Recent Research on Antimicrobial Interventions and their Validation to Control STEC in Beef Products

Gary R. Acuff
The successful implementation of HACCP requires validation.

International Commission on Microbiological Specifications for Foods (ICMSF), 2011, Microorganisms in Foods 8
Validation

- Validation involves obtaining evidence that control measures, if properly implemented, are capable of controlling the identified hazards. (Codex)
Obtaining Evidence

▶ Multiple approaches:
  ▶ Predictive modeling
  ▶ Scientific literature
  ▶ Microbiological challenge studies
    ▶ Laboratory
    ▶ In-plant
  ▶ “Safe Harbors”
▶ Often combined for sufficient data
Validation
ICMSF, 2011, *Microorganisms in Foods 8*

- Validation generally begins with microbiological studies on a laboratory scale, progresses to a pilot plant scale and ends with full validation on a commercial scale when possible or necessary.
Ideal Approach

- Validate Process Control
  - Measure hazard (pathogen) presence
    - Pre- and post-CCP (enumeration)
    - No detection in final product
  - Requires sufficient levels of pathogen
Validation of Interventions

- Pathogen presence on carcass surface
  - Inconsistent
  - Low levels
  - Insufficient for confirmation of process control
- A measureable alternative?
  - Indicators
Indicator Organisms

- Validation studies (Enumeration)
  - Total Plate Counts
    - Varied and unpredictable microbiota
  - Enterobacteriaceae, coliforms, *E. coli*
    - Close correlation to salmonellae, STEC
    - Numbers high enough for validation?
    - Consistent populations?
A More Consistent Alternative

- **Surrogates**
  - Similar heat/acid resistance
    - Slightly more resistant preferred
    - Underpredict reduction or control
  - Similar growth characteristics
  - Not pathogenic
  - Easy to detect and enumerate
Surrogates

- Biotype I *E. coli* strains
  - Evaluate effectiveness of carcass interventions for control of STEC and *Salmonella*
- American Type Culture Collection (ATCC)
  - www.atcc.org
  - Biosafety Level 1
  - Accession numbers
    - BAA-1427 to BAA-1431
Surrogates

Before

After

Log\textsubscript{10} CFU/cm\textsuperscript{2}
“Predictors of Eating Raw or Undercooked Meat, Poultry, Seafood, and Eggs...”
Validation of Antimicrobial Interventions for Small and Very Small Processors: A How-to Guide to Develop and Conduct Validations

CONSORTIUM OF FOOD PROCESS VALIDATION EXPERTS (CFPVE) —

The CFPVE is a collaborative effort comprising experts from various universities and institutions. Members include:

- Colorado State University
- Iowa State University
- Kansas State University
- Michigan State University
- Oklahoma State University
- The University of Arizona
- The University of Illinois
- The University of Maryland
- The University of Missouri
- The University of Nebraska
- The University of Oklahoma
- The University of Tennessee
- The University of Wisconsin
- The University of Wyoming
- The University of New Mexico

SUMMARY

It is essential to ensure that antimicrobial interventions are utilized effectively to control pathogenic microorganisms and achieve the intended goal of preventing, reducing, or eliminating spoilage and losses associated with a defined food product. This approach is necessary to avoid the potential for ineffective food safety interventions or to ensure that the implemented food safety measures are effective in reducing the risk of foodborne illness. The appropriate food safety measures at different stages of food production must be carefully considered to ensure that the investment in food safety is justified and is not a burden to the food industry. Validation is an integral component of the HACCP system, or that these interventions correctly remove or reduce the hazards, current or potential, to an acceptable level. This manual provides a practical approach for developing validation protocols to evaluate the effectiveness of antimicrobial interventions, especially for small and very small processors.

Collaborative Food Process Validation

Address: 10501 W. 96th St., Lenexa, KS 66214 USA
Phone: 866-339-7678, Fax: 913-396-8200
Email: info@cfpve.org
Website: www.cfpve.org

Copyright © 2013 International Association for Food Protection
All rights reserved.
STEC CAP Interventions Team

- **University of Nebraska – Lincoln** (Harshavardhan Thippareddi, Emie Yiannaka, Dennis Burson, Jeyam Subbiah, Galen Erickson, Terry Klopfenstein)
- **Kansas State University** (Randy Phebus, Sean Fox, Glynn Tonsor, Ted Schroeder, Sara Gragg)
- **Texas A&M University** (Gary Acuff, Matt Taylor, Alejandro Castillo)
- **North Carolina State University** (Ben Chapman)
- **USDA-ARS ERRC** (John Luchansky, Anna Porto-Fett)
- **University of California, Davis** (Christine Bruhn, Jim Cullor)
- **Alabama A&M** (Armitra Jackson-Davis)
Hide-on Bob Veal Interventions

- Experimental Objectives
  - Quantify STEC surrogate reductions on hide-on veal carcasses using combinations of:
    - Scalding
    - 180°F water wash
    - 4.5% lactic acid spray
Dressed Bob Veal Antimicrobial Sprays

Experimental Objectives:

- Quantify *E. coli* reductions on veal carcasses using water, 4.5% Lactic Acid, Citrilow and Beefxide sprays
- Hot and chilled carcass applications
- Chilled carcass color impacts
Dressed Bob Veal Interventions

- 4.5% Lactic Acid (pH 2.0)
- Beefxide (pH 2.3) – blend of lactic and citric acid
- Citrilow (pH 1.2) – blend of HCl and citric acid
- Water Wash – 120-130°F
Lauric Arginate Validation Study

In-bag lauric arginate application (vacuum-packaged subprimals) followed by a post-chill per oxyacetic acid spray
Non-Intact STEC Validation Studies

Key features/significance:

- Pathogenic strains of *E. coli*, not surrogates
- Pilot scale commercial food processing equipment, not laboratory scale and/or scientific apparatus
Non-Intact STEC Validation Studies

Key features/significance:

- Entire subprimals, steaks, cutlets, beef patties etc., not simulated or re-structured beef products
- Industry guidance and participation (planning & executing)
Validation of antimicrobials for surface treatment of beef subprimals to control STEC-8 using ESS

<table>
<thead>
<tr>
<th>Inoculation levels</th>
<th>~2.5 and 6.5 CFU/gram</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>Meat</strong></td>
<td>Top butt subprimals</td>
</tr>
<tr>
<td><strong>Delivery system</strong></td>
<td>Air-assisted electrostatic spraying system</td>
</tr>
</tbody>
</table>
| **Antimicrobials** | Lactic acid (2.5% or 5%)  
Peroxyacetic acid (0.02% and 0.04%)  
Lauric Arginate (2.5% or 5%) |
| **Microorganisms** | O157:H7 and Non-O157 STEC cocktail |
| **Conditions**     | 30 sec application of <5 mls (-7.8 mC/kg) |
Validation of antimicrobials for surface treatment of beef subprimals to control STEC-8 using ESS
Control of STEC in Ground and Non-Intact Beef & Veal

Processes:
- Blade tenderization
- Chemical Injection
- Vacuum tumbling
- Cooking – flip & hold
- Fermentation
- Electrostatic spray & SLIC

Products:
- Goetta & Jerky
- Ground patties & wafers
- Prime rib & cube steaks
- Beef steaks & veal cutlets
- Fermented dry-sausage
Control of STEC in Ground and Non-Intact Beef & Veal

Recent Peer-Review Publications:
- Prime Rib – J. Food Prot. 76:405-412 (2013)
- Frozen Ground Beef Patties - J. Food Prot. 76:1500-1512 (2013)
- Veal cutlets – J. Food Prot. (accepted February 2014)
- Fermented dry-sausage - J. Food Prot. (in preparation)
STEC and ECOH behave similarly…
“If it works for O157:H7, it will work for The Big Six”

- No discernible differences in translocation between ECOH and STEC following blade tenderization or chemical injection of beef subprimal
- Majority of cells to top-most 1 cm, but distributed throughout subprimal/steak
STEC and ECOH behave similarly... “If it works for O157:H7, it will work for The Big Six”

- No discernible differences in thermal resistance between STEC and ECOH following cooking of blade tenderized or chemically-injected steaks, frozen ground beef patties, goetta slices, veal cutlets, and/or prime rib
- Higher temperatures generated greater lethality (1.5 to 4.5 log reductions)
STEC and ECOH behave similarly…
“If it works for O157:H7, it will work for The Big Six”

- Greater risk of illness for consumption of blade and chemical tenderized steaks compared to otherwise similar, but intact, steaks
- 2-fold for blade tenderized steaks and 4-fold for chemically-injected
Validation of Fermentation and Cooking of Dry-Fermented Sausage to Control STEC

- Involvement and resources from industry partners
- STEC 8 targets – Post-fermentation heating step
- Provides more processing options to deliver a 2- or 5-log reduction as required by FSIS and validated by the NCBA Blue Ribbon Task Force

<table>
<thead>
<tr>
<th>Fermentation parameters</th>
<th>96°F and 85% RH</th>
</tr>
</thead>
<tbody>
<tr>
<td>Target final pH</td>
<td>pH 4.6 and pH 5.2</td>
</tr>
<tr>
<td>Post-fermentation heating temps</td>
<td>110°, 120°, 130°F 0.5 h to 10 h at 95% RH</td>
</tr>
</tbody>
</table>
Validation of Fermentation and Cooking of Dry-Fermented Sausage to Control STEC

- Higher temperatures and lower pH delivered greater reductions of STEC
- Regardless of the target end-point pH, fermentation delivered ca. 0.7- to 1.6-log reductions of STEC
- CUT to target cooking temperatures of 110° to 130°F delivered an additional ca. 0.2 to 3.6 log CFU/g
Validation of Fermentation and Cooking of Dry-Fermented Sausage to Control STEC

5-log reduction achieved:

- pH 4.6: in 8 h at 110°F and during CUT at 120° or 130°F
- pH 5.2: in 10 h at 110°F, in >4 h at 120°F, and during CUT at 130°F

These validated processes provide manufacturers with lower temperature processing options for dry fermented sausage
STEC Attachment: Chilled, Non-Chilled Beef

- Brisket chilling pre-STEC inoculation significantly impacted STEC8 attachment (p=0.017)
- Chilling produced greater numbers attached cells
- STEC attached cells declined during post-inoculation storage
Predictive Growth Model of STECs in Irradiated, Raw Ground Beef

1. Primary models (Baranyi and Roberts's)

   - STEC growth as a function of time, at a constant temperature

   10 °C (Rep 1)
   
   45 °C (Rep 1)

2. Secondary model (modified Ratkowsky)

   - STEC growth rate as a function of temperature

3. Model validation

   - RMSE 0.2 log CFU/g vs. 0.7 log CFU/g (Huang’s model)

   http://foodsafety.unl.edu
Growth of STECs in Ground Beef at Low Temperatures
Thermal Destruction

STEC *E. coli* O45 Ground Beef (93:7)

- 54.4°C
- 60.0°C
- 65.6°C
- Luchansky et al. 54.4
- Line et al. 54.4

Log CFU/g vs. Time (min)
Training Impacts

- 16 Interns
- 160 Externs
- Undergraduate projects
  - Minority research project
  - Undergraduate honors project
STEC CAP Interventions Team

- **University of Nebraska — Lincoln** (Harshavardhan Thippareddi, Emie Yiannaka, Dennis Burson, Jeyam Subbiah, Galen Erickson, Terry Klopfenstein)
- **Kansas State University** (Randy Phebus, Sean Fox, Glynn Tonsor, Ted Schroeder, Sara Gragg)
- **Texas A&M University** (Gary Acuff, Matt Taylor, Alejandro Castillo)
- **North Carolina State University** (Ben Chapman)
- **USDA-ARS ERRC** (John Luchansky, Anna Porto-Fett)
- **University of California, Davis** (Christine Bruhn, Jim Cullor)
- **Alabama A&M** (Armitra Jackson-Davis)