

# *E. coli* O157:H7 Laboratory Methods and Sampling

Public Meeting

October 14-15 L'Enfant Plaza Hotel

# Purpose of Presentation

- Laboratory Method
  - Technology
  - Media
  - Supporting studies
- Sample Collection
  - Risk-based sampling
- Sample Processing
  - N60 protocol

# Regulatory Sampling Results

- CY07 (MT03)
  - 12263 samples and 29 were positive (0.24%)
- CY08 (MT43)
  - 8405 samples and 32 were positive (0.38%)

	Negative	Positive	Total
CY2007	12263	29	12292
CY2008*	8405	32	8437
	20668	61	20729

P-value = 0.06

Odds ratio (95%) = 0.97-2.66

\* Data through September 14<sup>th</sup>

# Regulatory Sampling Results

- What has changed
  - Sample selection methodology
  - Enrichment media
  - Pathogen prevalence?

# Laboratory Method

- MLG Chapter 5
  - Screen
    - Rapid Check™, Transia™, BAX™
  - Confirmation
    - Morphology, agglutination, toxin, genetic
  - Quantification
    - MPN

# E. coli O157:H7 Method

- Day 0
  - Collect two pounds of sample
- Day 1
  - Received at lab, weigh 5-65g sub-samples
  - Add enrichment broth and incubate
- Day 2
  - Perform screen (BAX or lateral flow device)
  - Immuno-magnetic capture
  - Streak to Rainbow agar, incubate
- Day 3
  - Pick typical colonies
  - Streak to SBA, incubate
- Day 4
  - RIM latex agglutination test, biochemical (VITEK), EHEC kit
- Day 5
  - Shiga toxin/ *fliCh<sub>7</sub>* gene PCR confirmation (if EHEC or agglut test is H7 neg)

# Enrichment Media

- Shorter incubation time
  - mEC+n → 20-24 hours
  - TSB+n+casaminoacids → 15-22 hours
- Sample size appropriate for objective
  - 5 samples, 3 labs, 2 inoculation levels for each substrate (gb, trim, cooked patty, sausage, swab)

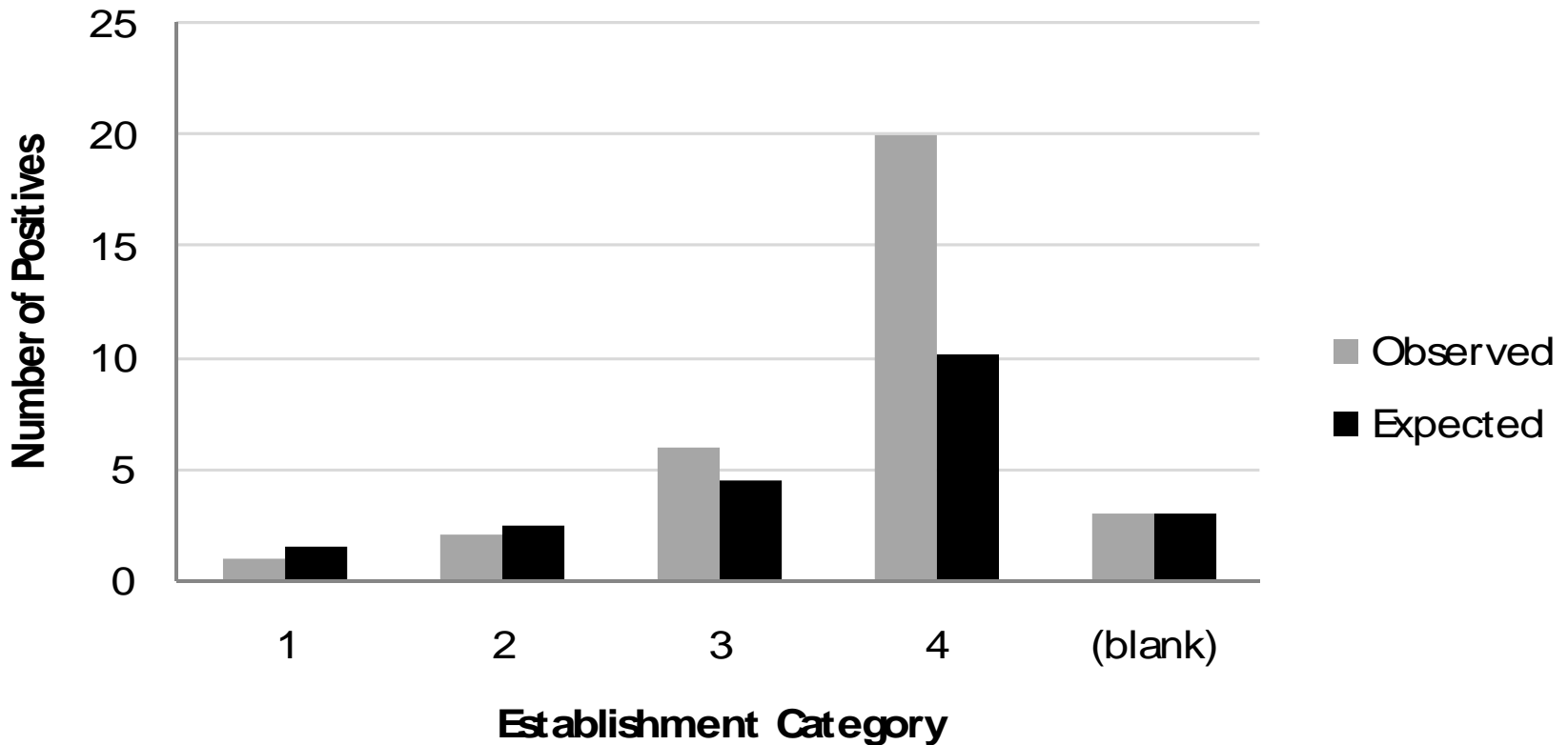
# Enrichment Media Study Results

Substrate	Actual CFU	Inoculum	Percent Confirmed Samples		
			15-16H mTSB+n	21-22H mTSB+n	20-24HmEC+n
Swab	21.1	20	100	100	100
	4.5	4	100	100	100
	0	0	0	0	0
Sausage	22.3	20	40	40	40
	4.4	4	10	10	10
	0	0	0	0	0
Beef trim	27.5	20	88.9	88.9	33.3
	5.5	4	93.3	93.3	0
	0	0	0	0	0
Ground beef	24.6	20	100	100	100
	4.9	4	100	100	86.7
	0	0	0	0	0
Beef patty	26	20	88.9	100	100
	5.2	4	93.3	100	100
	0	0	0	0	0



# Sample Collection Methodology

Number of positives expected in 2008 based on the new sampling algorithm



# Regulatory Sampling Results

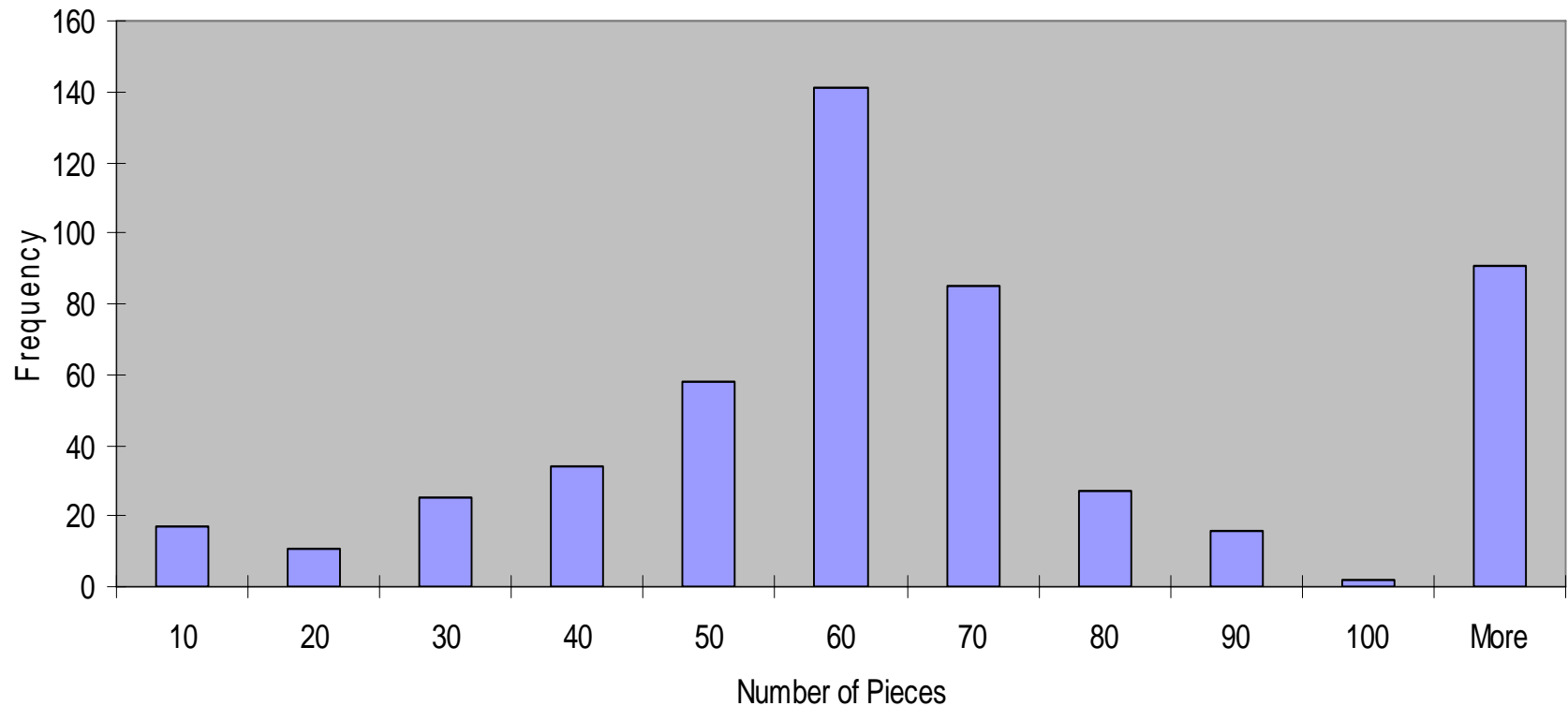
- What has changed
  - Sample selection methodology
    - Risk-based sampling does not seem to be related to increase in percent positive samples
  - Enrichment media
    - No statistical difference was observed in the media verification work. Study design was not constructed to differentiate performance
  - Pathogen prevalence?
    - Data only available from industry

# Sample Processing

- Sample purpose is verification testing
  - HACCP assessment
- Follow N60 protocol
- Analyze 5-65g (total 325g of tissue)

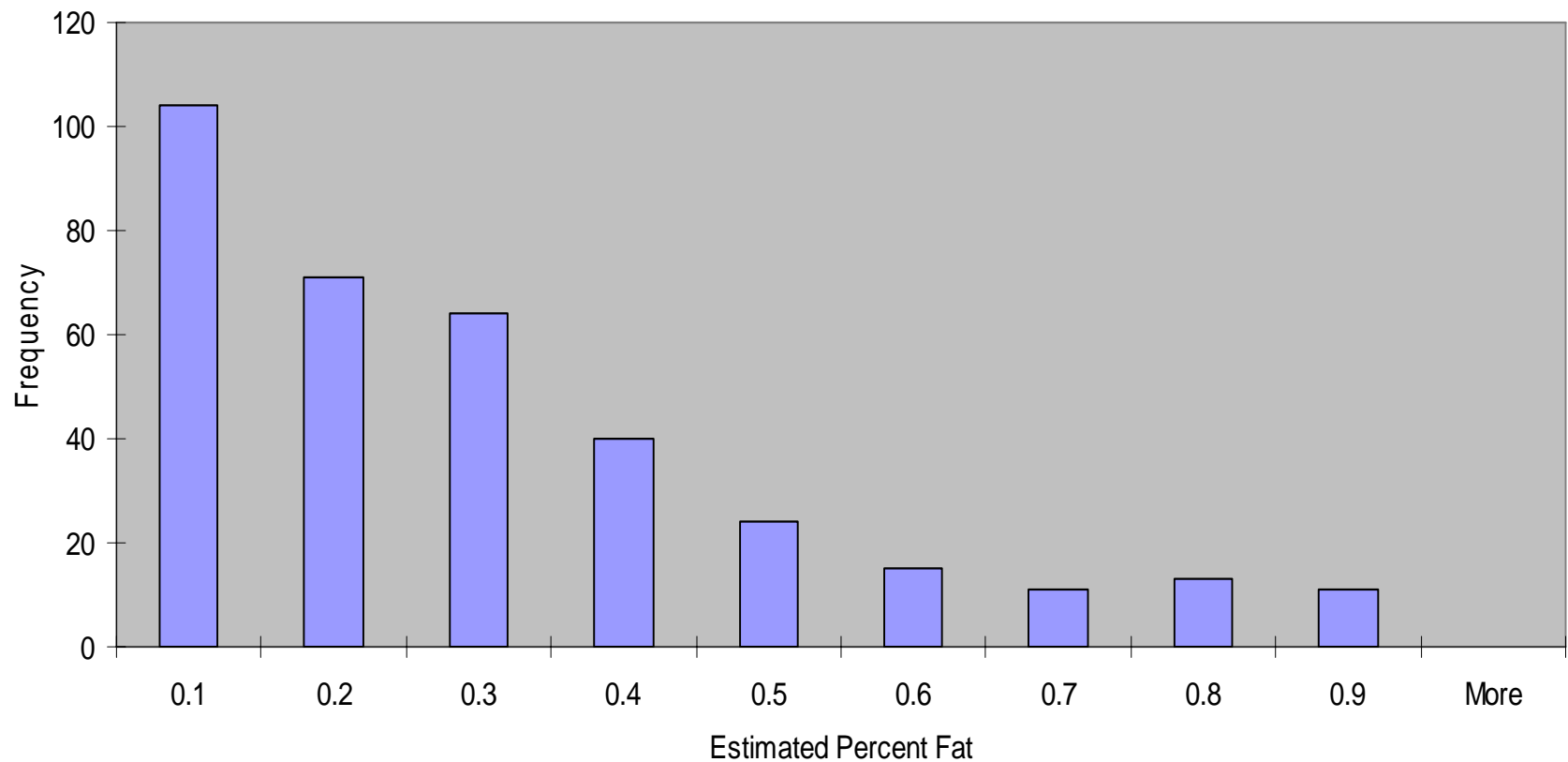
# Samples Received at Laboratory

Histogram of the Number of Pieces in Each Trim Sample Submitted for Analysis to the Field Services Laboratories (April to September 2008)

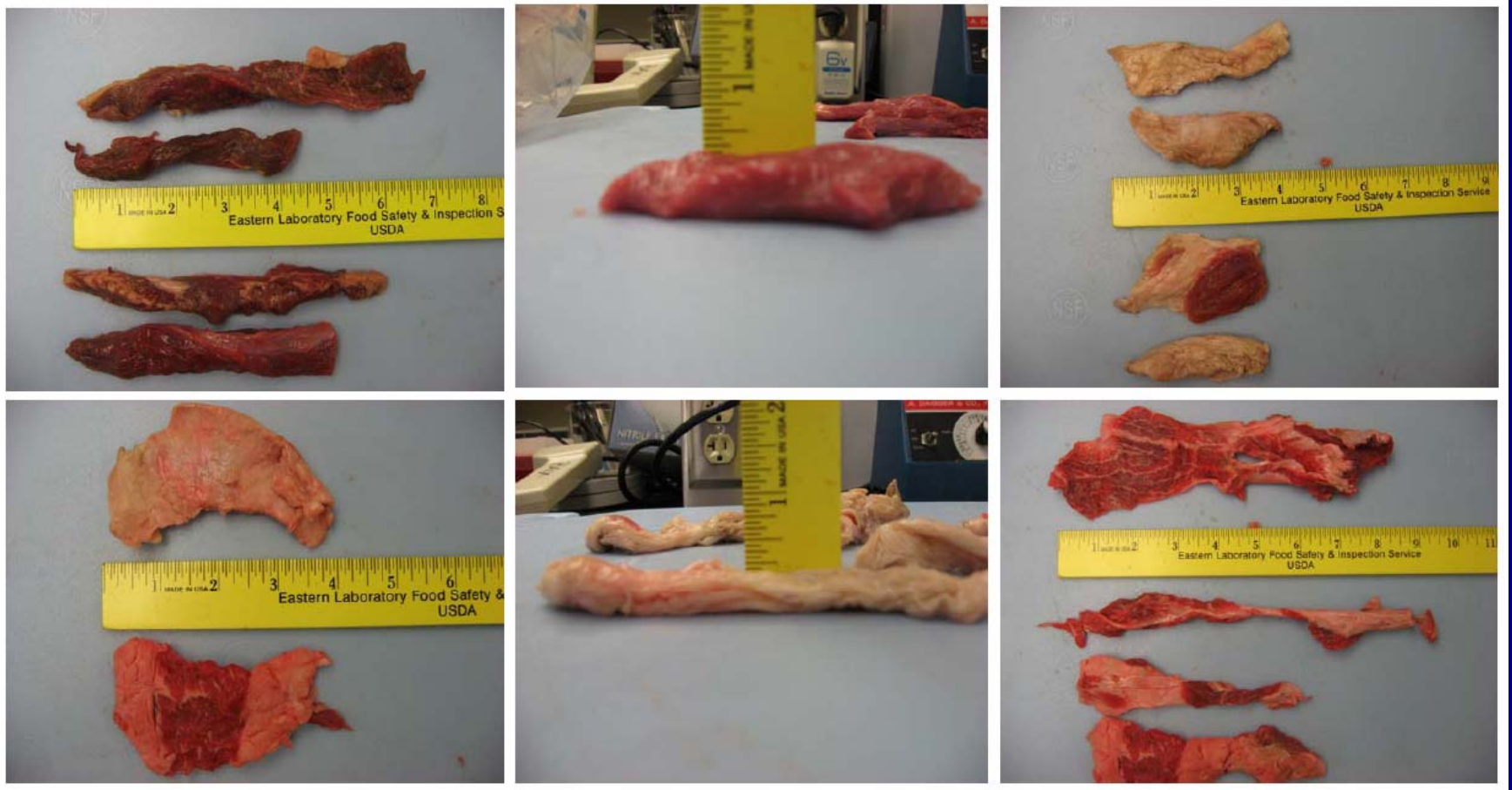


# Samples Received at Laboratory

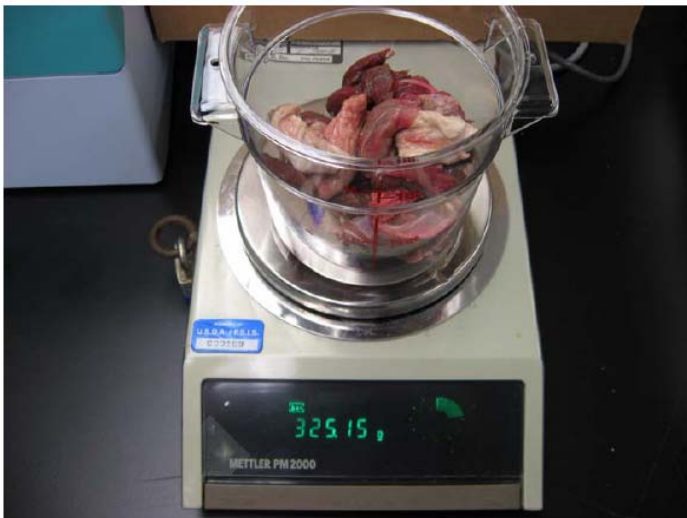
Histogram of Estimated Percent Fat Content from Trim Samples Submitted to the Field Services Laboratories (April to September 2008)



# Trim Pieces Received at Labs



# Work in Progress



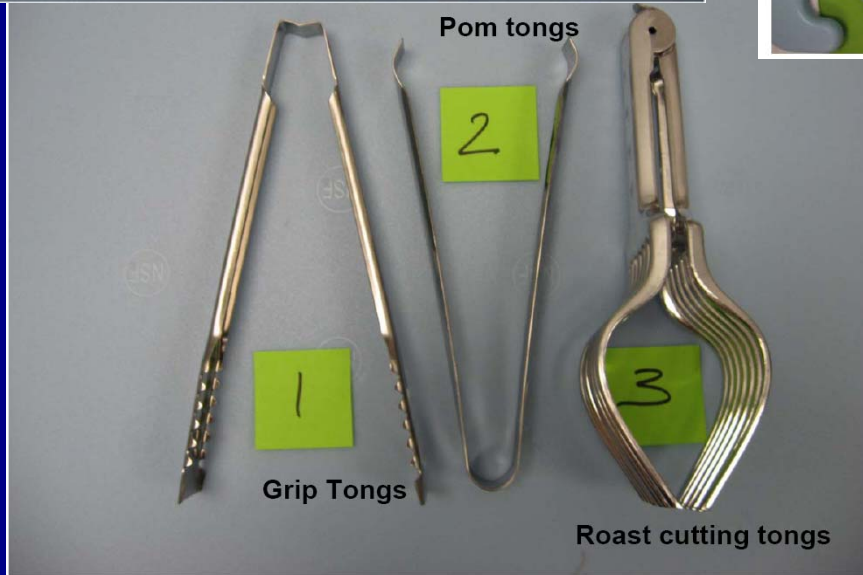
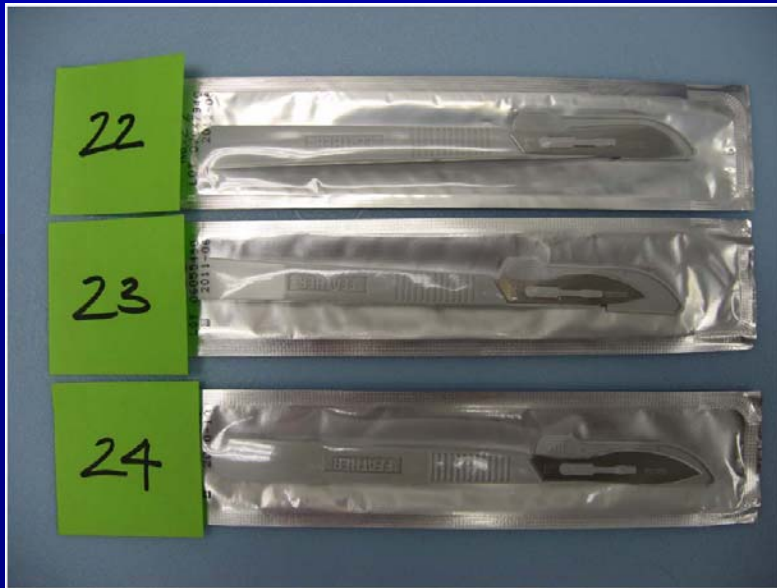
**Note: 325g of Beef trim was  
Approximately 20 precut pieces.  
Sample above used in all proceeding slides**

# Work in Progress





# Work in Progress



Other?

# Work in Progress

- Verification work to eliminate the sub-sample analysis. One 325g sample rather than 5-65g sub-samples.