In-Process Validation of Anti-microbial Interventions in Beef Processing Plants

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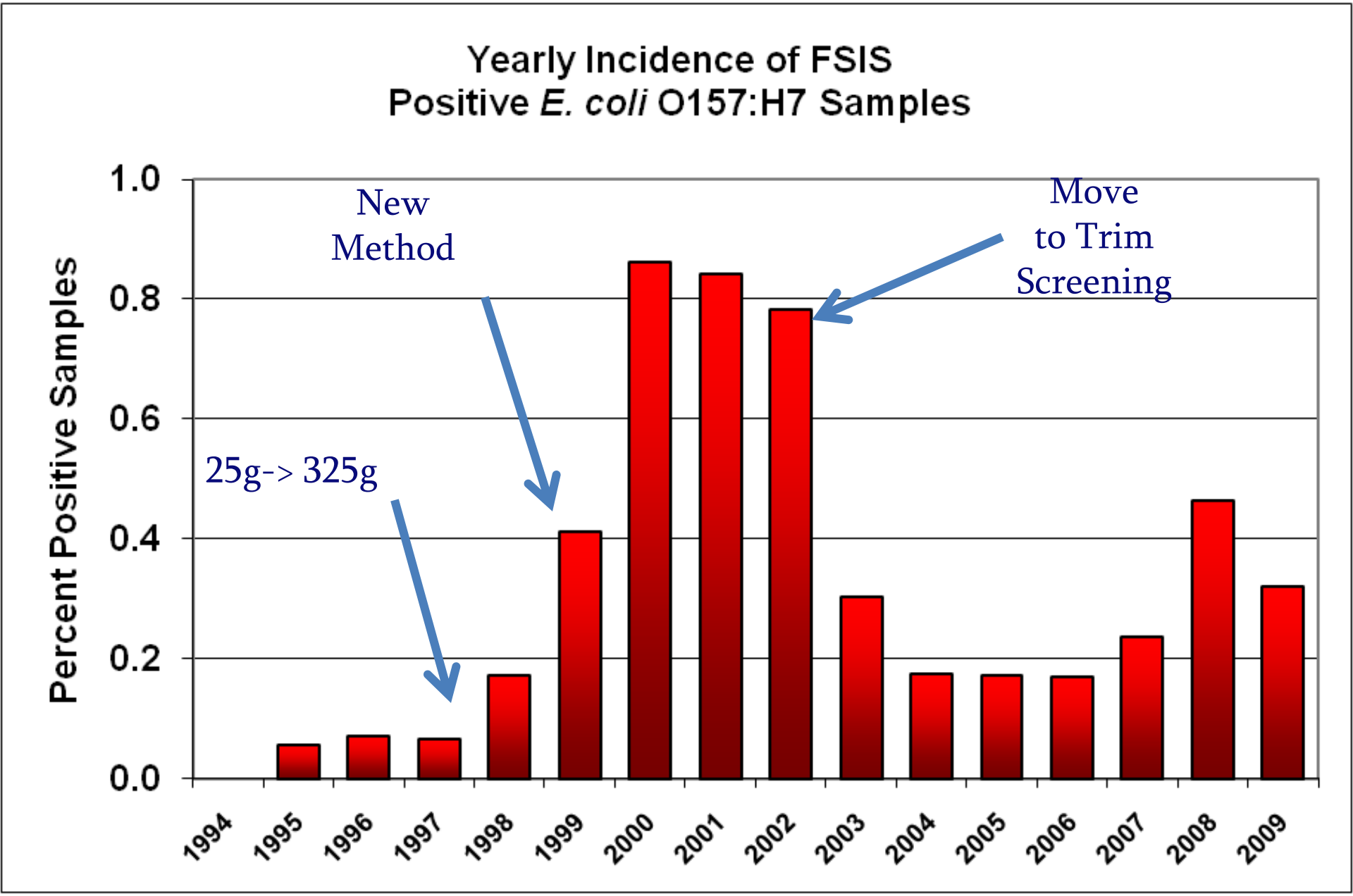
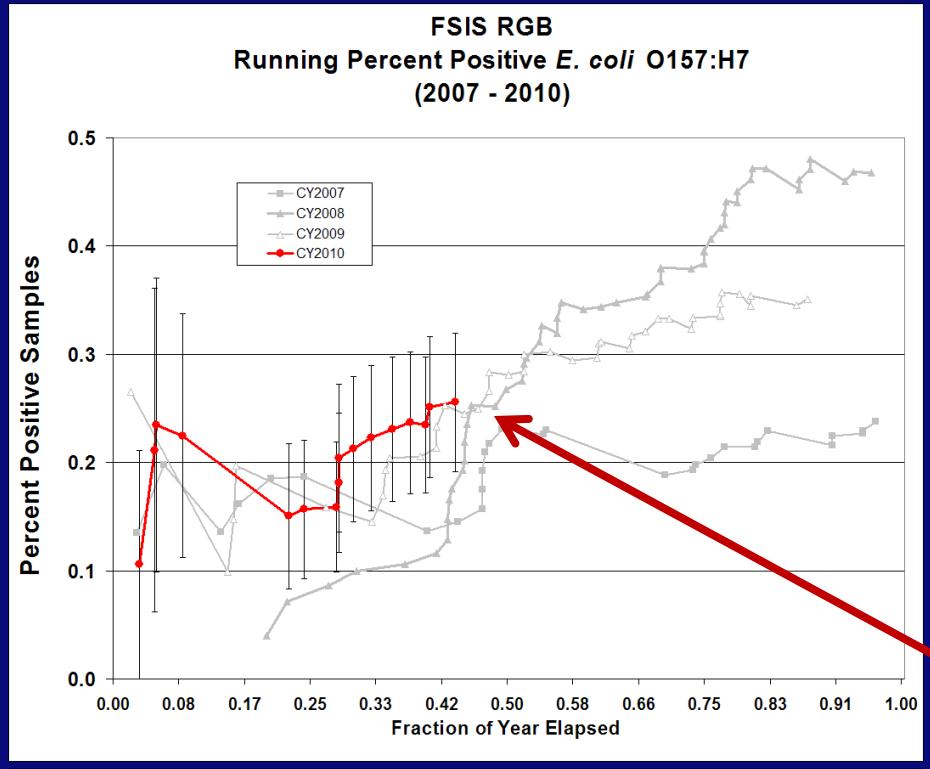
Presentation Outline

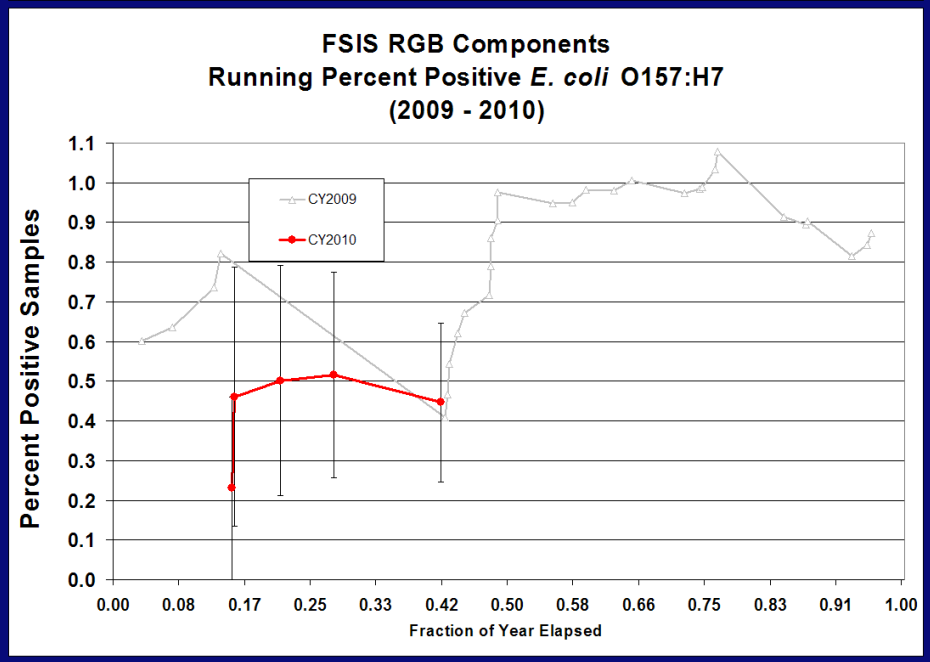
- Review of FSIS DRAFT guidance document
- A detailed validation protocol
- Examples of validation projects
- Summary and conclusions

Putting the Issue in Proper Prospective


Yearly Incidence of FSIS
Positive E. coli O157:H7 Samples
Percent Positive Samples
1.0
0.8
0.6
0.4
0.2
0.0
New
Method
Move
to Trim
Screening
25g-> 325g
1994
1995
1996
1997
1998
1999
2000
2001
2002
2003
2004
2005
2006
2007
2008
2009


FSIS RGB
Running Percent Positive E. coli O157:H7
(2007 - 2010)
Percent Positive Samples
0.5
0.4
0.3
0.2
0.1
0.0
CY2007
CY2008
CY2009
CY2010
0.00
0.08
0.17
0.25
0.33
0.42
0.50
0.58
0.66
0.75
0.83
0.91
1.00
Fraction of Year Elapsed

0.25%


FSIS RGB Components
Running Percent Positive E. coli O157:H7
(2009 - 2010)
Percent Positive Samples
1.1
1.0
0.9
0.8
0.7
0.6
0.5
0.4
0.3
0.2
0.1
0.0
CY2009
CY2010
0.00
0.08
0.17
0.25
0.33
0.42
0.50
0.58
0.66
0.75
0.83
0.91
1.00
Fraction of Year Elapsed

Healthy People 2010 Goal

- Goal of 1.0 case of human illness per 100,000 population

- For 2009 there were 459 cases of STEC O157 (0.99 cases per 100,000)

Draft Guidance: HACCP Systems Validation

- Issued by USDA-FSIS on March 19, 2010
- Guidance on HACCP system in general and intervention validation specifically.
- Out for comments – extended till June 19, 2010
- As of June 9th, FSIS had received approximately 2000 comments
- FSIS will revise the DRAFT guidance document and send out for comments again.

HACCP Systems (Interventions) Validation

- Interventions are used, as part of the HACCP plan, to control the microbiological hazard.

- Validation is the confirmation that the selected intervention effectively controls the hazard.

Validation of HACCP Systems

Validation is required in HACCP regulations, 9 CFR 417.4(a)(1). *FSIS is not imposing any new requirements.*

Why Now?

- Food Safety Assessment

The HACCP System – FSIS Document

- "The HACCP system is defined as the HACCP plan in operation, including *the HACCP plan* itself. *The HACCP plan in operation* includes the hazard analysis, the supporting documentation including prerequisite programs supporting decisions in the hazard analysis and the HACCP records."

Why Validate? HACCP Final Rule

- "FSIS believes that validation data for any HACCP plan must include *some practical data* or information reflecting an establishment's actual early experience in implementing the HACCP plan. *This is because validation must demonstrate not only that the HACCP plan is theoretically sound, but also that this establishment can implement it and make it work*."

Why validate? HACCP Final Rule

- "For example, steam vacuuming has been *scientifically demonstrated* to be effective in removing visible contamination and associated bacteria from carcass surfaces. A slaughtering establishment using the technology as a control measure at a CCP, *however, would still have to demonstrate its ability to use the technology effectively at the CCP*."

Components of a Sound Validation – FSIS Document

1) Scientific Support:
   - an article from a peer-reviewed scientific journal
   - a documented study
   - data underlying published guidelines
   - in-house data.

Scientific Support – FSIS Document

- The process should also be implemented in the establishment as described in the supporting documentation.
- Failure to take these steps *would raise questions on whether the HACCP system has been adequately validated*.

- According to FSIS plants are deviating from the support document was observed during FSA.

Components of a Sound Validation

1) Scientific Support
2) In-Plant Validation or in-process

An example – Slaughter plant (FSIS Document)

Initial Process Flow Diagram

- Receiving Cattle
- Pre-slaughter wash
- Stunning/bleeding
- Head & Shank removal
- Hide removal
- Evisceration
- Variety meat processing
- Splitting Carcasses
- Trim Zero tolerance
- Final Washes (water and organic acid)
- Chilling

- The Hazard analysis has identified *E. coli* O157:H7 as a biological hazard reasonably likely to occur.
- CCPs
  1. Trim off any visible fecal/ingesta with zero tolerance. Monitor trimming by visual inspection
  2. Organic acid spray (2% LA @43-54 C). Monitor concentration and temperature
  3. Carcass temperature of <45 F within 24 hr

These intervention strategies are implemented and documented in the supporting document.

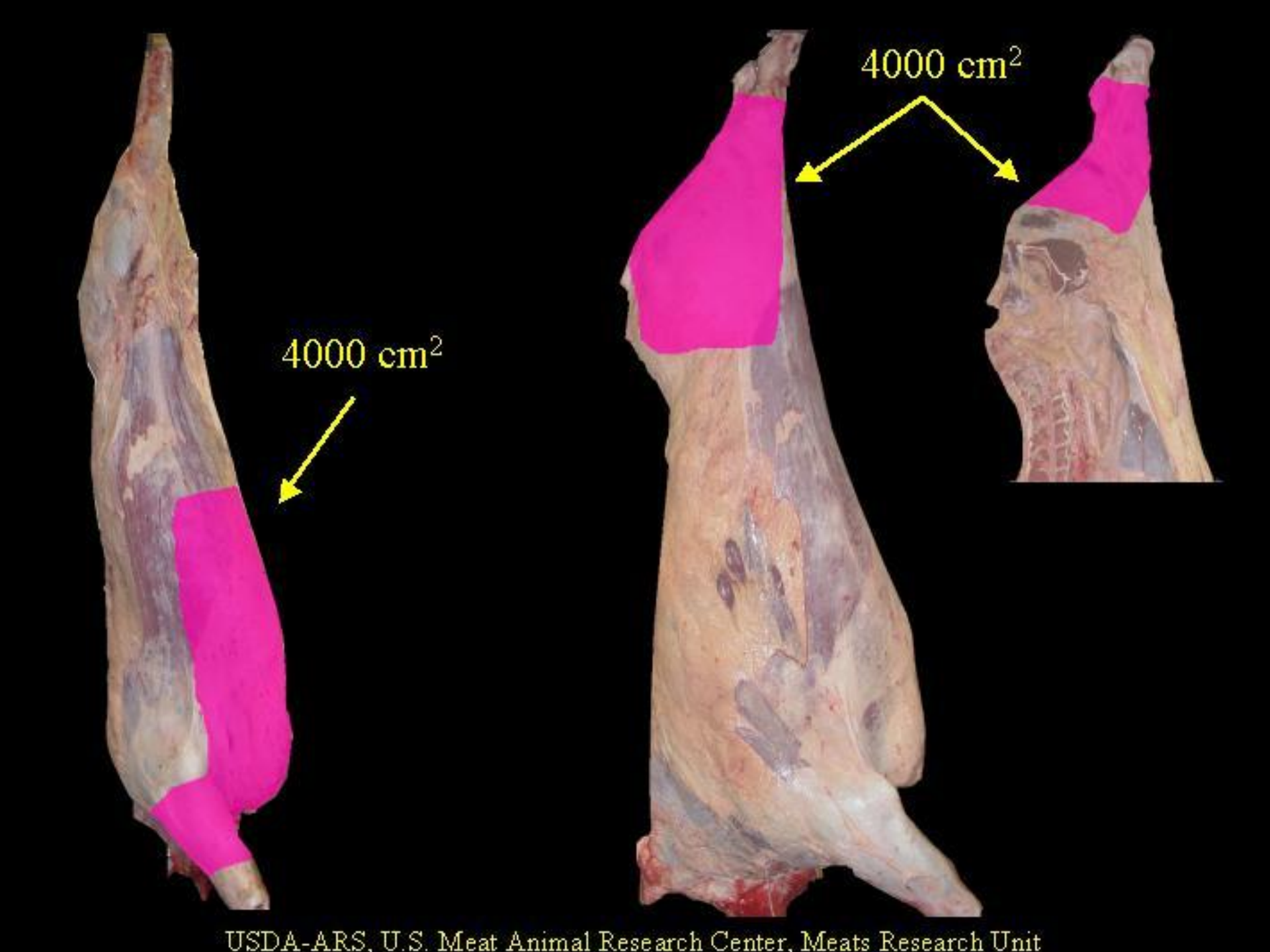
In-plant Validation

- Collect data to demonstrate that the plan that you have chosen *will actually* lead to the control of the hazard.

In-plant Validation – FSIS Document

- Collect microbiological data using a sampling scheme published by USMARC (Arthur et al., 2004).
- Data should show that the selected interventions used *do* reduce *E. coli* O157:H7 to an acceptable level as described in the hazard analysis.

USDA-ARS, U.S. Meat Animal Research Center, Meats Research Unit



USDA-ARS, U.S. Meat Animal Research Center, Meats Research Unit

In-plant Validation – FSIS Document

- FSIS suggests sampling (for a small plant) one carcass per week for 13 weeks
- Analyze samples for:
  - Aerobic Plate Count (FDA BAM)
  - Generic *E. coli* enumeration (FDA BAM)
  - *E. coli* O157:H7 (USDA-FSIS MLG)

Results – FSIS Document

Carcass Number	APC CFU/cm ^{2}		Genric *E. coli* CFU/cm		*E. coli* O157:H7	
	Dehided	**Chilled**	**Dehided**	**Chilled**	**Dehided**	**Chilled**
1	2.2×10^5	2.2×10^2	210	3	NEG	NEG
2	1.7×10^5	8.8×10^1	75	<3	NEG	NEG
3	4.7×10^5	3.6×10^2	240	3	NEG	NEG
4	2.5 x 10	5.6×10^2	1,100	3	POS	NEG
5	5.2×10^4	4.3×10^2	210	3	NEG	NEG
…..						
Mean	1.04×10^6	4.3×10^2	210	3		
Log	5.513	2.412	428	2.4		

In-plant Intervention(s) Validation

- Establishments request in-plant validations for variety of reasons:
  - FSA
  - NOIE
  - Installation of a new intervention
  - Hot Day Event (HDE)
  - Getting ready for the high season
  - Deciding what intervention to use

Components of a Validation Study

- Sampling:
  - Representative sampling to give true picture of the effect of the intervention
  - Acceptance of the results by USDA-FSIS
  - Method of sampling (sponge, excision, etc.)
  - Number of observations
- Microbiological analysis
- Conditions (parameters) of application

Components of a Validation Study – Representative Sampling

- Carcass – portion of the carcass
- Offal – all exposed surfaces
- Subprimals – with most external surface
- Trim - random

Components of a Validation Study – # of Observations

- The number of samples to be collected is determined by:
  - the desired "power" (i.e., the likelihood that the study will identify a significant difference (effect) when one exists.
  - the anticipated standard deviation of the transformed data.
  - the desired degree of resolution (i.e., the anticipated difference between "before" and "after" mean log values).

Components of a Validation Study – # of Observations

- The number of samples to be collected is determined by:
  - Following common convention the power is selected as 80%.
  - An anticipated standard deviation for log transformed counts of 0.80 is used. Source: 1) Internal company data and 2) ICMSF (2002)
  - The desired degree of resolution :

Components of a Validation Study – # of Observations

Desired Resolution in Separation of Log Transformed Means	N = Estimated Number of Samples Required (per case, i.e., Before and After)
0.25 log units	162
0.50 log units	42
1.00 log units	12

Components of a Validation Study – Microbiological Analysis

- Microbiological analysis:
  - Indicator Organisms
    - Aerobic Plate Counts (APC)
    - Total Coliforms Counts (TCC)
    - Generic E. coli Counts (ECC)
    - Pathogens ?
    - Pathogenic Index - Molecular Markers, a measure of microorganisms which carry one or more genetic virulence factors. Samples will first be incubated in enriched media and then analyzed by a qualitative polymerase chain reaction (PCR) method looking for selected marker gene fragments.

Components of a Validation Study – Operating Parameters

- The operating parameters
  - Intervention :
  - Method of application
  - Concentration
  - Pressure
  - Temperature
  - Equipment used
  - Other relevant parameters

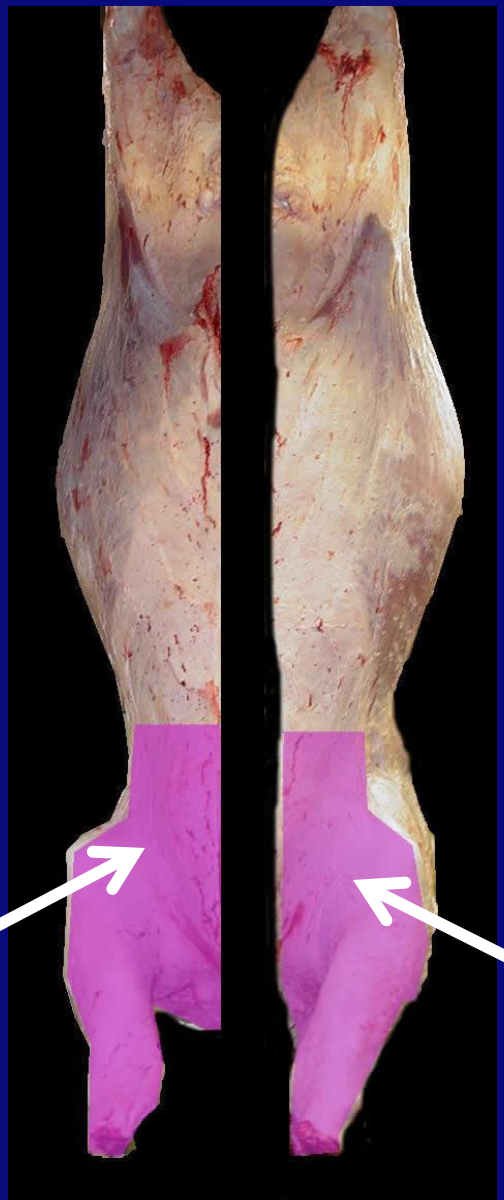
Some Actual Field Examples

- Hot Water Validation
- Subprimals Intervention Validation

Hot Water Validation

- Sampling – Sponge
- Carcass during the routine operation
- Number of samples – 45 before and 45 after
- Microbiological Analysis –
  - APC
  - TCC
  - ECC
  - Molecular Markers

Hot Water Validation



Leading Side

Trailing Side

Results

Table 3. Mean ± SD of APC, TCC and ECC (Log CFU/sponge) and percentage of molecular markers from samples taken before and after the application of carcass hot water pasteurization cabinet.

	APC Log CFU/sponge	TCC Log CFU/sponge	ECC Log CFU/sponge	Molecular Markers, %
Before (n=44)	4.5 ± 0.5[a]	1.7 ± 1.0[a]	1.1 ± 1.0	14.1
After (n=45)	2.2 ± 0.8[b]	0.4 ± 0.1[b]	0.4 ± 0.0	2.2
Reduction	2.3	1.3	0.7	11.9

[a] Means, within column, lacking common superscript letters, differ ($P \leq .05$).

Results

Table 3. Mean ± SD of APC, TCC and ECC (***Log CFU/sponge***) and percentage of molecular markers from samples taken before and after the application of carcass hot water pasteurization cabinet.

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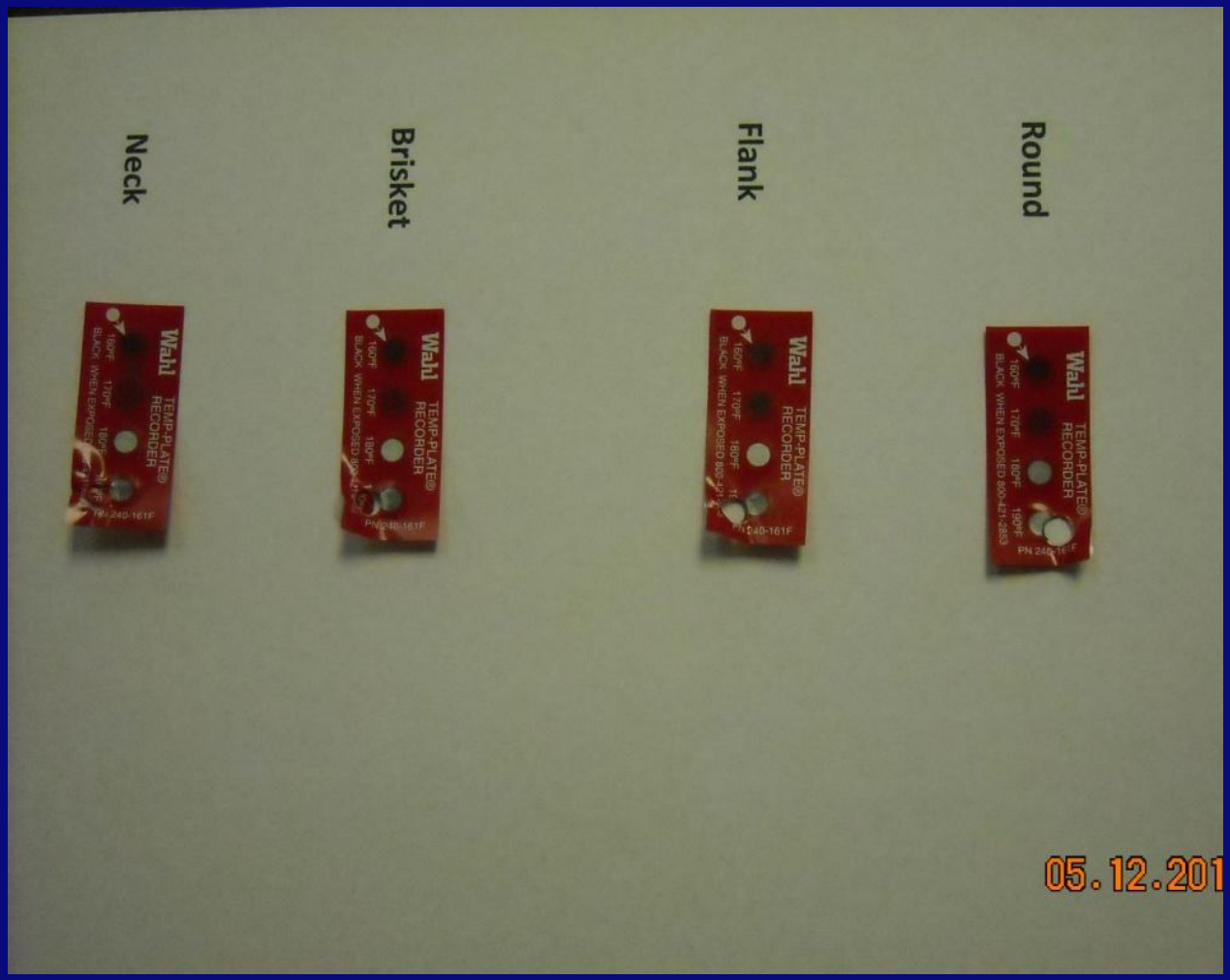
Operating Parameters

- Chad Hot Water Cabinet
- Water temperature:
  - 195°F using temperature gauge for water temperature delivered to the hot water cabinet
  - 170°F on the carcass surface as measured by Wahl tags

Surface Carcass Temperature



Validation Study for Treatment of Beef Subprimals using Compound X, Compound Y, and Compound Z

Subprimals Validation

- Sampling – Sponge
- Shoulder clod
- Number of samples – 45 before and 45 after
- Microbiological Analysis –
  - APC
  - TCC
  - ECC
  - Molecular Markers

Results

Table 1. Mean ± SD of TPC, TCC and ECC (Log CFU/sponge) and percentage of molecular markers from subprimal samples taken before and after the application of Compound X.

	TPC Log CFU/sponge	TCC Log CFU/sponge	ECC Log CFU/sponge	Molecular Markers, %
Before (n=50)	3.96 ± 0.38[a]	1.18 ± 0.90[a]	0.40 ± 0.76[a]	18.8
After (n=50)	3.91 ± 0.56[a]	1.05 ± 0.91[a]	0.07 ± 0.32[b]	24.4
Reduction	**0.05**	**0.13**	**0.33**	**-5.6**

[ab] Means, within column, lacking common superscript letters, differ ($P \leq 0.05$).

Results

Table 2. Mean ± SD of TPC, TCC and ECC (Log CFU/sponge) and percentage of molecular markers from subprimal samples taken before and after the application of Compound Y.

	TPC Log CFU/sponge	TCC Log CFU/sponge	ECC Log CFU/sponge	Molecular Markers, %
Before (n=50)	4.37 ± 0.36^{a}	1.75 ± 0.81^{a}	0.22 ± 0.61^{a}	19.2
After (n=50)	4.62 ± 0.29^{b}	2.14 ± 0.57^{b}	0.61 ± 0.83^{b}	19.2
Reduction	**-0.25**	**-0.39**	**-0.39**	**0**

[ab] Means, within column, lacking common superscript letters, differ ($P \leq 0.05$).

Results

Table 3. Mean ± SD of TPC, TCC and ECC (Log CFU/sponge) and percentage of molecular markers from subprimal samples taken before and after the application of Compound Z.

	TPC Log CFU/sponge	TCC Log CFU/sponge	ECC Log CFU/sponge	Molecular Markers, %
Before (n=50)	4.36 ± 0.37^{a}	1.99 ± 0.75^{a}	0.81 ± 0.91^{a}	17.2
After (n=50)	3.92 ± 0.26^{b}	0.84 ± 0.62^{b}	0.06 ± 0.29^{b}	6.4
Reduction	**0.44**	**1.15**	**0.75**	**10.8**

[ab] Means, within column, lacking common superscript letters, differ ($P \leq .05$).

Validation of the Efficacy of Compound Z as a Subprimal Intervention by other Establishments

Methods

- Sampling – Sponge
- Loin tail
- Number of samples – 50 before and 50 after
- Microbiological Analysis –
  - APC
  - Molecular Markers

Results

Table 1. Log mean (SE) aerobic plate counts and molecular index of loin tails before and after compound Z treatment.

Stage	APC (CFU/sample)	Molecular index (%)	No. Molecular Signals
Before (n=50)	3.33[a] (0.05)	14.0 [a]	35
After (n=50)	1.78 [b] (0.11)	2.0 [b]	5
Reduction	**1.55**	**12**	**30**

[a] Values in the same column bearing the same letter do not differ significantly at $P \leq 0.05$

Results – Weight Gain

Table 4. Weight gain of beef trim after acidified sodium chloride treatment

Stage	Weight (g)	% weight gain
Before (n=45)	42.0	0.47%
After (n=45)	42.2	

Methods

- Sampling – Sponge
- Ball tip
- Number of samples – 50 before and 50 after
- Microbiological Analysis –
  - APC
  - Molecular Markers

Results

Table 1. Mean[1] (Log CFU/Sponge) (SE) aerobic plate, anaerobic plate, total coliform, and *E. coli* counts of ball tips before and after lactic acid intervention.

Stage	APC	AnPC[2]	TCC	ECC
Before (n=48)	3.65[a] (0.03)	3.06[a] (0.03)	1.89[a] (0.07)	1.31[a] (0.09)
After (n=49)	0.71[b] (0.10)	1.06[b] (0.11)	0.40[b] (0.00)	0.40[b] (0.00)
Difference	**2.94**	**2.00**	**1.49**	**0.91**

[1] Values in the same column bearing the same letter do not differ significantly at $P \leq 0.05$

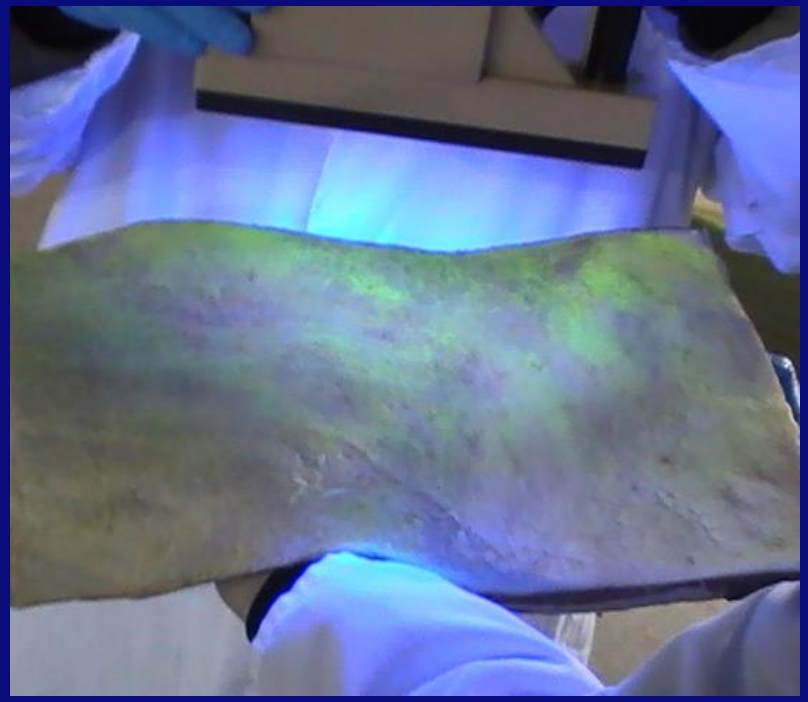
[2] Anaerobic Plate Counts

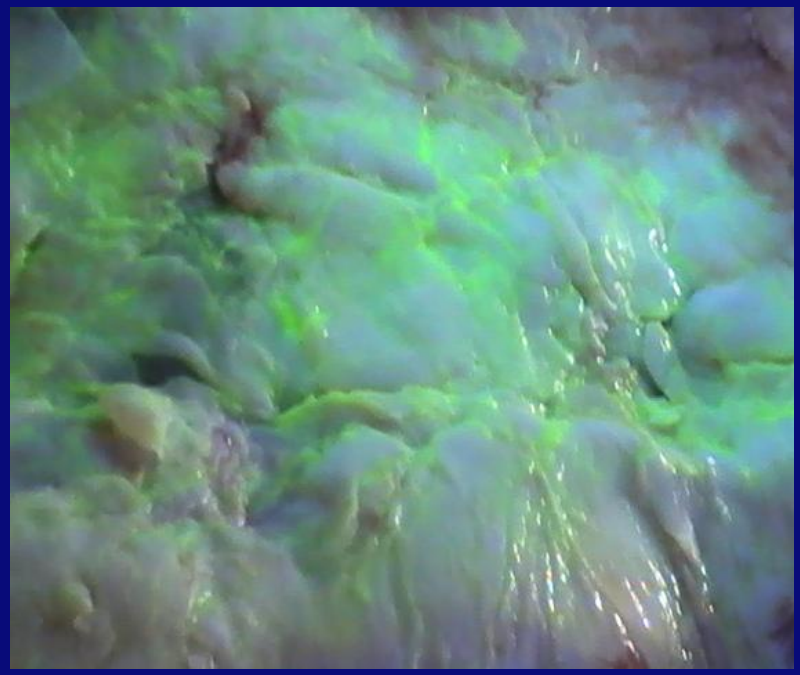
What is the Reason for Different Results?

- Deviation from relevant parameters?

Complete Treatment

- A verification of treatment must be put in place to ensure complete and adequate coverage.
- The pictures to the right depict a method of fluorescent dye to check coverage.





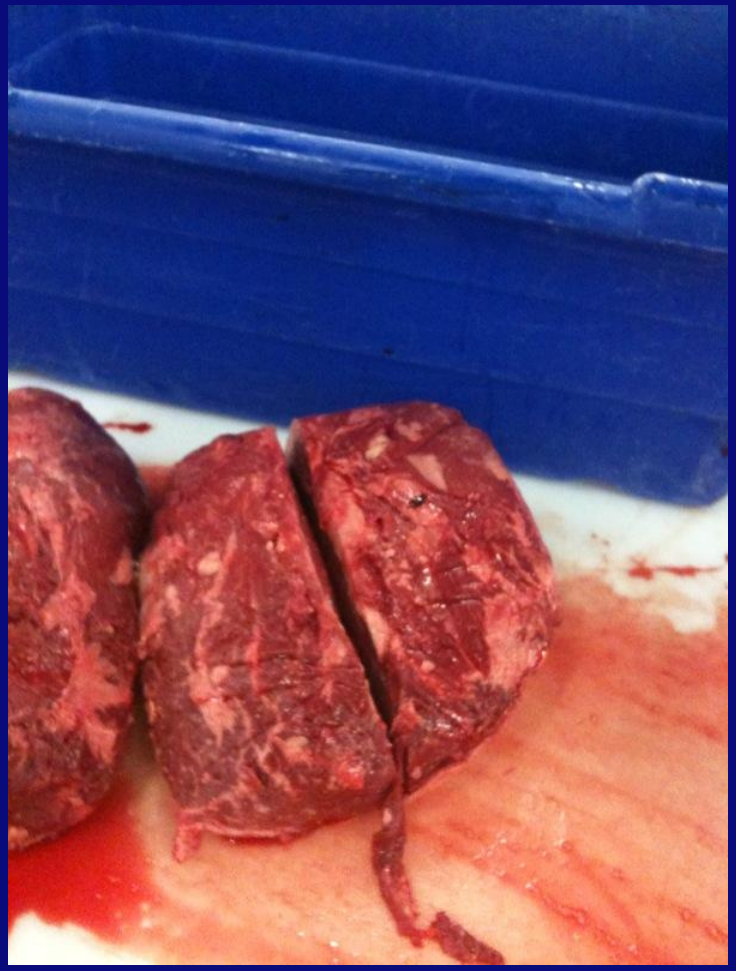
Validation Study for Treatment of Beef Subprimal Using an Approved Intervention

- Very small processor
- Wanted to validate the use of Lactic Acid as an intervention for subprimal before needle tenderization.

Methods

- Sampling – Sponge
- Top sirloin
- Number of samples – 50 before and 50 after
- Microbiological Analysis –
  - APC
  - TCC
  - ECC
  - Molecular Markers

Protocol



Protocol

Protocol

- Allow to drain for 15 minutes
- Sampled the other half for the “after” sample
- Weigh another 50 half “before” and “after”

Conditions of Subprimal Intervention

The operating parameters of the intervention cabinet during the validation study were:

1. Intervention used: Lactic acid
2. Method of application: Spray
3. Concentration of application: 4.0-4.3%
4. Pressure of application: 40 psi
5. Mechanical explanation of equipment used: Mist using two bars, on top and spray nozzles from the bottom
6. Temperature of the application: 49°F
7. Exposure time: products were exposed to lactic acid for approximately 20 seconds before tenderization

Results

Table 1. Mean (SE) APC, TCC, and ECC (Log CFU/sponge) of subprimal treated with and without lactic acid intervention.

Stage	APC	TCC	ECC
After (LA off, n=50)	6.99[a] (0.09)	1.69[a] (0.11)	0.40 (0.00)
After (LA on, n=50)	5.00[b] (0.10)	0.73[b] (0.08)	0.40 (0.00)
Difference (Off-On)	1.99	0.96	NA

[a] Values in the same column bearing the same letter do not differ significantly at $P \leq 0.05$

Summary & Conclusions

- Validations are essential part of the HACCP Systems.
- Validation has two components:
  - Scientific (Evidence for efficacy of an intervention)
  - In-plant (will work in *this specific plant)*
- FSIS will soon reissue another DRAFT document or a proposed rule (Federal Register)
- Regardless of FSIS expectation, it is in the best interest of the plant to ensure that the interventions are “working” as intended.

Summary & Conclusions

- False sense of security
- Validations for slaughter plant interventions or HACCP system should be conducted on an ongoing basis.
- At the minimum, interventions with a CCP designation will have to be validated annually and preferably prior to “high season.”

Thank you for listening

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