post-Chill Control		
1	820	2.91
2	26	1.41
3	55	1.74
4	74	1.87
5	99	2.00
6	53	1.72
7	270	2.43
8	35	1.54
9	49	1.69
10	38	1.58
Average	151.90	1.89

	Total Aerobic Count	
	CFU/ml	LOG
AFTEC 3000 Dip (30sec, pH 1.61-1.72, Buffered Peptone)		
1	37	1.57
2	21	1.32
3	40	1.60
4	8	0.90
5	37	1.57
6	12	1.08
7	22	1.34
8	18	1.26
9	14	1.15
10	5	0.70
Average	21.40	1.25

Study III: Evaluation of AFTEC 3000 as an antimicrobial in a post-chill spray cabinet, Plant 3.

	Total Aerobic Count		Total E.coli Count		Salmonella
	CFU/ml	LOG	CFU/ml	LOG	1 = +, 0 = -
Post-Chill Control					
1101	25	1.40	1	0.00	0
1102	260	2.41	12	1.08	0
1103	160	2.20	4	0.60	0
1104	32	1.51	1	0.00	1
1105	16	1.20	1	0.00	0
1106	340	2.53	2	0.30	0
1107	74	1.87	1	0.00	0
1108	6400	3.81	270	2.43	0
1109	180	2.26	62	1.79	0
1110	80	1.90	16	1.20	1
Average	756.70	2.11	37.00	0.74	20.00%
Post-Chill AFTEC 3000 Spray (pH 1.6)					
1111	6	0.78	1	0.00	0
1112	2	0.30	1	0.00	0
1113	6	0.78	2	0.30	0
1114	11	1.04	1	0.00	0
1115	7	0.85	1	0.00	1
1116	7	0.85	1	0.00	0
1117	11	1.04	1	0.00	0
1118	1	0.00	1	0.00	0
1119	4	0.60	1	0.00	0
1120	8	0.90	1	0.00	0
Average	6.30	0.71	1.10	0.03	10.00%

Study V: Sulfuric Acid/Sodium Sulfate blend as pre-chill dip/spray combination

Sample Description	APC		*Salmonella* sp.
	Count	log_{10}	
Pre-Dip Control	1910	3.3	Positive
Pre-Dip Control	2140	3.3	Positive
Pre-Dip Control	1650	3.2	Negative
Pre-Dip Control	700	2.8	Negative
Pre-Dip Control	590	2.8	Negative
Pre-Dip Control	2200	3.3	Positive
Pre-Dip Control	1790	3.3	Negative
Pre-Dip Control	4200	3.6	Positive
Pre-Dip Control	16700	4.2	Positive
Pre-Dip Control	2800	3.4	Negative
Pre-Dip Control	7600	3.9	Negative
Pre-Dip Control	8900	3.9	Negative
Pre-Dip Control	3800	3.6	Negative
Pre-Dip Control	4600	3.7	Negative
Pre-Dip Control	5000	3.7	Negative
Pre-Dip Control	20300	4.3	Negative
Pre-Dip Control	3300	3.5	Negative
Pre-Dip Control	6400	3.8	Negative
Pre-Dip Control	31000	4.5	Negative
Pre-Dip Control	49000	4.7	Negative
Pre-Dip Control	12600	4.1	Negative
Pre-Dip Control	11100	4.0	Negative
Pre-Dip Control	3300	3.5	Negative
Pre-Dip Control	9500	4.0	Negative
Pre-Dip Control	1860	3.3	Negative
Pre-Dip Control	3800	3.6	Negative
Pre-Dip Control	4500	3.7	Negative
Pre-Dip Control	5100	3.7	Positive
Pre-Dip Control	3700	3.6	Positive
Pre-Dip Control	2600	3.4	Positive
Pre-Dip Control	114000	5.1	Negative
Pre-Dip Control	7100	3.9	Negative
Pre-Dip Control	2500	3.4	Negative
Pre-Dip Control	6800	3.8	Negative
Pre-Dip Control	2310	3.4	Positive
Pre-Dip Control	2270	3.4	Negative
Pre-Dip Control	3300	3.5	Positive
Pre-Dip Control	34000	4.5	Negative
Pre-Dip Control	1080	3.0	Negative
Pre-Dip Control	265000 Est.	5.4	Negative
Pre-Dip Control	2350	3.4	Positive
Pre-Dip Control	20200	4.3	Negative
Pre-Dip Control	10800	4.0	Negative
Pre-Dip Control	2900	3.5	Negative
Pre-Dip Control	3500	3.5	Negative

Pre-Dip Control	2900	3.5	Negative
Pre-Dip Control	41000	4.6	Positive
Pre-Dip Control	12700	4.1	Positive
Pre-Dip Control	1790	3.3	Positive
Pre-Dip Control	2100	3.3	Negative
Pre-Dip Control	3900	3.6	Negative
Pre-Dip Control	990	3.0	Negative
Pre-Dip Control	4900	3.7	Negative
Pre-Dip Control	2700	3.4	Negative
Pre-Dip Control	5000	3.7	Negative
Pre-Dip Control	4200	3.6	Negative
Pre-Dip Control	4100	3.6	Positive
Pre-Dip Control	1510	3.2	Negative
Pre-Dip Control	6500	3.8	Negative
Pre-Dip Control	10000	4.0	Negative
	AVG. =	**3.7**	**15/60 = 25.0%**
Post-Dip Treated	175	2.2	Negative
Post-Dip Treated	71	1.9	Negative
Post-Dip Treated	217	2.3	Negative
Post-Dip Treated	97	2.0	Negative
Post-Dip Treated	75	1.9	Negative
Post-Dip Treated	1210	3.1	Negative
Post-Dip Treated	35	1.5	Negative
Post-Dip Treated	78	1.9	Negative
Post-Dip Treated	67	1.8	Negative
Post-Dip Treated	178	2.3	Negative
Post-Dip Treated	480	2.7	Negative
Post-Dip Treated	4	0.6	Negative
Post-Dip Treated	182	2.3	Negative
Post-Dip Treated	180	2.3	Negative
Post-Dip Treated	1450	3.2	Negative
Post-Dip Treated	1770	3.2	Negative
Post-Dip Treated	10800	4.0	Negative
Post-Dip Treated	520	2.7	Negative
Post-Dip Treated	960	3.0	Negative
Post-Dip Treated	540	2.7	Negative
Post-Dip Treated	250	2.4	Negative
Post-Dip Treated	122	2.1	Negative
Post-Dip Treated	330	2.5	Negative
Post-Dip Treated	3300	3.5	Negative
Post-Dip Treated	164	2.2	Negative
Post-Dip Treated	1060	3.0	Negative
Post-Dip Treated	270	2.4	Negative
Post-Dip Treated	39000 Est.	4.6	Negative
Post-Dip Treated	370	2.6	Negative
Post-Dip Treated	500	2.7	Negative
Post-Dip Treated	260	2.4	Negative
Post-Dip Treated	171	2.2	Negative

Post-Dip Treated	55	1.7	Positive
Post-Dip Treated	95	2.0	Positive
Post-Dip Treated	118	2.1	Negative
Post-Dip Treated	136000	5.1	Negative
Post-Dip Treated	225	2.4	Negative
Post-Dip Treated	105	2.0	Negative
Post-Dip Treated	310	2.5	Positive
Post-Dip Treated	111	2.0	Negative
Post-Dip Treated	2200	3.3	Negative
Post-Dip Treated	750	2.9	Negative
Post-Dip Treated	220	2.3	Negative
Post-Dip Treated	800	2.9	Negative
Post-Dip Treated	98	2.0	Negative
Post-Dip Treated	400	2.6	Negative
Post-Dip Treated	540	2.7	Negative
Post-Dip Treated	480	2.7	Positive
Post-Dip Treated	260	2.4	Negative
Post-Dip Treated	140	2.1	Negative
Post-Dip Treated	1250	3.1	Negative
Post-Dip Treated	69	1.8	Negative
Post-Dip Treated	71	1.9	Negative
Post-Dip Treated	340	2.5	Negative
Post-Dip Treated	81	1.9	Negative
Post-Dip Treated	780	2.9	Negative
Post-Dip Treated	43	1.6	Negative
Post-Dip Treated	33	1.5	Negative
Post-Dip Treated	63	1.8	Negative
Post-Dip Treated	195	2.3	Negative
	AVG. =	**2.5**	**4/60 = 6.7%**
Post-Chill	67	1.8	Negative
Post-Chill	28	1.4	Negative
Post-Chill	19	1.3	Negative
Post-Chill	34	1.5	Negative
Post-Chill	9	1.0	Negative
Post-Chill	17	1.2	Positive
Post-Chill	12	1.1	Negative
Post-Chill	65	1.8	Negative
Post-Chill	16	1.2	Negative
Post-Chill	7	0.8	Negative
Post-Chill	9	1.0	Negative
Post-Chill	13	1.1	Negative
Post-Chill	7	0.8	Negative
Post-Chill	12	1.1	Negative
Post-Chill	3	0.5	Negative
Post-Chill	1490	3.2	Negative
Post-Chill	90	2.0	Negative
Post-Chill	27	1.4	Negative
Post-Chill	310	2.5	Negative

Post-Chill	13	1.1	Positive
Post-Chill	2	0.3	Negative
Post-Chill	1	0.0	Negative
Post-Chill	7	0.8	Negative
Post-Chill	15	1.2	Negative
Post-Chill	87	1.9	Negative
Post-Chill	106	2.0	Negative
Post-Chill	<1	0.0	Negative
Post-Chill	34	1.5	Negative
Post-Chill	123	2.1	Negative
Post-Chill	95	2.0	Negative
Post-Chill	28	1.4	Negative
Post-Chill	460	2.7	Negative
Post-Chill	45	1.7	Negative
Post-Chill	66	1.8	Negative
Post-Chill	210	2.3	Negative
Post-Chill	460	2.7	Negative
Post-Chill	81	1.9	Negative
Post-Chill	420	2.6	Negative
Post-Chill	49	1.7	Negative
Post-Chill	29	1.5	Negative
Post-Chill	250	2.4	Negative
Post-Chill	32	1.5	Negative
Post-Chill	79	1.9	Negative
Post-Chill	96	2.0	Negative
Post-Chill	38	1.6	Negative
Post-Chill	26	1.4	Negative
Post-Chill	37	1.6	Negative
Post-Chill	950	3.0	Negative
Post-Chill	46	1.7	Negative
Post-Chill	75	1.9	Negative
Post-Chill	19	1.3	Negative
Post-Chill	182	2.3	Negative
Post-Chill	290	2.5	Negative
Post-Chill	47	1.7	Negative
Post-Chill	4	0.6	Negative
Post-Chill	290	2.5	Negative
Post-Chill	33	1.5	Negative
Post-Chill	47	1.7	Negative
Post-Chill	530	2.7	Negative
Post-Chill	18	1.3	Negative
	AVG. =	**1.6**	**2/60 = 3.3%**

Study VII--cabinets

NEW YORK RINSE CABINET TEST

CONTROL SAMPLES

	Total Aerobic Count		E.coli	
	CFU/ml	LOG	CFU/ml	LOG
NYR Control (pulled post-NYR, cabinet OFF)				
1	120000	5.08	480	2.68
2	40000	4.60	610	2.79
3	160000	5.20	100	2.00
4	86000	4.93	40	1.60
5	10000	4.00	40	1.60
6	42000	4.62	0	0.00
7	22000	4.34	170	2.23
8	18000	4.26	100	2.00
9	30000	4.48	0	0.00
10	48000	4.68	0	0.00
Average	57600	4.62	154	1.49

TREATED SAMPLES

	Total Aerobic Count		E.coli	
	CFU/ml	LOG	CFU/ml	LOG
NYR Treated (pulled post-NYR cabinet ON at pH 1.5-1.6)				
1	560	2.75	30	1.48
2	290	2.46	0	0.00
3	710	2.85	0	0.00
4	11300	4.05	40	1.60
5	10000	4.00	170	2.23
6	2500	3.40	0	0.00
7	6000	3.78	50	1.70
8	600	2.78	0	0.00
9	8000	3.90	0	0.00
10	14000	4.15	40	1.60
Average	5396	3.41	33	0.86

SIMULATED OLR CABINET TEST

pre-OLR Control (NYR OFF)				
1	63000	4.80	250	2.40
2	28000	4.45	10	1.00
3	8000	3.90	270	2.43
4	90000	4.95	190	2.28
5	9600	3.98	10	1.00
6	46000	4.66	20	1.30
7	5200	3.72	110	2.04
8	100000	5.00	0	0.00
9	4700	3.67	10	1.00
10	20000	4.30	190	2.28
Average	37450	4.34	106	1.57

post-OLR Treated (pH 1.5-1.6)				
1	1200	3.08	10	1.00
2	800	2.90	0	0.00
3	210	2.32	0	0.00
4	1500	3.18	40	1.60
5	90	1.95	0	0.00
6	1200	3.08	0	0.00
7	370	2.57	0	0.00
8	900	2.95	0	0.00
9	1100	3.04	0	0.00
10	1200	3.08	0	0.00
Average	857	2.82	5	0.26

VI. Applicable prior approvals

United States Department of Agriculture | Food Safety and Inspection Service | Washington, D.C. 20250

Mr. Dennis Smithyman
President
Advanced Food Technologies, LLC
11230 Magnolia Glen
Shreveport, LA 71106

NOV 0 4 2008

Dear Mr. Smithyman:

This is in response to your October 30, 2008 email (Log number 08-NT-0387-N-A) requesting a letter from the Food Safety and Inspection Service (FSIS) stating that AFT Clear 3000 is the same as sodium bisulfate and under FSIS Directive 7120.1, "Safe and Suitable Ingredients Used in the Production of Meat and Poultry Products" can be used as an acidifier in meat and poultry plants.

After reviewing your submitted information, FSIS has determined that AFT Clear 3000 is considered the same as sodium acid sulfate (SAS) or sodium bisulfate and, thus, would not be considered new technology. SAS or sodium bisulfate is already permitted by FSIS to be used as a pH control agent (acidifier) in water used in meat and poultry processing sufficient for the purpose.

This letter should not be considered as validation that your process will be effective in any particular FSIS establishment. Your technology, as described in your notification, will need to be factored into an establishment's hazard analysis and, if appropriate, incorporated into its HACCP Plan or SSOP, or other prerequisite program, validated for its application, and verified on an ongoing basis for its effectiveness. If the establishment does not address the effects of using your technology in its hazard analysis, FSIS would be unable to determine that the product processed using your technology is safe, including microbiologically, not adulterated; and therefore, the product would not be eligible to bear the mark of inspection.

If you have any questions, please contact Dr. David Zeitz at (202)690-3556 or david.zeitz@fsis.usda.gov.

Sincerely,

(b) (6)

Dr. John Hicks, Director
Risk and Innovations Management Division
Office of Policy and Program Development

FSIS FORM 2630-9 (8/96)

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VII. References

1. **Dickens, J. A., and A. D. Whittemore**, 1994. The effect of acetic acid and air injection on appearance, moisture pick-up, microbiological quality, and *Salmonella* incidence on processed poultry carcasses. Poultry Sci. 73:582-586.

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VIII. Responses to questions posed by Dr. David Zeitz, USDA/FSIS/OPPD/RIMD, regarding these background studies for use of AFTEC 3000 (Log#10-OLR-0514-N-A)

The questions posed by USDA-FSIS are emboldened and the responses to the questions are posted below.

1. Please explain the reason for the variation of the 7 study designs,

i.e., the selection of carcass numbers, the microorganism selections,

and number of days.

AFTEC 3000 has been approved for use as an acidifier since November, 2008. Numerous studies have been conducted by various groups to ascertain applicability and effectiveness of AFTEC 3000 from several poultry interventions. Some of these have been laboratory studies, some have been quick in-plant assessments, and some were full in-plant validation studies. Sometimes the studies and methodologies were conducted by UGA professor Dr. Scott Russell, and other times the testing was directed and paid for by the local plant with their in-house corporate laboratory services.

Cost effectiveness and statistical significance was also usually considered. Thus, a small sample size for post-chill application usually did not include salmonella testing. Incidence levels would be expected to be low and therefore not have statistical significance in a small sample size.

Study 1 was for a commercial post-chill spray at a plant in Delmarva. UGA directed the study and validated the results. Study 3 was a month later re-test to ensure performance was maintained, which the short study confirmed (same methodologies).

Study 2 was a short APC test for a finishing chiller in a plant in GA that was switching to AFTEC 3000 from one of the hydrochloric acid blends. As this plant already had low salmonella incidence post-finishing chiller, the subsequent salmonella inoculation study (Study 4) was conducted at UGA labs on their behalf.

Studies 5 and 6 were conducted by the corporate laboratory of a major processor at one of their plants in AR. AFTEC 3000 was initially introduced as a post-OLR/pre-chill dip (Study 5). A new finishing chiller was later installed using AFTEC 3000 and the finishing chiller overflow was filtered and then recycled back to the post-OLR/pre-chill dip. AFTEC 3000 was also then used as the chlorine acidifier for the chiller. Study 6 biomapped the net results.

Study 7 was a quick APC study for a plant considering the use of AFTEC 3000 in the NYR cabinets and OLR cabinets (once AFTEC 3000 is approved for OLR application).

2. When tap water was used, do you know what antimicrobials were in the tap water and at what levels?

No specifics tests were conducted. All the plants were in districts where the city water is chlorinated. No tests were conducted in plants where the local water district uses mainly ammonia or chloramines. However, no difficulties would be expected from a chemical interaction standpoint.

3. This mirrors question 1 above. In Study 2 why were only 20 total carcasses selected and why was only APC's tested?

As mentioned above, Study 2 was a short APC test for a finishing chiller in a plant in GA that was switching to AFTEC 3000 from one of the hydrochloric acid blends (FreshFx). The plant only requested an APC test to prove reductions across the finishing chiller. As this plant already had low salmonella incidence post-finishing chiller, the subsequent salmonella inoculation study (Study 4) was conducted at UGA labs on their behalf.

4. Were controls and AFTEC testing done with both the NYR cabinet and pre-OLR cabinet running at the same time? If both were used at the same time during testing, it appears that the APC's and E. coli counts went up after the NYR cabinet and before the pre-OLR cabinet (Table 7).

No. Both cabinets were OFF for the Control samples. Thus, there was a greater than 1 log reduction in APC's across the treated (ON) NYR cabinet. With the NYR cabinet OFF, the pre-OLR controls were log 4.34 and after the OLR cabinet they were log 2.82. Thus, both AFTEC cabinets reduced APC's by over 1 log. A test was not conducted with both cabinets ON. We're sorry for the confusion in the write-up.

Attachment 5

Analysis of On-Line Reprocessing Results with Aftec 3000™ as an Antimicrobial Agent

Purpose: An on-line reprocessing (OLR) trial was conducted under a USDA waiver, (log # 10-OLR-0514-N-A) to determine if Aftec 3000™ is an effective antimicrobial agent in an OLR application. Aftec consists of sulfuric acid blended with sodium sulfate. The sulfate is a natural buffering salt that makes the acid solution easy to handle and prevents organoleptic damage to meats. Over a 10 day period, 400 carcasses were identified as being either visually uncontaminated (VC) or visually contaminated (VF). One hundred carcasses, ten per day per condition, were rinsed before and after the OLR.

Statistical Analysis: Aerobic plate count APC and *E. coli* colony forming units data were $\log_{10}$ transformed prior to analysis. Prior to transforming the numbers, values of zero or none detected were converted to the detection limit for that sample. For all cases, a 1:10 dilution was the lowest dilution level used, so a 10 replaced all zero or none detected observations. Count data were analyzed using an analysis of variance (ANOVA) with the aid of SAS (Cary, N.C.).

The statistical model used was:

$$Y_{ijkl}=\mu + D_i + C_j + L_k + C*L(_{jk}) + \varepsilon_{ijkl}$$

Where: Y_{ijkl} is the result of the overall mean μ, plus the effect of the ith Day[1], (I = 1, 2,..10), plus the effect of the jth Carcass condition (Visually uncontaminated (VC) or visually contaminated (VF)), plus the effect of the kth location (pre-OLR or post-OLR), plus the effect of the jk^{th} carcass condition by location interaction (pre-OLR, VC; pre-OLR, VF; post-OLR, VC: and post-OLR, VF), plus the effect of the $ijkl^{th}$ whole bird carcass rinse.

For *Salmonella spp.* only six positive samples were identified, four pre-OLR and two post-OLR. No differences would be detectable from such low incidence so no statistical tests were conducted. For this reason, only a descriptive analysis is presented for *Salmonella spp.*

Results: Carcass condition at a location did not have a significant impact on colony forming unit levels of APC or *E. coli* (Table 1). However, across the OLR system there were significant reductions in both APC and *E. coli;* both microbial class means pre-OLR were higher than post-OLR means (Figure 1).

For *Salmonella spp.*, few positive carcasses were detected; however, from a descriptive standpoint the trend was favorable. Four positive carcasses were detected pre-OLR and two positive carcasses were detected post-OLR, which demonstrates a 50 percent reduction in incidence. For both the VC and the VF carcasses the number of positive carcasses post-OLR was reduced by one from pre-OLR carcasses (Figure 2).

1. The original protocol stated data would be collected over a 9 day period, however, during the time from when the protocol was submitted to the time the data collection began, facilities were trying to get approved for Russian export. If the facility had to switch to an approved PAA the protocol was a ten day data collection period. The ten day collection period was mistakenly used for this protocol as well. The additional data collection enhances the trial.

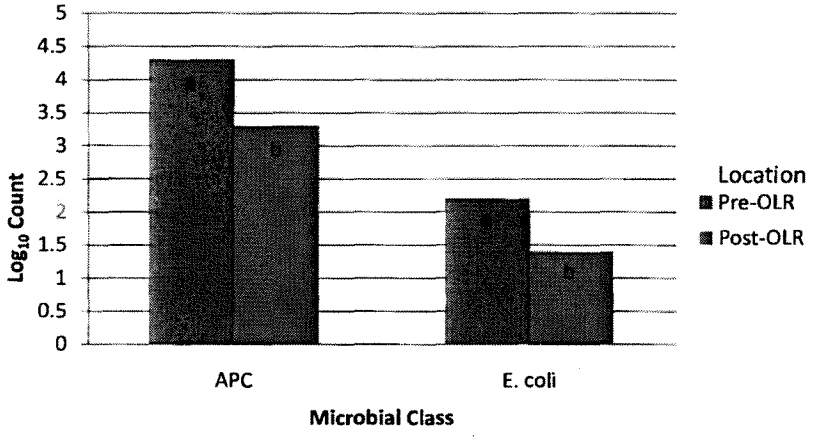
Table 1. Mean $\log_{10}$ APC and *E. coli* counts by location and carcass condition[1]

Microbial Group and Carcass Condition

	APC		*E. coli*	
Location	VC	VF	VC	VF
Pre-OLR	4.28	4.35	2.17	2.30
Post-OLR	3.33	3.30	1.42	1.39

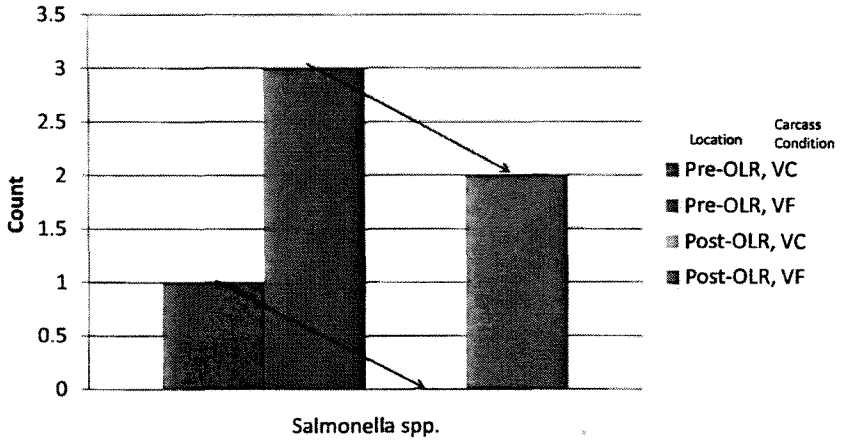
[1] For a microbial class at a location means were not significantly different ($p > 0.05$).

Figure 1. Mean $\log_{10}$ counts of APC and *E. coli* by location



[ab] Mean counts for a location within a microbial class were statistically different ($p \leq 0.05$).

Figure 2. Number of *Salmonella spp*. carcasses detected out of the 100 sampled per location and condition



Discussion: The APC and *E. coli* count numbers showed 90 and 85 percent statistically significant reductions from pre-OLR to post-OLR, respectively. *Salmonella spp.* showed a low positive incidence, so even though there was a 50 percent reduction in positive incidence post-OLR compared to pre-OLR, the reduction could not be shown to be significant. A similar trend was observed among all three microbial classes examined in this trial, which illustrates the importance of measuring pathogenic organisms and broader class indicator organisms.

Conclusion: The Aftec 3000™ product met the conditions for the waiver and is shown by these results to be an effective antimicrobial agent in the OLR. Additionally, since these data are satisfactory, the product should be granted waivers for two additional in-plant trials.

Attachment 6

United States Department of Agriculture
Food Safety and Inspection Service

Office of Policy and Program Development

Risk and Innovations Management Division
George Washington Carver Center
5601 Sunnyside Ave: STOP 5271
Beltsville, MD 20705-5271

March 8, 2011

Dennis Smithyman
Advanced Food Technologies, LLC
11230 Magnolia Glen
Shreveport, LA 71106

Dear Mr. Smithyman:

This letter is in response to your February 7, 2011, revised notification requesting to conduct additional in-plant trials to "Evaluate the application of AFTEC 3000 (AFT Clear 3000) in commercial poultry on-line reprocessing for elimination of pathogenic and indicator populations of bateria. (Log No. 10-OLR-0514-N-B,C).

You have requested a waiver, pursuant under Title 9 of the Code of Federal Regulations 9 CFR §381.3 (b), to use AFTEC 3000 (AFT Clear 3000) On-Line Reprocessing (OLR) system on prechill poultry carcasses to conduct the second and third in-plant trials, pending Agency amendment of 9CFR §381.91(b) (1) [off-line reprocessing regulation].

In your notification, you requested permission to conduct simultaneously the second in-plant trial at Pilgrim's Pride, establishment # P6638, Enterprise, AL and the third in-plant trial at Gold'n Plump, establishment # P322, Cold Spring, MN following the revised February 3, 2011 protocol. You intend to conduct the study to test AFTEC 3000 (AFT Clear 3000) using a pH level of 1.8 (+/– 0.4).

FSIS has completed its review of your first in-plant trial data collected at Tyson Foods, Inc. establishment # P164, Forest, MS. The first in-plant trial data showed that the number of aerobic plate count (APC) bacteria, *Escherichia coli*, and *Salmonella* positive samples was statistically reduced after passage through the AFTEC 3000 (AFT Clear 3000) OLR system. The data showed that there was no statistical microbiological difference between carcasses marked visibly clean and those marked visibly contaminated after decontamination with the AFTEC 3000 (AFT Clear 3000) OLR treatment. Therefore, FSIS is granting you permission in lieu of 9CFR §381.91(b)(1) to conduct the second in-plant trial at establishment # P6638 and the third in-plant trial at establishment # P322, provided that:

1. Risk, Innovations, and Management Division (RIMD) receives data comparing microbiological levels of Aerobic Plate count (APC), *Escherichia coli* and *Salmonella* prevalence on two groups, marked visibly clean carcasses and

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marked visibly contaminated carcasses **BEFORE** both have been treated with the AFTEC 3000 (AFT Clear 3000) OLR system.

2. RIMD receives data comparing microbiological levels of aerobic plate count (APC), *Escherichia coli* and *Salmonella* prevalence on two groups of marked visibly clean carcasses and marked visibly contaminated carcasses **AFTER** decontamination and both have been treated with the AFTEC 3000 (AFT Clear 3000) OLR system before they enter the chiller.

3. Data collected throughout the in-plant trial should be provided to the RIMD Project Manager (PM) for the Agency to examine using an Excel format. Final report on the results must be submitted in an Excel format to RIMD PM at the completion of the trial. The data must show results that are consistent with reduced microbiological and pathogen levels.

4. The parameters set forth in your revised February 3, 2011, OLR protocol, are followed. Operational parameters include:

   a. The dip tank or spray cabinet is fed continuously with tap water dosed with AFTEC 3000 (AFT Clear 3000) to a target pH level of 1.8 (+/- 0.4).

   b. For dip tanks, the fresh mixture will enter the tank at the bird exit end and counter flow through the tank to an overflow drain at the entrance end of the tank. The dip tank system will flow between 5 gal/minute and 10 gal/minute.

   c. For spray cabinets, the fresh mixture will be delivered to spray bars at a minimum pressure of 10 psi and flows between 5 gal/minute and 10 gal/minute.

5. The OLR system is validated in-plant to demonstrate that the establishment can apply it to obtain the anticipated effect under actual in-plant operational conditions as stated in the aforementioned protocol.

6. Establishment discusses the waiver and OLR system with the inspection program personnel (IPP), at the weekly meeting, prior to its implementation. The alternative procedures must be addressed in the Hazard Analysis and Critical Control Point (HACCP) plan, Sanitation Standard Operating Procedures (Sanitation SOPs), or a prerequisite program. In addition, establishments will need to explain how they intend to use the OLR system and where in the establishment's food safety system the procedures will be located.

   NOTE: The IPP will verify the procedures according to their location in the food safety system and verify whether the establishment is monitoring and documenting these parameters as described above. Once a week, IPP will use an appropriately scheduled PBIS procedure to verify one or more parts of the alternative procedures or parameters. IPP will verify whether the OLR

technology is operating in a manner that is consistent with this grant permission letter.

7. Establishment that does not have an existing OLR regulatory waiver under 9 CFR §381.3 (b) to use the OLR system must apply to the *Salmonella* Initiative Program (SIP), as detailed in Federal Register Notice 73FR4767, January 28, 2008, before a waiver for the use of the OLR system may be granted.

8. After the completion of the in-plant trials, establishment # P164, establishment # P6638, and establishment # P322 agrees to submission of ongoing microbiological monitoring results to RIMD at each quarter. RIMD will review the monitoring data to assess the ongoing effectiveness of your OLR system. Data should be submitted in an Excel format to RIMD.OLRD@fsis.usda.gov.

FSIS IPP will have access to FSIS intranet at RIMD NT & NI Summaries postings on the web page that describes the alternative procedures (parameters) for this OLR technology.

Carcasses extensively affected with contamination or mutilation are condemned by FSIS inspectors (9CFR §381.91) and these carcasses will not be allowed to enter the OLR system. Sanitary dressing of carcasses on the line must be maintained in a manner to minimize contamination, including internally contaminated carcasses going through the OLR system.

Carcasses that are normally subject to off-line reprocessing (OLR) can be reprocessed on-line and are subject to compliance with 9CFR §381.65 (e) and 9CFR §381.76 for Finished Product Standards (FPS). IPP will continue to conduct zero fecal tolerance and FPS checks.

This letter should not be considered as validation that your chemical or process would be effective in any particular official establishment.

Be aware that if establishment # P164, establishment # P6638, and establishment # P322 produces product that conflicts with the provisions of the Poultry Products Inspection Act (21 U.S.C. 451, et seq.) or has repeated Noncompliance Records (NRs) documenting failure to maintain the alternative procedures associated with this waiver, the waiver could be revoked.

Continuation of the in-plant trials will be granted based on evidence of a timely start, adherence to the schedule in the protocol, and appropriate progress towards the purpose stated in your protocol. If the in-plant trials do not commence within 90 calendar days of receipt of this letter, then the "Permission" status to start the in-plant trials will be withdrawn. You will need to submit in writing for an extension of time to commence the in-plant trials.

If you have any questions, please contact the PM, Dr. David Zeitz, at (321) 327-2576 or by e-mail at David.Zeitz@fsis.usda.gov. If you have any questions on SIP, please contact Dr. Isabel Arrington at Isabel.Arrington@fsis.usda.gov.

Sincerely,

(b) (6)

William K. Shaw, Jr., Ph.D.
Director
Risk, Innovations, and Management Division
Office of Policy and Program Development

Attachment 7

Study title: Integral Anti-microbial Solution Application Systems Using a Ross Blade Tenderizer—Phase 1 and 2 results

Conducted by: Peter M. Muriana, Ph.D., Professor, Food Microbiology, Dept. of Animal Science, Oklahoma State University

Study dates: Report completed on April 2, 2011

Objective: to demonstrate significant reductions of E. coli O157:H7 in non-intact meat by spraying anti-microbial solutions directly on the surface of the meat just prior to blade tenderization.

Methodology: Phase 1—evaluate 14 submitted anti-microbial solutions by spraying E. coli O157:H7 inoculated beef discs in the Ross machine spray system and measuring reductions versus controls. Phase 2—utilizing the best 7 performing anti-microbial solutions, spray inoculated whole muscle cuts just prior to blade tenderization. Core out the meat and slice to create meat discs at measurable depths from the surface. Check for E. coli incidence in each meat disc to measure performance vs. untreated controls.

Results and discussion: In Phase 1, there were 5 chemical blends that achieved a 1.0 Log cfu/cm2 reduction (90%) from inoculated controls when measured in less than 1 hour from treatment. AFTEC was one of the 5 best. A total of 7 treatment chemicals, including AFTEC, were selected for Phase 2 testing.

In Phase 2, each surface inoculated whole muscle cut was cored in 4 places and each core was sliced into 4 sections below the surface. This yields a total of 16 samples for each treatment plus 16 for the untreated controls. When analyzed for E. coli presence, the control cut had 15 of 16 samples positive for E. coli O157:H7. The 7 treated cuts had incidence levels from 1 (best) to 10 (worst). AFTEC performed in the middle group (with the other acids) with a total of 8.

Thus, the study did confirm that there is a real risk of contaminated meat carrying the E.coli into the interior of the meat with blade tenderization. However, spraying the meat with an anti-microbial solution just prior to tenderization can significantly reduce the contaimination risk to the general poupulation (AFTEC 3000 being one of these solutions). Continuing studies will look to optimize the systems and improve the quantification of risks and reductions.

Technical Report

Integral Antimicrobial Solution Application on the Ross Blade Tenderizer

---

Phase 1 & Phase 2 Results

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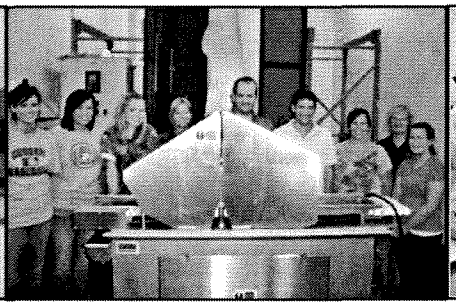
Graduate Students: Jackie Eager (Meat Science)
Brent Wellings (Meat Science)
Preetty Pranatharthiharan (Food Micro)
Dinesh Babu, PhD (Food Micro)



Industry Issue: A current concern with blade tenderized beef is the potential to carry surface contamination (i.e., *E. coli* O157:H7) into steak cuts that may be prepared rare for consumption, presenting a potential health risk to consumers. The USDA-FSIS distinguishes such tenderized beef as 'non-intact' beef and declares that there should be antimicrobial interventions in place to eliminate (or reduce) surface *E. coli* O157:H7 prior to blade tenderization. Most recently, an outbreak linked to a supplier of blade tenderized beef has raised concern for this issue even further within the industry and the regulatory agency.

Objective(s):

Phase 1: To use sufficiently high inoculation levels of *E. coli* O157:H7 that we can determine process effectiveness of various antimicrobial spray treatments on beef surfaces.

Phase 2: To use practical inoculum levels of *E. coli* O157:H7 on subprimal beef surfaces followed by spray treatment and blade tenderization to demonstrate that entry does not occur, or is minimized, after interventions are applied (complements Phase 1).

Major Equipment. A Ross Industries Inc. blade tenderizer, equipped with a multi-nozzle spray system (integral product tank and positive displacement pump) will be supplied for use in 228 FAPC. The advantage of using this system is that it is the same equipment that is currently being developed for the meat industry involved with blade tenderization and therefore there is no question as to whether data obtained in this study will reflect spray treatment of commercial systems (i.e., identical spray system, number of nozzles, same water pressure, operating speed, and dosing rate).

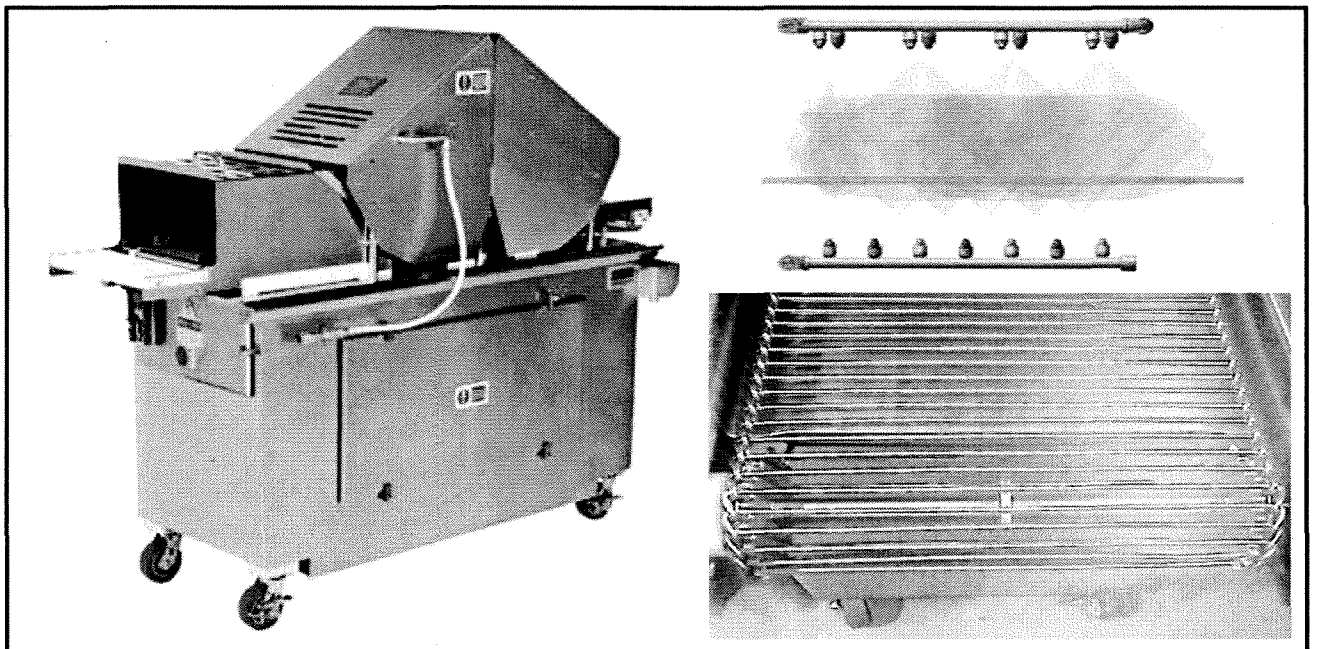
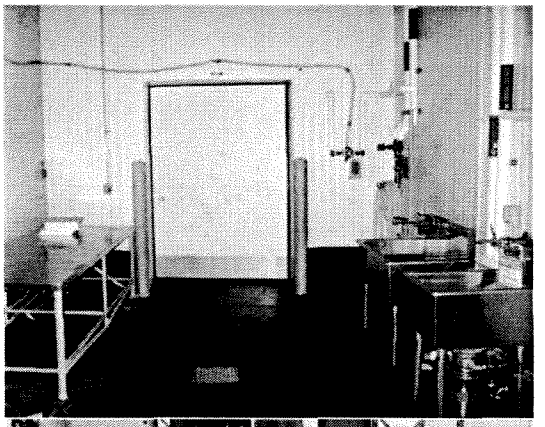
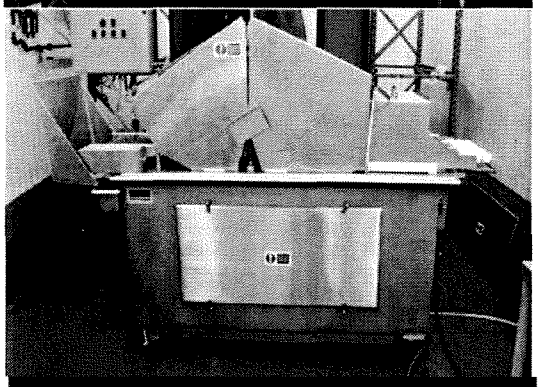


Figure 1. Photo and schematics of Ross tenderizer (TC700MC) with full coverage (top and bottom) spray system including the sani-grid conveyor belt.

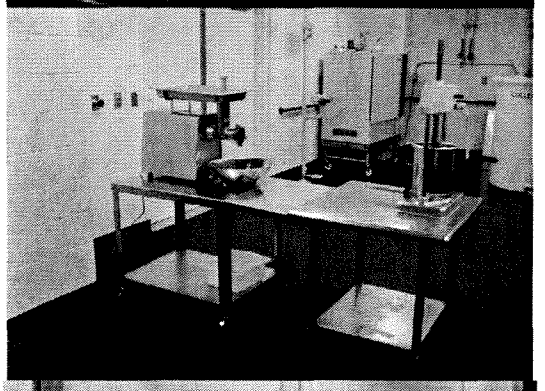
Process Location 1 (228 FAPC). Initial processing was performed in 228 FAPC Bldg. Prior to the use of *E. coli* O157:H7 surface inoculants on beef products, protocols detailing experimentation and worker safety were submitted and approved by the Institutional Biosafety Committee (IBC) which designated 228 FAPC as a BSL-2 facility.

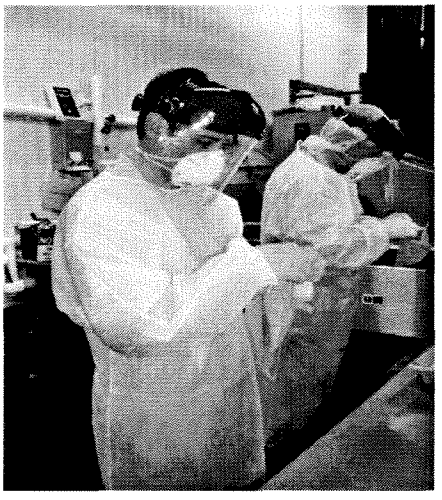


The use of inoculated beef samples (Phase 1: lean sirloin wafers; Phase 2: 10-15 lb sirloin top butts) and the potential for spray displacement of the inoculated organisms required the placement of the equipment in a secured and safe use location. Spray contact was minimized due to a shielded housing of the basic tenderizer machine. Personnel must wear designated PPE (personal protection equipment) when working with pathogens and sanitation regimens after each trial includes hot water spray, followed by hypochlorite spray and finally fogging of the entire room with a pressurized canister containing a quat sanitizer. BSL-2 status has been approved based on the safety precautions we implemented. This room is secured, is compatible with wall-to-floor spray sanitation, and has the necessary electrical, water, sink, drainage, and space requirements to perform the intended testing.



Process Location 2 (302/307 FAPC). After processing was performed in 228 FAPC (and even while it was ongoing), samples were transported to our microbiology laboratory or microbiology processing lab for microbial sampling (Phase 1) or for thermal sanitation of cores, followed by blending, incubation, and final microbial sampling (Phase 2). Rms 302/307 FAPC are also BSL-2 designated labs. Rm 307 FAPC is a traditional microbiology lab with autoclave, benches, incubators where microbial dilutions/plating is performed; 302 FAPC is a 'pathogen processing pilot plant' that is often used for processing of pathogen-inoculated samples for evaluation of thermal or chemical antimicrobial intervention. Both labs are adjoining to facilitate microbial plating when inoculated samples are handled/processed in the pathogen lab.





Protocols & Results: Phase 1.

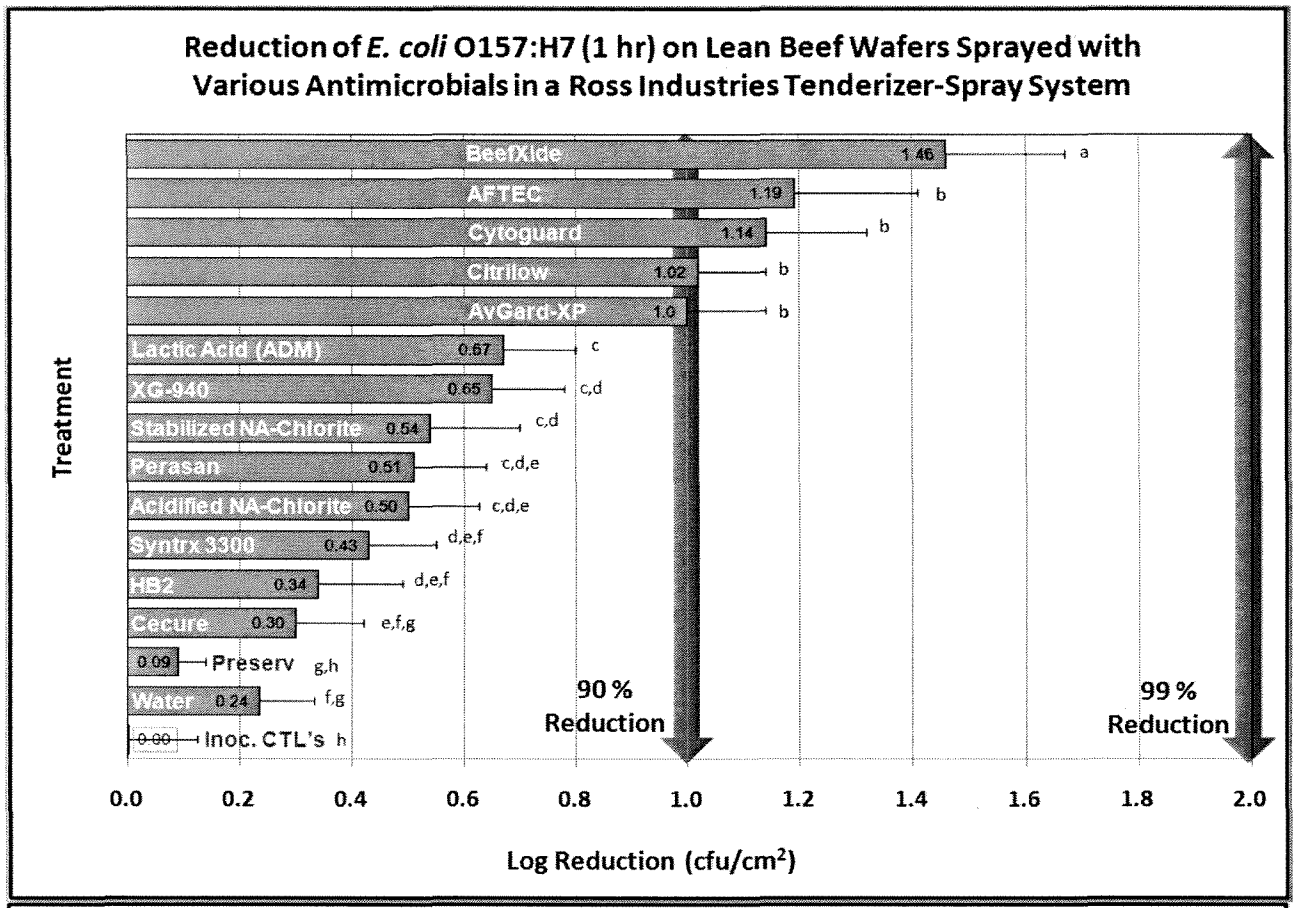
Cultures. Bacterial cultures used in this study included *E. coli* O157:H7 PMM53150, ATCC 43890 (California outbreak isolate from human feces), ATCC 43894 (Michigan outbreak isolate from human feces), and ATCC 43895 (hamburger isolate implicated in human outbreak). Resistant variants of each strain was recovered to both Rifamycin S/V (10 ug/ml) and Gentamycin (10 ug/ml) whereby they were selectively recovered from non-sterile sample/meat environments in the presence of indigenous microbial backgrounds by plating on media containing these antibiotics.

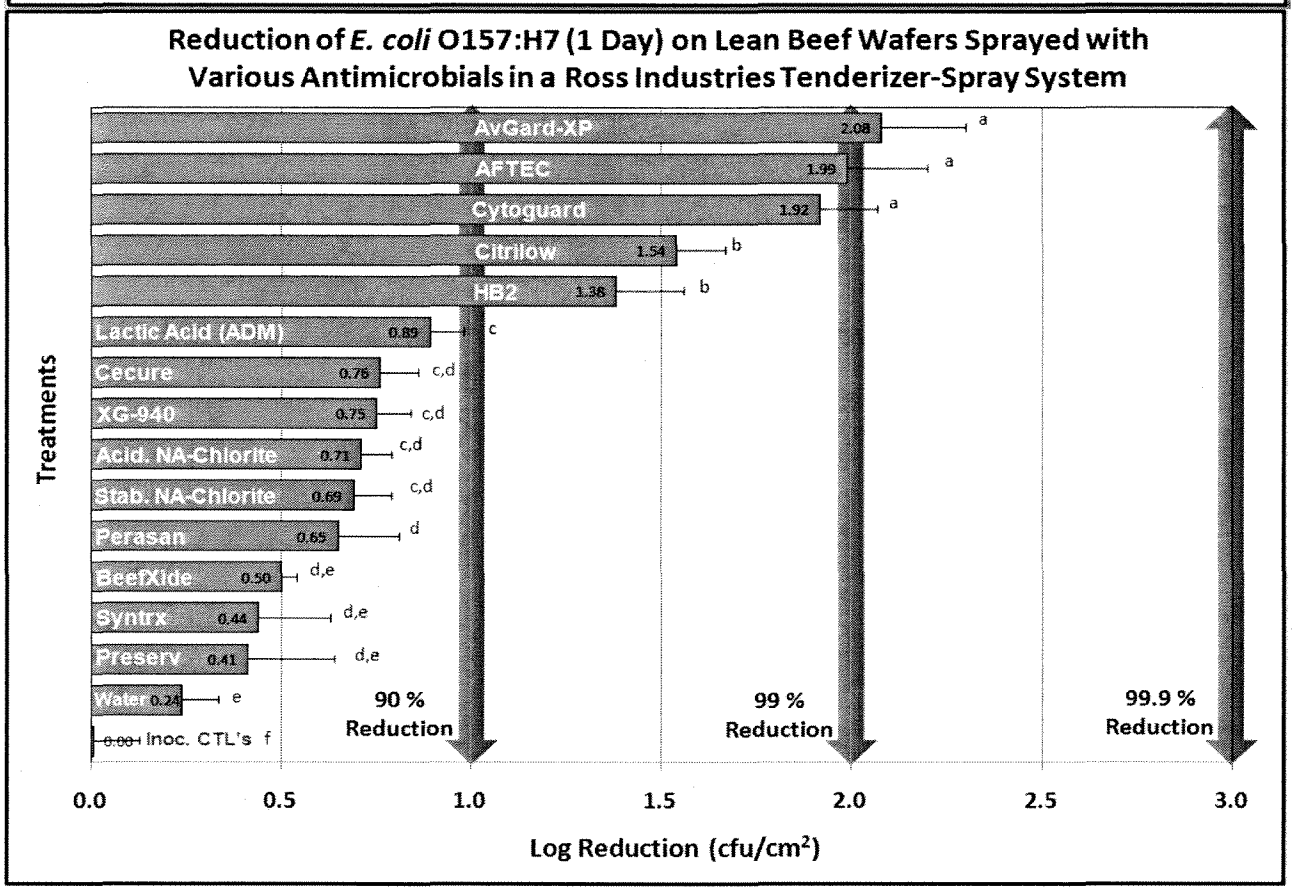
Assessment of antimicrobial spray treatment efficacy using lean sirloin wafers. We obtained numerous 'lean beef discs' from beef sirloins using a 2-inch diameter drill bit to 'core' a circular core from intact beef. Individual beef wafers, or discs (i.e., 20.25 cm^2), were then sliced from the cores for subsequent inoculation (~1 x 10^6 cfu/cm^2), 30 min holding, and then spray treatment with water or antimicrobials. Un-inoculated controls were also tested against our selection media as well as inoculated, but un-treated, samples that served as the basis of our microbial inoculation baseline. Samples inoculated and spray treated were kept on ice or refrigerated and processed for residual microbial counts. All samples were eventually stomached with DE Neutralizing broth and dilutions made in 0.1% buffered peptone water for plating. Samples not plated immediately after processing were held in bags in the refrigerator and then DE Neutralizing broth was added prior to microbial processing.

Sampling times:
a. 1 hr, as soon as possible after spray treatment (18 samples)
b. 1 day (18 samples)
c. 7 days(18 samples)
d. 14 days (18 samples)

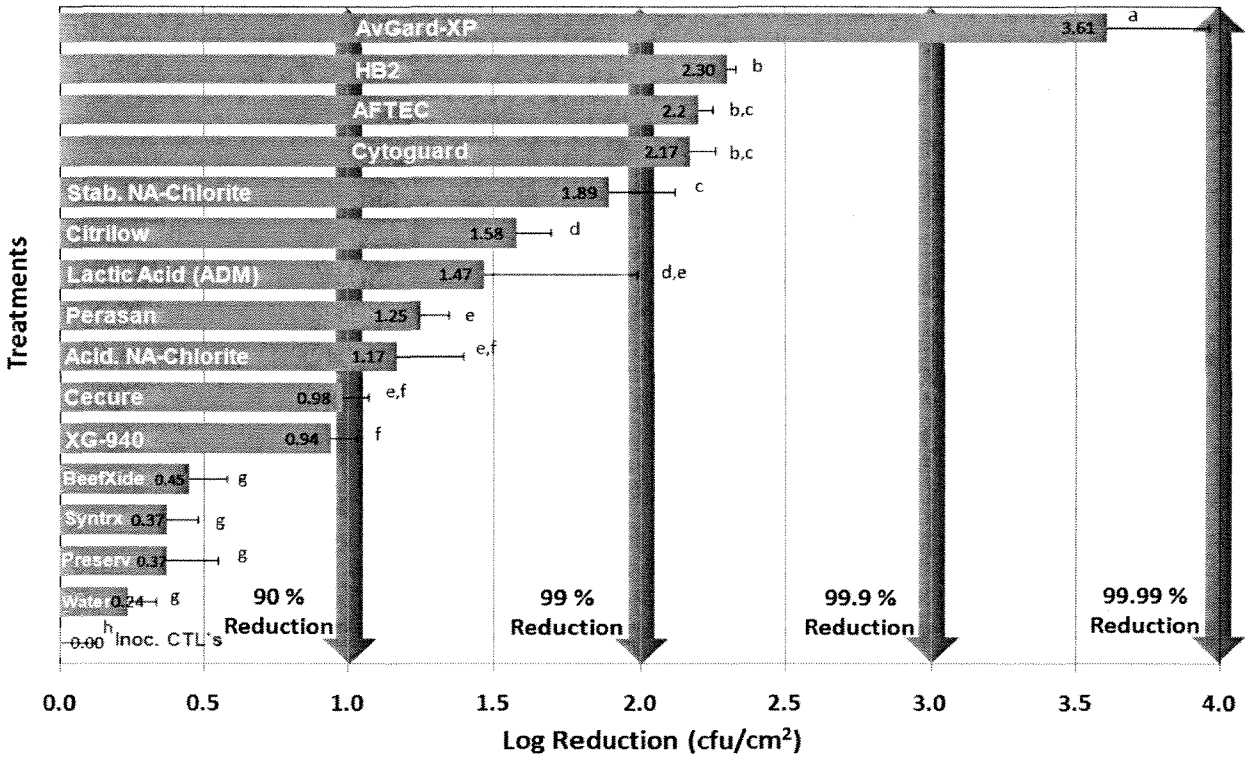
List of Antimicrobials Used in Phase 1			
Trade Name	**Active(s)**	**pH**	**Application Strength**
1. AvGard-XP	Disodium Metasilicate	13.1	60,000 ppm SMS
2. HB2	Hydrobromic Acid	7.5	300 ppm Br
3. Cecure	Cetylpyridinium Chloride	7.0	4,000 ppm
4. Preserv	Copper Sulfate Pentahydrate	6.8	*30% dilution of concentrate
5. Stabilized Na Chlorite	Na Chlorite/Citric Acid/Na Hydroxide	6.5	*<1%, <1%, <1% each
6. XG-940	Acidified Sodium Chlorite	6.5	200 ppm
7. Perasan MP2	Peroxyacetic Acid	3.2	220 ppm
8. Cytoguard PLUS	Lauric Arginate & Peroxyacetic Acid	3.0	5,000 ppm LAE; 220 ppm PAA
9. Acidified Na Chlorite	Na Chlorite acidified with Citric Acid	2.7	1,100 ppm
10. BeefXide	Lactic and Citric Acids	2.1	*2.4% dilution of concentrate
11. Lactic Acid	Hydroxypropanoic Acid	1.9	50,000 ppm LA
12. Syntrx 3300	HCl and Citric Acids	1.2	*3% dilution of concentrate
13. AFTEC 3000	Buffered Sulfuric Acid	1.0	17,500 ppm
14. Citrilow	HCl and Citric Acids	0.8	*18% dilution of concentrate

***Note:** For proprietary reasons the actual concentrations have not been disclosed; the 'application strength' listed is the dilution level of the concentrate provided by the manufacturer or approximate level of active.

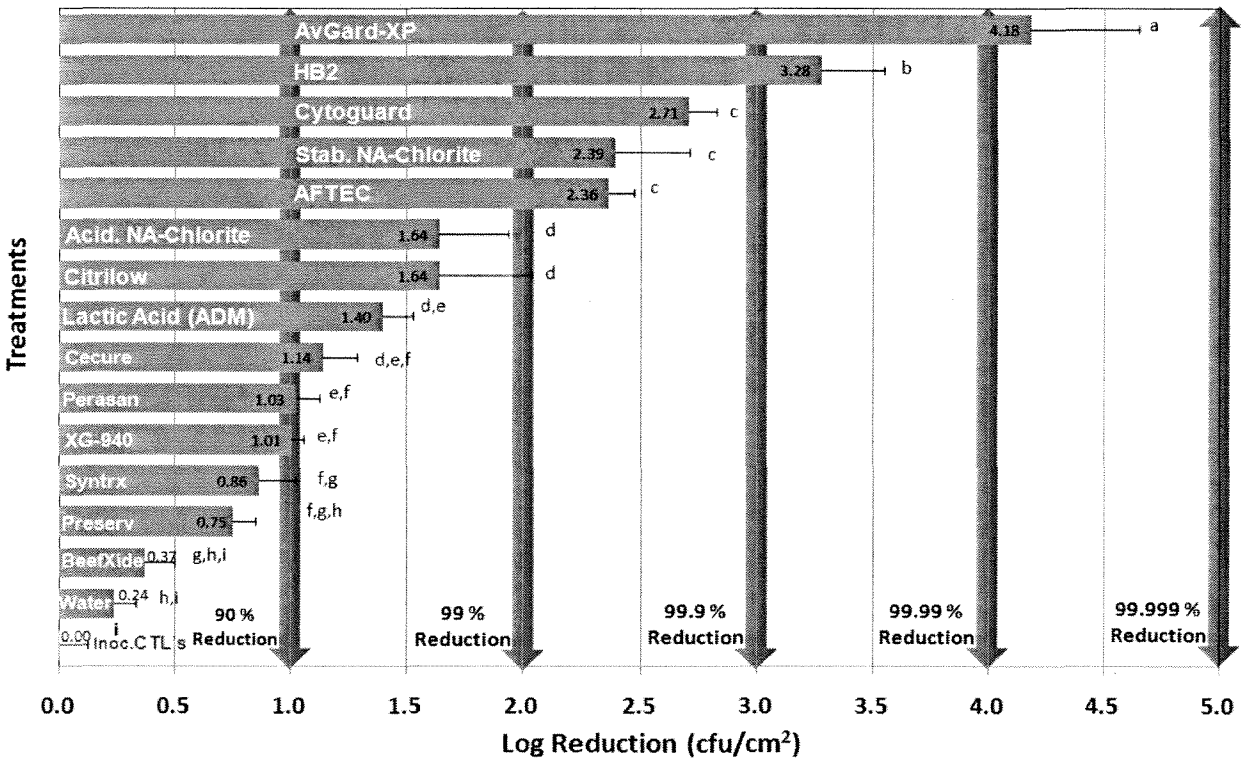

Reduction of E. coli O157:H7 (1 hr) on Lean Beef Wafers Sprayed with Various Antimicrobials in a Ross Industries Tenderizer-Spray System
Treatment
BeefXide 1.46 a
AFTEC 1.19 b
Cytoguard 1.14 b
Citrilow 1.02 b
AvGard-XP 1.0 b
Lactic Acid (ADM) 0.67 c
XG-940 0.65 c,d
Stabilized NA-Chlorite 0.54 c,d
Perasan 0.51 c,d,e
Acidified NA-Chlorite 0.50 c,d,e
Syntrx 3300 0.43 d,e,f
HB2 0.34 d,e,f
Cecure 0.30 e,f,g
0.09 Preserv g,h
Water 0.24 f,g
0.00 Inoc. CTL's h
90 % Reduction
99 % Reduction
0.0 0.2 0.4 0.6 0.8 1.0 1.2 1.4 1.6 1.8 2.0
Log Reduction (cfu/cm2)


Reduction of E. coli O157:H7 (1 Day) on Lean Beef Wafers Sprayed with Various Antimicrobials in a Ross Industries Tenderizer-Spray System
Treatments
AvGard-XP 2.08 a
AFTEC 1.99 a
Cytoguard 1.92 a
Citrilow 1.54 b
HB2 1.36 b
Lactic Acid (ADM) 0.89 c
Cecure 0.76 c,d
XG-940 0.75 c,d
Acid. NA-Chlorite 0.71 c,d
Stab. NA-Chlorite 0.69 c,d
Perasan 0.65 d
BeefXide 0.50 d,e
Syntrx 0.44 d,e
Preserv 0.41 d,e
Water 0.24 e
0.00 Inoc. CTL's f
90 % Reduction
99 % Reduction
99.9 % Reduction
0.0 0.5 1.0 1.5 2.0 2.5 3.0
Log Reduction (cfu/cm2)

Reduction of *E. coli* O157:H7 (7 Day) on Lean Beef Wafers Sprayed with Various Antimicrobials in a Ross Industries Tenderizer-Spray System



Reduction of *E. coli* O157:H7 (14 Day) on Lean Beef Wafers Sprayed with Various Antimicrobials in a Ross Industries Tenderizer-Spray System



Discussion. The data obtained for the various antimicrobials demonstrated differences in microbial reduction of *E. coli* O157:H7 for both short-term and longer-term sampling intervals. Although the short term intervals may be more relevant to blade tenderization, the longer-term intervals could provide information for applications whereby products may be sprayed, packaged, and then delivered to final use sources (i.e., the reduction is obtained during transportation/storage before use). It is also not clear if the longer-term intervals may apply to *E. coli* O157:H7 that could become translocated into blade channels in beef along with the respective antimicrobial, thereby eliciting a reduction over time *internally.* In hindsight, it was also apparent that antimicrobials (some which were similar to others, and some that were not), were applied at respective use levels specified by each manufacturer as opposed to targeting a specific concentration (and not all concentrations in the supplied solutions were openly identified). So in that respect, comparisons can only be made for the use level specified (by the manufacturers), some concentrations were not identified due to 'proprietary reasons' and likely resulted in different concentrations of similar agents provided by the different suppliers. In one instance, two similar actives were tested from different suppliers: Acidified Sodium Chlorite (+citric acid; 1,100 ppm) gave slightly better results than XG-940 (200 ppm acidified sodium chlorite) during the longer hold time, which can be attributed to the higher concentration of ASC used and possibly because of the citric acid in the blend used to acidify the product. Additional studies with any respective supplier may be needed to investigate enhancement of reduction levels different from those obtained in this study, since the nature of this study was simply a one-off testing and not an optimization for any given antimicrobial. Initially, I (Dr. Muriana) was under the assumption that we were only going forward with the best 2-3 antimicrobials into Phase 2, but subsequently that was broadened at the suggestion of Dr. Morgan/Wayne Spillner into the best 7 (of the 14) from the 7-day data that would proceed forward into Phase 2. This would give a larger evaluation for half of the antimicrobials examined in Phase 1 and accommodate more sponsors than was originally intended for Phase 2.

Phase 1 determined the efficacy of reduction of *E. coli* O157:H7 on the surface of lean beef discs as would occur if the *E. coli* were on the surface of beef subprimals. The reasoning is that reduction of the surface bacteria by the antimicrobial(s) reduces the chances for translocation (internalization) during blade tenderization. However, solution strengths that can be used commercially are limited by federally-approved limits for specific compounds (i.e., FDA-approved for foods based on safety, and USDA-FSIS approved for meat and poultry products based on efficacy) as well as costs, but spray dosage levels can be modified. Another factor that can play an important role to enhance the effect of antimicrobials is the solution temperature that is applied. In our study, we examined solutions applied at room temperature to comply with the least complicated, and likely, the most prevalent commercial situation. However, solutions applied at warmer temperatures may provide better access of the antimicrobials to the surface bacteria (bacteria may be protected by fatty film on the surface of meats at room temperature) and/or enhance their lethality by short-term temperature-enhanced inhibition. Other approaches that are yet to be examined include combinations of antimicrobials, although some of which have already been included in this study, such as Cytoguard PLUS (lauric arginate + peroxyacetic acid) which outperformed peroxyacetic acid alone in this study. There is still the possibility that those antimicrobials that were not chosen for Phase 2, could still perform sufficiently well for commercial applications given attention to details that were not examined in this study: different concentration, application temperature, and/or possible synergistic effects when applied in combination with other antimicrobials that have different modes of action.

Protocols & Results: Phase 2.

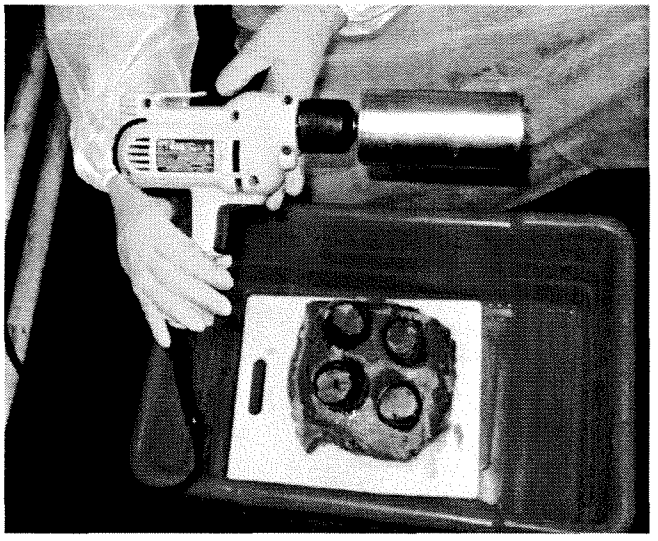
Cultures. The *E. coli* O157:H7 cultures were the same as those used in Phase 1 and were handled similarly (freshly grown for the morning of use, washed, inoculated and allowed 30-min attachment time before proceeding with treatments).

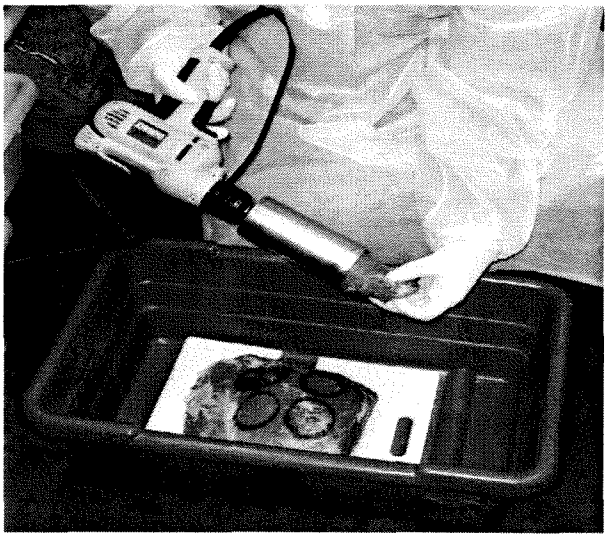
Beef subprimals. Beef subprimals were obtained fresh from a local processor, the day prior to use and the cap was removed for Phase 2 so that a contiguous intact core could be obtained.

Inoculation of subprimals. Beef samples were inoculated by marking a circle on the surface of the beef suprimals with an imprint using edible ink that was a smaller diameter than our 2-inch coring device. After inoculation (~ 1.0×10^4 cfu/cm^2), samples were allowed a 30-min attachment time. Several regimens were utilized for each antimicrobial solution:

a) Inoculation, water spray (no blade treatment), and core removal,
b) Inoculation, water spray, blade tenderization, and core removal,
c) Inoculation, antimicrobial spray, blade tenderization, and core removal.

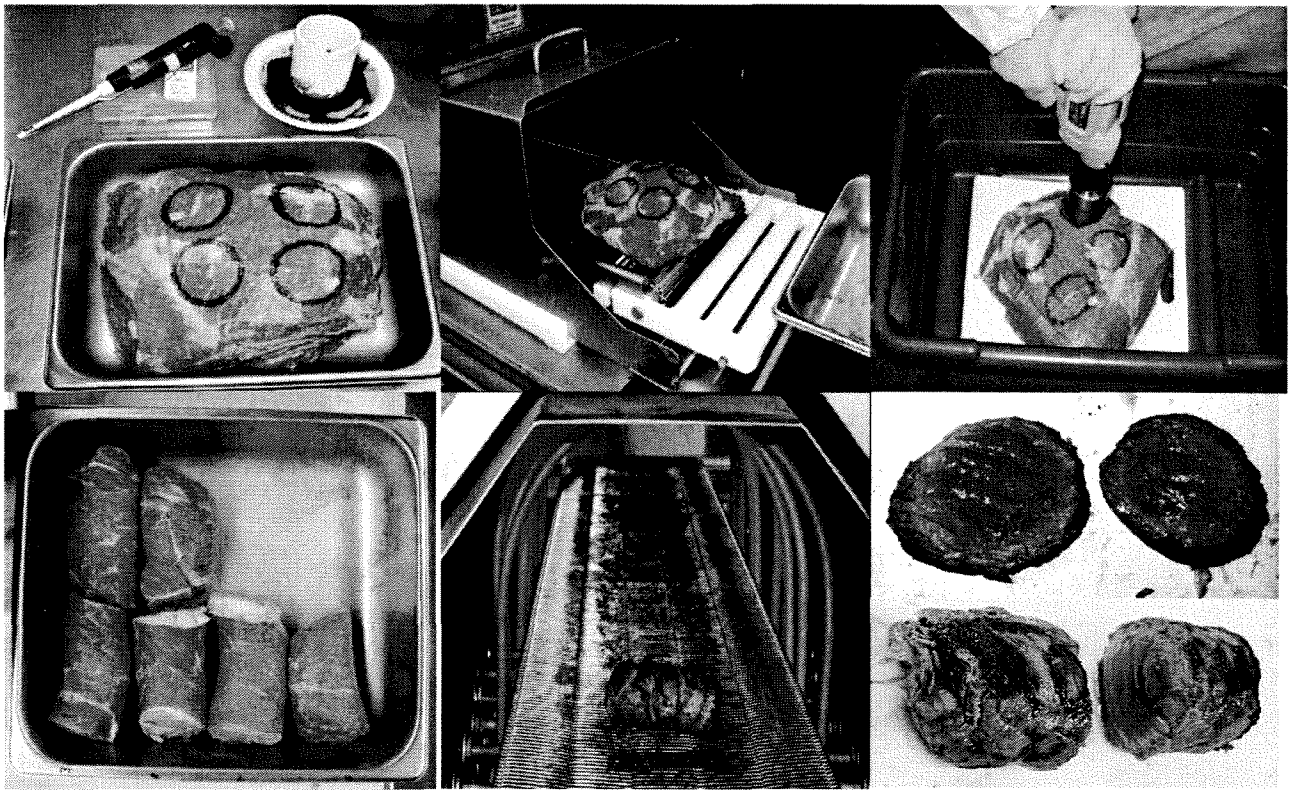
Phase 2 Antimicrobials			
Trade Name	**Active Ingredient(s)**	**Application Strength**	**pH**
AvGard-XP	Disodium Metasilicate	60,000 ppm SMS	13.1
HB2	Hydrobromic Acid	300 ppm Br	7.5
Stabilized Na Chlorite	Na Chlorite/ Citric Acid/Na Hydroxide	*<1%, <1%, <1% each	6.5
Cytoguard PLUS	Lauric Arginate & Peroxyacetic Acid	5,000 ppm LAE; 220 ppm PAA	3.0
Lactic Acid (FCC 88%)	Hydroxypropanoic Acid	50,000 ppm	1.9
AFTEC 3000	Buffered Sulfuric Acid	17,500 ppm	1.0
Citrilow	Hydrochloric & Citric Acids	*18% dilution of concentrate	0.8
***Note:** For proprietary reasons the actual concentrations have not been disclosed; the 'application strength' listed is the dilution level of the concentrate provided by the manufacturer or approximate level of active.			



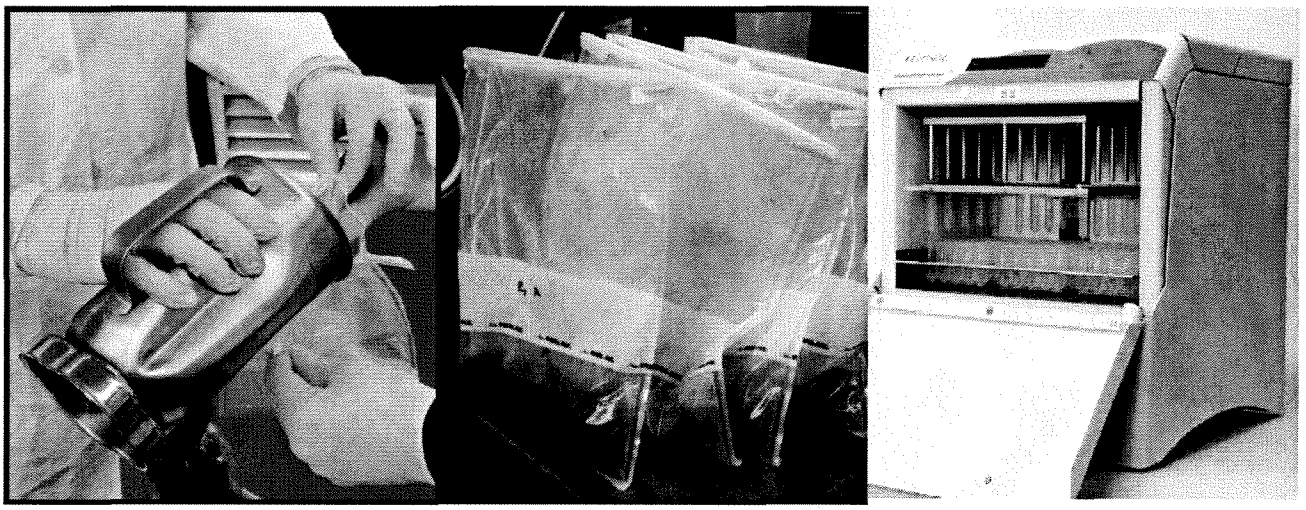


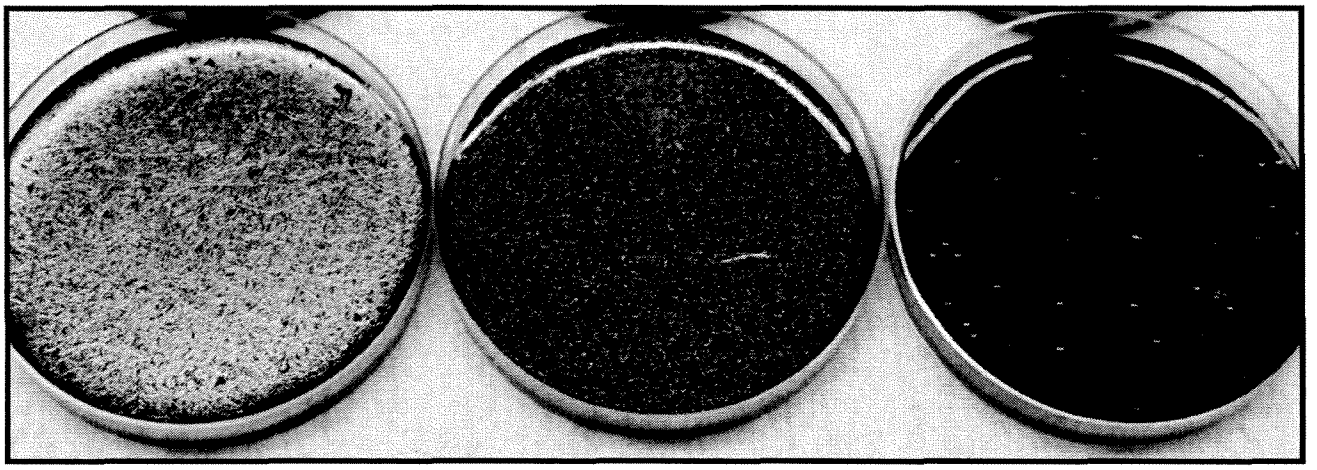
Drill and 2-inch diameter circular drill bit used to excise core samples from inoculated, sprayed, and blade-tenderized subprimals.

Core sample recovery. After exiting the Ross Integrated Tenderizer, beef cores were obtained using a 2-inch circular drill bit along the inoculated surface areas. In order to eliminate confusion of the source of the microbes after blending and plating (i.e., whether they were translocated internally or were from the core-surface contamination), we took several steps to eliminate non-translocated contaminants from the core surface. The ¼-inch of the inoculated surface of the cores was cut off after coring.

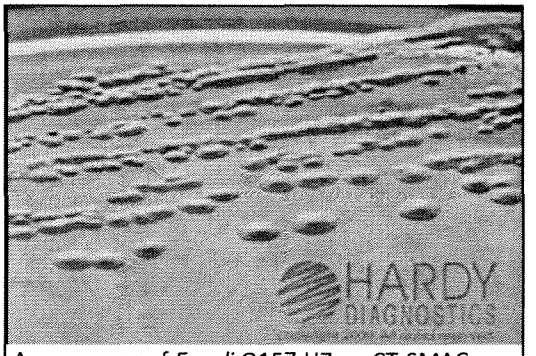


To further eliminate surface contamination from recovered beef cores we used a radiant-heat oven (i.e., IR Grill) to surface heat all post-treatment beef cores before sectioning and blending. Dr. Muriana has used IR Grill heating as a pre-package antimicrobial intervention for RTE meats that is accepted by USDA-FSIS as a post-process antimicrobial intervention. We used this same process to eliminate incidental contamination on the surface of the beef cores prior to sectioning, blending, and enrichment.





After careful aseptic sectioning (1-inch segments), individual core sections were blended in a minimal volume (2:1) of enrichment broth and incubated for 1 day at 30°C. After incubation, 1-ml of the blended samples was then extracted with *E. coli* O157:H7-specific immunomagnetic beads (i.e., magnetic beads coated with antibodies specifically to *E. coli* O157:H7). This allowed selective recovery by use of immunomagnetic beads that was facilitated by an automated 'Bead Retriever'. The entire amount of recovered magnetic beads after extraction of the 1-ml enrichment sample was plated onto EMB agar containing Rifamycin (to which our inoculated strains were resistant). This provided selective recovery by antibodies (beads), selective recovery by antibiotic resistance (plates), and differential detection (green sheen of colonies) of *E. coli* O157:H7 from EMB-Rif agar for our positive samples. Negative samples did not have the green sheen or did not have any colonies.

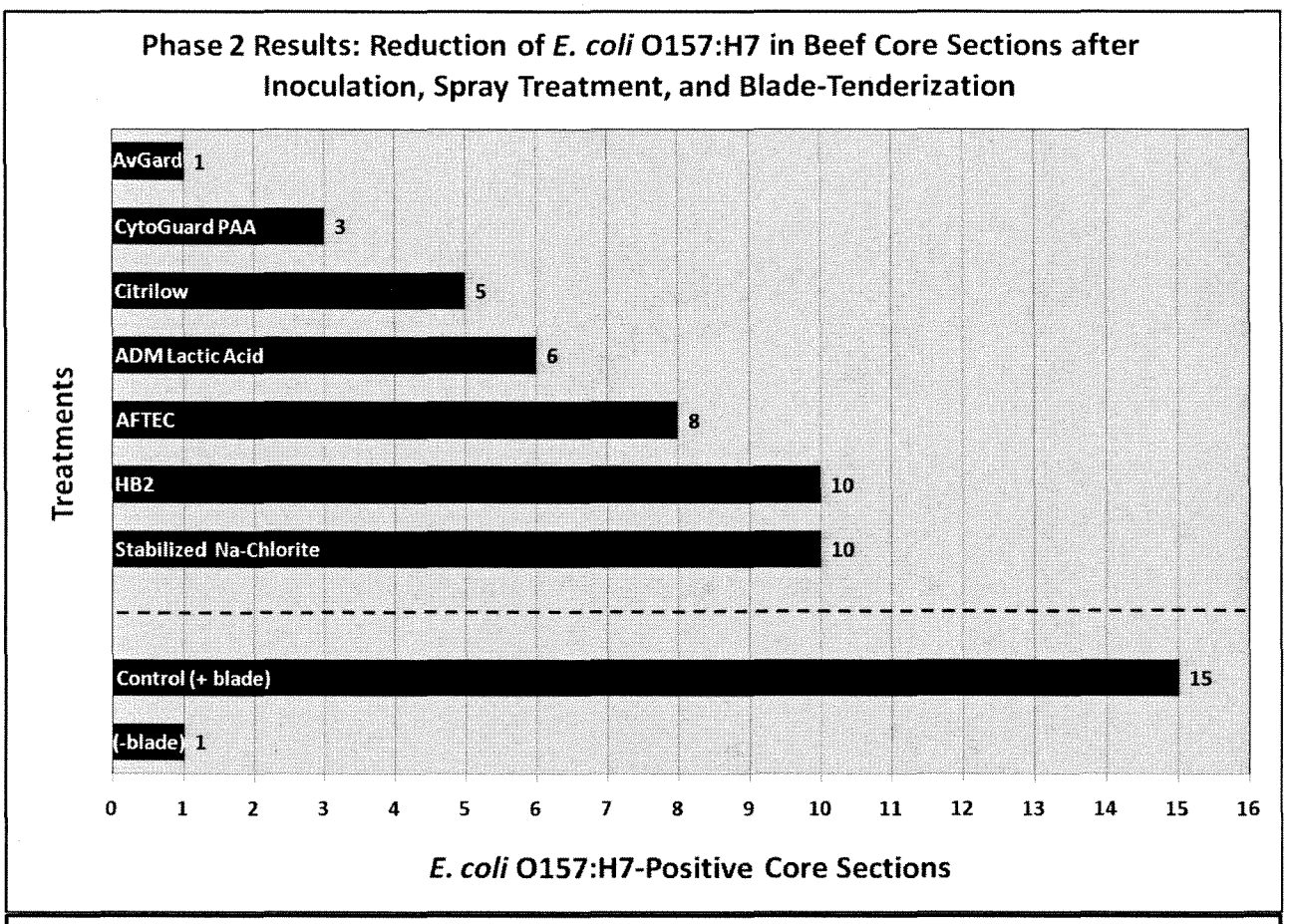


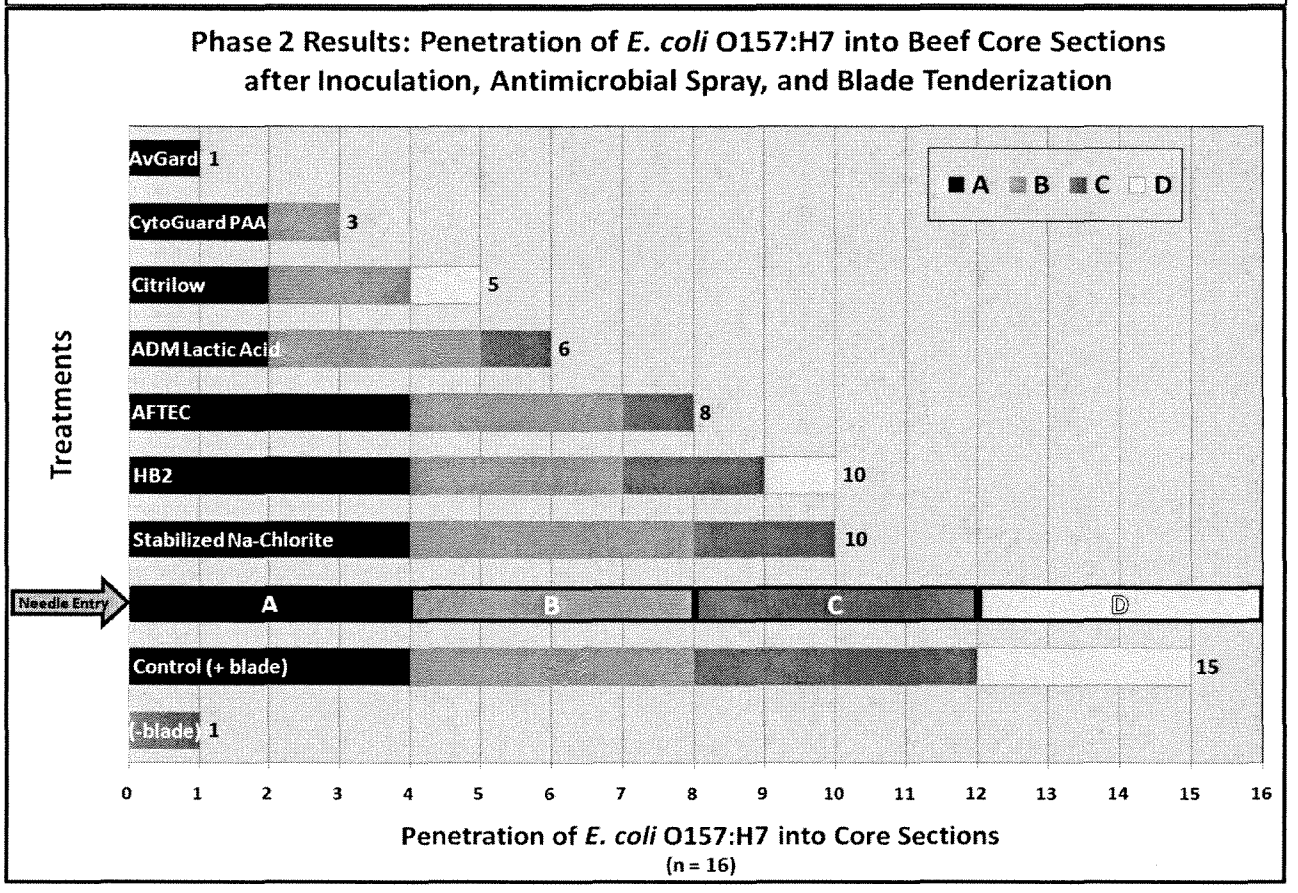
Appearance of *E. coli* O157:H7 on CT-SMAC agar.

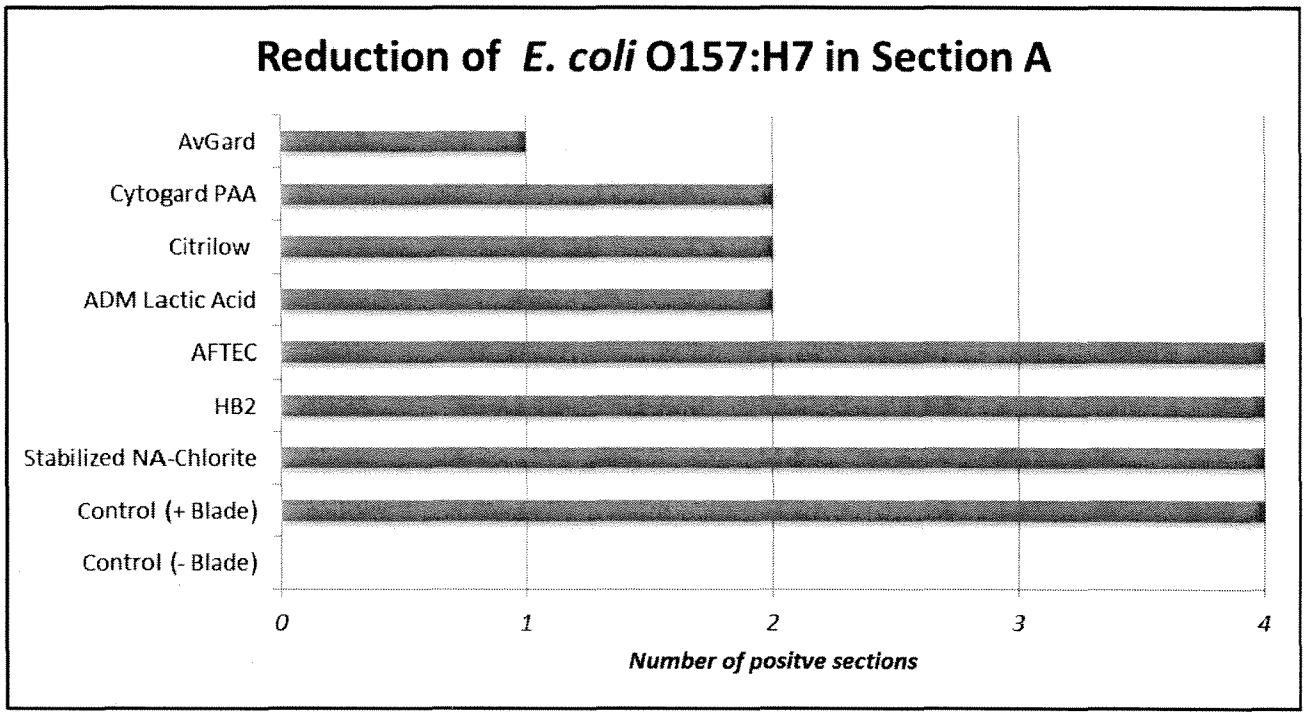
We first examined the possibility of using CT-SMAC (Sorbital-MacConkey Agar + cefixime & tellurite) as the selective media for *E. coli* O157:H7, however, the colonies were very pale and not distinctive, and after extended incubation changed color a bit. Since this part of our process was not heavily dependent on the medium for selection from a diverse background flora as occurs during ground beef testing (i.e., the broth enrichment followed by immunomagnetic bead enrichment gave us overhwelming levels of our *E. coli* O157:H7 recovered), we decided to use EMB medium containing one of the antibiotics for which the strains were resistant to as a positive visual score for Rifamycin-resistant *E. coli*. All of our EMB-Rifamycin plates for Phase 2 had in excess of 1,000 colonies or higher and the 'green sheen' was an easy visual score for presence after the double enrichment (broth medium & immunomagnetic bead recovery using 1-ml of enrichment broth, wash, and plating).

For each antimicrobial tested, we obtained 4 core samples, each sectioned into 4 sections. Results were tabulated as total sections positive per core as well as sections positive for each depth penetration that the sections represented (1-, 2-, 3-, 4-inches).

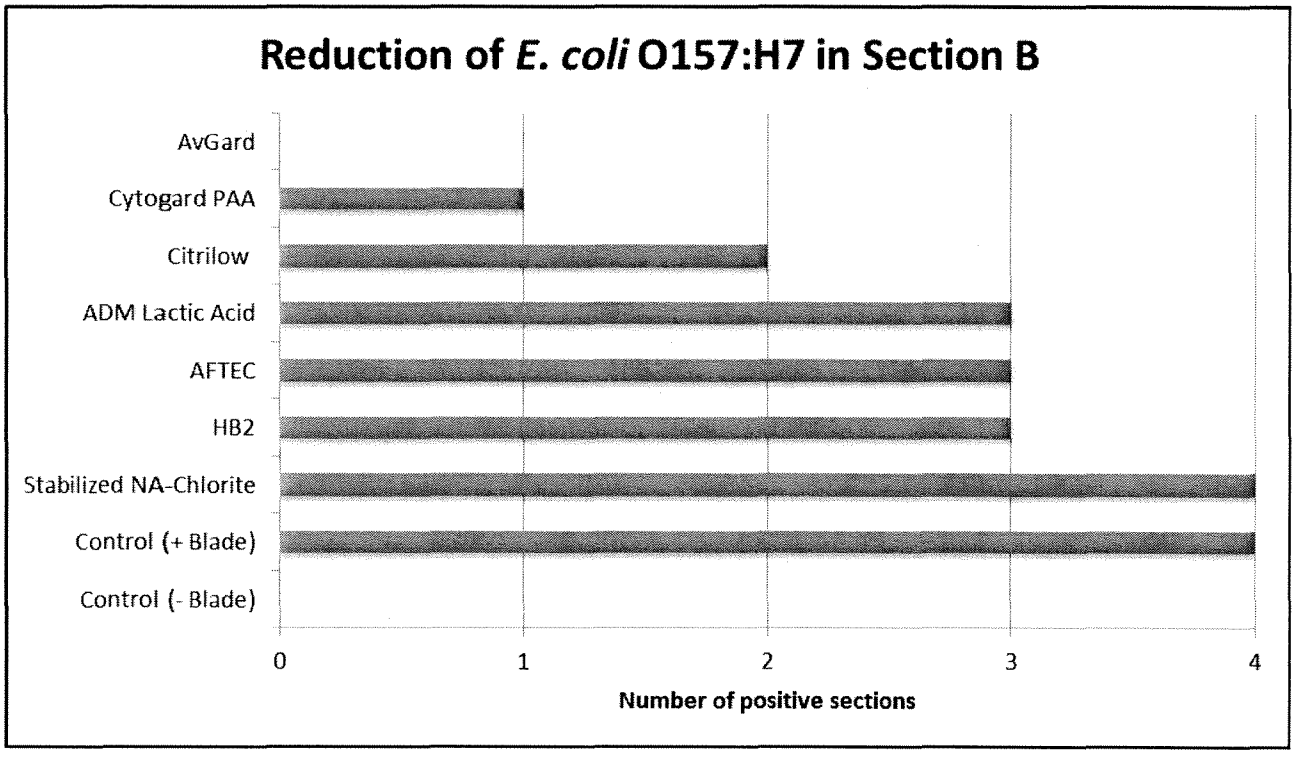
Discussion. The data obtained shows that all antimicrobial interventions reduced the number of segments containing *E. coli* O157:H7 relative to controls (top graph). Some interventions were more efficacious then others (at the levels used). The antimicrobials showing lower efficacy could likely be improved with further testing and tweaking, however this study did not provide that opportunity. As we look as the *E. coli* O157-H7-positive samples within each depth interval, it appears that as antimicrobials were more effective in reducing the number of sections that were positive, the sections that were being eliminated were those that were deeper. For instance, *E.* coli-positive samples were only recovered from the top-most 1-inch of penetration with AvGard which demonstrated the greatest efficacy. As efficacy increased, *E. coli* O157 was eliminated from the deeper depths in meat samples.


Phase 2 Results: Reduction of E. coli O157:H7 in Beef Core Sections after Inoculation, Spray Treatment, and Blade-Tenderization
Treatments
AvGard 1
CytoGuard PAA 3
Citrilow 5
ADM Lactic Acid 6
AFTEC 8
HB2 10
Stabilized Na-Chlorite 10
Control (+ blade) 15
(-blade) 1
0 1 2 3 4 5 6 7 8 9 10 11 12 13 14 15 16
E. coli O157:H7-Positive Core Sections

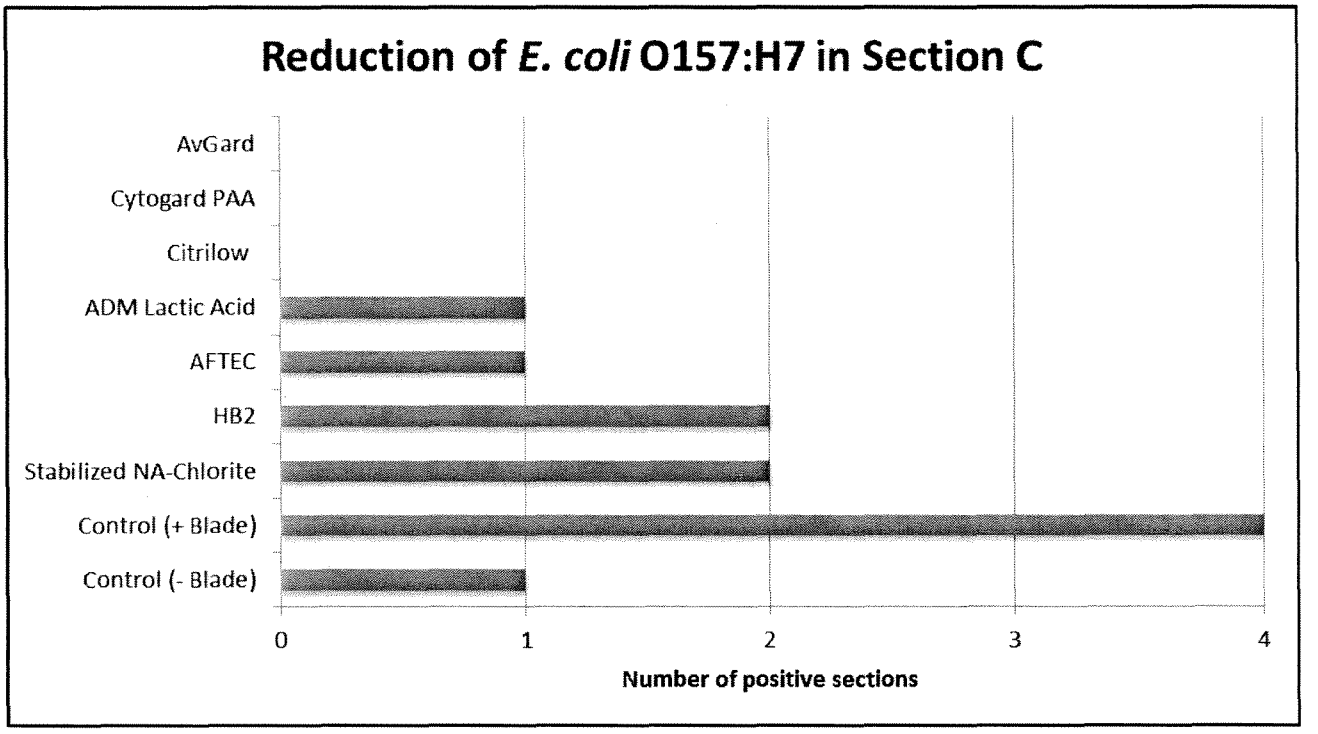

Phase 2 Results: Penetration of E. coli O157:H7 into Beef Core Sections after Inoculation, Antimicrobial Spray, and Blade Tenderization
A B C D
Treatments
AvGard 1
CytoGuard PAA 3
Citrilow 5
ADM Lactic Acid 6
AFTEC 8
HB2 10
Stabilized Na-Chlorite 10
Needle Entry
A B C D
Control (+ blade) 15
(-blade) 1
0 1 2 3 4 5 6 7 8 9 10 11 12 13 14 15 16
Penetration of E. coli O157:H7 into Core Sections
(n = 16)



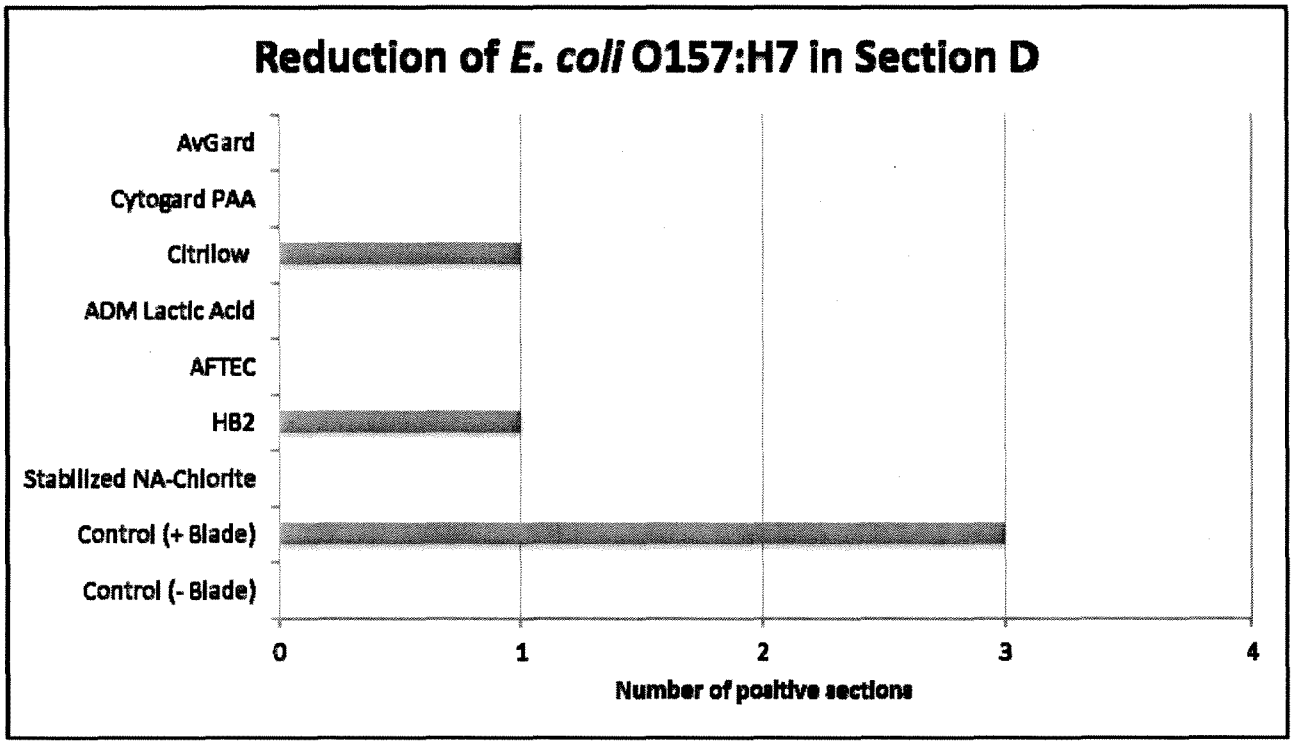
Section A. Recovery of *E. coli* O157:H7 from the top-most 1-inch inoculated side of the beef core. *E. coli* O157:H7 was recovered from all 4 control (+blade) sections, AFTEC, HB2, and Stabilized Na-Chlorite; from only 2 sections treated with Cytoguard PLUS, Citrilow, and Lactic Acid; and from only 1 section treated with AvGard-XP.



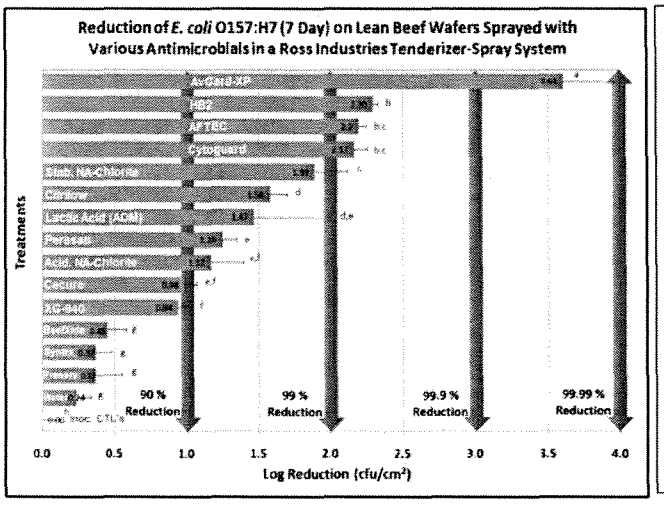
Section B. Recovery of *E. coli* O157:H7 from the second 1-inch layer of beef cores. *E. coli* O157:H7 was recovered from all 4 control (+blade) sections and Stabilized Na-Chlorite; from only 3 sections treated with Lactic Acid, AFTEC, and HB2; from 2 sections treated with Citrilow; and from only 1 section treated with Cytoguard PLUS, *E. coli* O157:H7 was not recovered from sections at this level when treated with AvGard-XP.

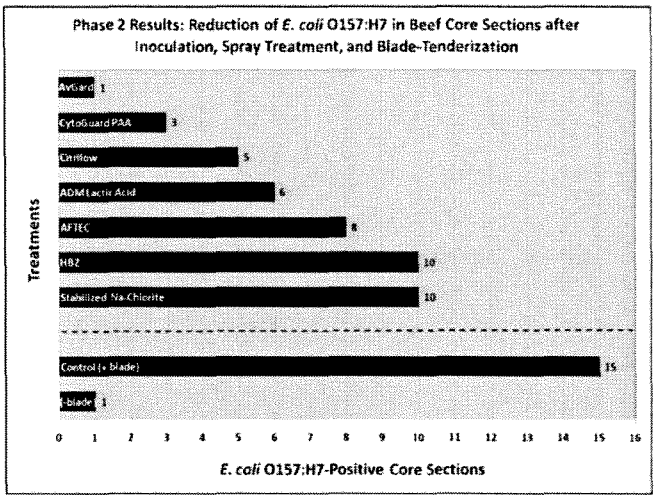


Section C. Recovery of *E. coli* O157:H7 from the 3-inch layer of beef cores. *E. coli* O157:H7 was recovered from all 4 control (+blade) sections but from only 2 sections when treated with Stabilized Na-Chlorite or HB2; from only 1 section when treated with Lactic Acid or AFTEC; *E. coli* O157:H7 was not recovered from this level when treated with Citrilow, Cytoguard PLUS, or AvGard-XP. *E. coli* O157:H7 recovered from 1 non-bladed control section, presumably due to external contamination.



Section D. Recovery of *E. coli* O157:H7 from the bottom (4-inch) layer of beef cores. *E. coli* O157:H7 was recovered from 3 of 4 control (+blade) sections and from only 1 section treated with Citrilow or HB2; no *E. coli* were recovered from this lowest section when treated with Stabilized Na-Chlorite, AFTEC, Lactic acid, Cytoguard PLUS, or AvGard-XP.





The two Phases of this study are somewhat different, yet are complimentary to each other.

Phase 1 deals with the application of an antimicrobial onto *E. coli* O157:H7 surface-inoculated beef discs and quantification of survivors to determine reduction of initial population on the surface.

Phase 2 also uses surface-inoculated beef discs that are sprayed with antimicrobials, but is different in that we perform blade tenderization and look for recovery of internalized *E. coli* which is proportional to the reduction of *E. coli* O157:H7 on the surface (i.e., the greater the reduction on the surface, the less likely you will recover it from internalized sections after blade tenderization). That is, you need a sufficiently high population on the surface to observe translocation to internal sections.

The data obtained in Phase 2 compliments that which was obtained in Phase 1 based on performance. For instance, AvGard-XP was shown in Phase 1 to have the greatest reduction of *E. coli* O157:H7 on lean beef discs (7-days) and similarly, in Phase 2 gave the fewest *E. coli* O157:H7-positive samples (only 1 of 16) in Phase 2 testing. This strongly confirms the efficacy of AvGard-XP as an antimicrobial in both types of testing. Phase 2 testing also complimented Phase 1 results with other antimicrobials. Cytoguard PLUS was close to 2nd (not significantly different) in Phase 1 and was 2nd in efficacy in Phase 2 (Cytoguard PLUS)

Conclusion: The use of antimicrobial spray interventions prior to blade tenderization (i.e., integral solution intervention) can reduce the population of *E. coli* O157:H7 to lower levels than those that occur prior to spray treatment, and therefore, can reduce the likelihood of translocation to beef internal sections concomitant with lower surface populations. The novelty of antimicrobial intervention as demonstrated herein, may be applied immediately prior to blade tenderization (Phase 2 data) or perhaps even further upstream in the sourcing process as our Phase 1 data demonstrated antimicrobial efficacy at 1 & 2 week holding time.

Additional Research: The data obtained for Phase 2 is certainly positive, but is limited in the amount of data generated for any one antimicrobial. We would need to establish greater degree of confidence by providing more detailed examination of which ever supplier would like to pursue additional, in-depth testing with their antimicrobial(s), including possible synergism with oxidative water solutions, different temperature of application, and/or different concentrations.

Attachment 8

Study title: Antimicrobial treatment of beef trimmings for control of *Escherichia coli* O157:H7 and *Salmonella spp.* using a Sulfuric Acid/Sodium Sulfate blend (Aftec)

Conducted by: James L. Marsden, Ph.D., Regent's Distinguished Professor—Food Safety & Security, Kansas State University

Study dates: October 2009

Objective: to establish the efficacy of treatment using Aftec on beef trimmings for control of *E. coli* O157:H7 and *Salmonella*. Also, evaluate effect on shelf life, color, and residues.

Methodology: Beef trimmings inoculated with a 5-strain cocktail of *E. coli O157:H7 or Salmonella* spp. were sprayed with a solution of *Aftec* adjusted to a pH of 1.3-1.5 for periods of 0, 10, 20 and 30 seconds. The target surface inoculation was 7.0 Log CFU/cm^2 . After each treatment, the trimmings were tested to determine reductions of each pathogen tested. Three replications were conducted. For sensory evaluation, ground beef manufactured using beef trimmings treated with Aftec at 10, 20, and 30 second time intervals were compared to a control in triangle tests using a trained sensory panel. Chemical tests were conducted for residuals.

Results and discussion: The 10 second treatments for pathogens *E. coli* O157:H7 and *Salmonella* spp. showed 0.7 and 1.1 Log CFU/cm^2 reductions, respectively. The highest lethality was achieved with the 30 second treatments for both pathogens, *E. coli* O157:H7 and *Salmonella* spp., which showed 1.5 and 1.6 Log CFU/cm^2 reductions, respectively.

The study conducted a sensory evaluation comparing a control to ground beef manufactured using beef trimmings treated with AFTEC at 10, 20, and 30 second time intervals in triangle tests using a trained sensory panel. No differences between the treated samples and the control were reported.

The study also examined the shelf life of treated products. Samples were evaluated daily for visual color and microbiological testing for total aerobic plate count. No differences in color stability were observed between treated samples versus the control. No significant differences in aerobic plate counts were reported between treated and control ground beef samples. Finally, no statistical differences in residual levels of sodium sulfate or sulfuric acid were reported in ground beef treated with the Aftec solution versus control samples. This would support the categorization of the treatments using Aftec as a processing aid.

Antimicrobial treatment of beef trimmings for control of *Escherichia coli* O157:H7 and *Salmonella* spp. using a Sulfuric Acid/Sodium Sulfate blend (*Aftec*)

Final Report

October 13, 2009

Submitted to:
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Introduction:

The control of *E. coli* O157:H7 and *Salmonella* in beef trimmings is essential for the production of safe ground beef products. An integrated food safety system for ground beef involves the application of control measures during the slaughter process and at other critical points in the process. An effective intervention applied to beef trimmings prior to grinding would provide an important reduction in risk. The evaluated chemistry in this study is a Sulfuric Acid/Sodium Sulfate blend adjusted to a pH of 1.3-1.5. The brand name for this product is *Aftec* and it is manufactured by Advanced Food Technologies, LLC. The ingredients are FDA GRAS and use of product as an acidifier has already been approved by USDA FSIS for meat and poultry applications. The purpose of this study was to establish the efficacy of treatment using *Aftec* on beef trimmings for control of *E. coli* O157:H7 and *Salmonella.*

An advantage of this approach is the ease of application and availability to small processors. The make-up of the antimicrobial solution and application can be conducted in operations of any size. Since the anti-microbial effect is not due to oxidation, it can be easily applied without adversely affecting the quality of the trimmings or ground beef manufactured from treated trimmings.

This study involved a spray application of *Aftec* at various durations on beef trimmings. Future studies will evaluate the efficacy of dipping applications and other methods of applying the product in various meat processing steps.

This study was designed to measure the effect of the treatment on ground beef shelf life, color, sensory characteristics and residues in order to support a request to USDA's Food Safety and Inspection Service that treatment of beef carcasses, beef subprimals and beef trimmings using Aftec would be considered as an anti-microbial use.

The beef industry and FSIS share the objective of reducing the risk of E. coli 0157:H7 and Salmonella in raw ground beef. The availability of effective interventions that may be applied at appropriate processing steps is essential to meeting that objective.

Materials and Methods

Bacterial Culture Preparation: The following strains from the Kansas State University culture collection were used to prepare the inoculum:

- *Escherichia coli* O157:H7: ATCC 43890 and ATCC 43889, obtained from Jackie Staats at KSU Veterinary School; USDA-FSIS 380-94, KSU 01, CDC (Patient outbreak), and KSU 03, CDC (Meat outbreak).
- *Salmonella* spp.: *Salmonella choleraesuis* subsp. *cholerasuis (S. enteriditis) (ATCC 4931,* and USDA-FSIS 15060*), S. seftenburg* subsp. *cholerasuis* (ATCC 43485), *S. newport* (Dr. Phebus, KSU), and *S. montevideo* (Dr. L. Beuchat, UGA).

To prepare the inoculum, stock cultures were cultivated by placing one impregnated bead into a 5 ml solution of Difco® Tryptic Soy Broth (TSB) and incubating for 24 h at 35°C. Next, a 0.05 ml loop of the respective culture was inoculated into a 10 ml solution of TSB and incubated for 24 h at 35°C. All five samples from each culture were mixed together to create a 50 ml cocktail containing 10^9 to 10^{10}CFU/ml of *E. coli* O157:H7 or *Salmonella* spp. The cell density of this suspensions was determined by plating appropriate dilutions on MSA (MacConkey Sorbitol Agar, Difco, Detroit, MI) for *E. coli* O157:H7 and XLD (Xylose Lysine Desoxycholate Agar, Difco, Detroit, MI) for *Salmonella* spp., and placed in the incubator for 48 hours at 35°C. Cultures were confirmed by cultivation on selective and differential media, and biochemical analysis of presumptive colonies using API 20E kits.

Sample Preparation: Beef trimmings were obtained from the KSU meat laboratory and cut into app. one inch square pieces. The trimmings were held at 40°F prior to treatment. Selected trimmings were inoculated with *Escherichia coli* O157:H7 or *Salmonella* spp. inside a "bio-containment" chamber by "misting" the surface of the meat with approximately 10 ml of the inoculum. This was done ensuring that all sides of each piece of meat received the same exposure to the inoculum. Samples were held for 30 min at room temperature to allow proper bacterial attachment to the surface of the meat. Immediately prior to treatment applications, the surfaces of the inoculated products were sampled and analyzed to establish the actual inoculum level of the attached organisms.

Application of Treatment: Beef trimmings inoculated with a 5-strain cocktail of *E. coli O157:H7 or Salmonella* spp. were sprayed with a solution of *Aftec* adjusted to a pH of 1.3-1.5 for periods of 0, 10 20 and 30 seconds. The target surface inoculation was 7.0 Log CFU/cm^2. After each treatment, the beef trimmings were tested to determine reductions of each pathogen tested. Three replications were conducted for each treatment.

Sampling Method: Individual pieces of beef trimmings were placed into a stomacher bag. The tissue samples were diluted with 90 ml of 0.1% sterile peptone water (PW) and homogenized in a stomacher for one minute. Samples were serially diluted in sterile PW and plated onto corresponding media for each pathogen tested. The plates were incubated at 37 °C for 48 hrs. The colony forming units were enumerated and calculated as the difference in log recovery.

Sensory Evaluation: Ground beef manufactured using beef trimmings treated with Aftec at 10, 20, and 30 second time intervals were compared to a control in triangle tests using a trained sensory panel. No differences between the treated samples and the control were reported.

Shelf Life Determination: Ground beef manufactured using beef trimmings treated with Aftec at 0 (control), 10, 20, and 30 second time intervals were packaged in overwrap oxygen permeable packages and placed in a commercial display case for a period of 5 days. Samples were evaluated daily for visual color and microbiological testing for total aerobic plate count. No differences in color stability were observed between treated samples versus the control. The total aerobic plate counts are listed in Table 3. No significant differences in APC's were reported between treated and control ground beef samples.

Results and Discussion:

Results from this study can be found in Tables 1, 2 and 3. Log CFU/cm^2 reductions were calculated as the difference in log recoveries from the inoculated products prior to treatment and the log recovery after treatment.

Table 1. Average recoveries (Log CFU/cm^2) of *Salmonella* spp and *E. coli* O157:H7 in boneless beef trimmings treated with a solution of *Aftec* Sulfuric Acid and Sodium Sulfate blend (pH 1.3-1.5) for periods of 0, 10, 20 and 30 seconds.

Sample	*Salmonella*	*E. coli* O157:H7
Control (0 Seconds)	**6.9**	**6.7**
10 Seconds	**5.8**	**6.0**
20 Seconds	**5.5**	**5.6**
30 Seconds	**5.3**	**5.2**

Table 2 shows the average reductions obtained from the boneless beef trimmings sprayed with a solution of *Aftec* Sulfuric Acid and Sodium Sulfate blend (pH 1.3-1.5) for periods of 0, 10, 20 and 30 seconds. The highest lethality was achieved with the 30 seconds treatments for both pathogens, *E. coli* O157:H7 and *Salmonella* spp., which showed 1.6 and 1.5 log cfu/cm2 reductions, respectively.

Table 2. Average reductions (Log CFU/cm^2) of *Salmonella* spp and *E. coli* O157:H7 in boneless beef trimmings treated using *Aftec* Sulfuric Acid and Sodium Sulfate blend (pH 1.3-1.5) for periods of 10, 20 and 30 seconds.

Sample	*Salmonella* spp.	*E. coli* O157:H7
10 Seconds	**1.1**	**0.7**
20 Seconds	**1.4**	**1.1**
30 Seconds	**1.6**	**1.5**

Table 3. Aerobic Plate Counts during 5 days Shelf Life Storage in Ground Beef manufactured using beef trimmings treated using *Aftec* Sulfuric Acid and Sodium Sulfate blend (pH 1.3-1.5) for periods of 0 (control), 10, 20 and 30 seconds.

	Control	***10 Seconds***	***20 Seconds***	***30 Seconds***
Day 1	**1.9×10^3 cfu/gm**	**1.0×10^3 cfu/gm**	**1.3×10^3 cfu/gm**	**1.2×10^3 cfu/gm**
Day 2	**2.3×10^3 cfu/gm**	**1.8×10^3 cfu/gm**	**2.0×10^3 cfu/gm**	**2.1×10^3 cfu/gm**
Day 3	**3.1×10^3 cfu/gm**	**2.9×10^3 cfu/gm**	**3.0×10^3 cfu/gm**	**2.9×10^3 cfu/gm**
Day 4	**5.7×10^3 cfu/gm**	**5.1×10^3 cfu/gm**	**5.3×10^3 cfu/gm**	**5.3×10^3 cfu/gm**
Day 5	**5.9×10^3 cfu/gm**	**5.8×10^3 cfu/gm**	**5.5×10^3 cfu/gm**	**5.6×10^3 cfu/gm**

The results of this study demonstrate that the treatments using *Aftec* Sulfuric Acid and Sodium Sulfate blend (pH 1.3-1.5) at 10, 20 and 30 seconds were effective at reducing levels of *Salmonella* and *E. coli* O157:H7 on inoculated beef trimmings. The greatest reductions were achieved with the 30 second spray.

There was no long term residual effect on the color, shelf life, or microbiological quality of ground beef manufactured from the treated trimmings versus the control. In addition, no statistical differences in residual levels of sodium sulfate or sulfuric acid were reported in ground beef treated with the *Aftec* solution versus control samples. This would support the classification of *Aftec* as an anti-microbial and categorization of the treatments using Aftec as a processing aid.

SUBMISSION END