



# Environmental Sampling and Rapid Techniques

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# Introduction



- In USA, foodborne pathogens have been estimated to cause 48 million illnesses and up to 3,000 deaths, annually.
- Among these pathogen, the bacteria *Listeria monocytogenes* cause a serious illness known as listeriosis.
- Between 1989 and 1993 the annual incidence of listeriosis decreased 44%. A study showed a 38% decreasing trend from 1996 to 2002. However, listeriosis outbreaks still occur.
- There is strong probability for the presence of *Listeria monocytogenes* in places where meat are sliced and packed.

# Control Methods for *Listeria monocytogenes*



- Contamination levels of raw ingredients
- Sanitation
- **Laboratory Analysis**
  - Environmental sampling
    - Non-contact surfaces with food
  - Equipment sampling
    - Food-contact surfaces
  - Product sampling

# Environmental Test Applications



- Information about the contamination sources
- Pathogenic contamination station
- Information about the equipment design and operation
- Identification of where the most probable cross contamination sites may occur



# Design of the Testing Program



- Purpose
- Microorganism
- Type of Assay
- Sampling Method
- Time



# Assay Types



- Environmental Surfaces



- Food-Contact Surfaces



- Product



*Vaccum-Packed  
Vegetables*

# Environmental Surface Testing



Positive environmental tests – Indicates that a problem may exist:

- and transferred to the product
- must be monitored by cleaning and product testing to ensure product safety

# Food Contact Surface Testing



- Positive tests on food contact surface:
  - *Listeria spp.* – Implies product contamination
  - *Lm* – Product in contact with contaminated surface is adulterated





# Product Testing



- A positive result of the product, *Lm*

- Product is adulterated



- Evidence that *Lm* is a biological hazard that is likely to occur





# Verification Sampling



# Sanitization Efficiency Verification



- Physical Verification
  - Organoleptic
- Microbiological Verification
  - Pre-operational, operational, post-operational
    - Bioluminescence / ATP (immediate results)
    - Aerobic Plate Count (APC) (results in 2-3 days)
    - *Listeria* spp. (several days)
    - Environmental Monitoring (results in 3-5 days)

# Food Safety Educational Programs in Spanish on the Control of *Listeria monocytogenes* in Retail Food and Deli Establishments and Other Topics



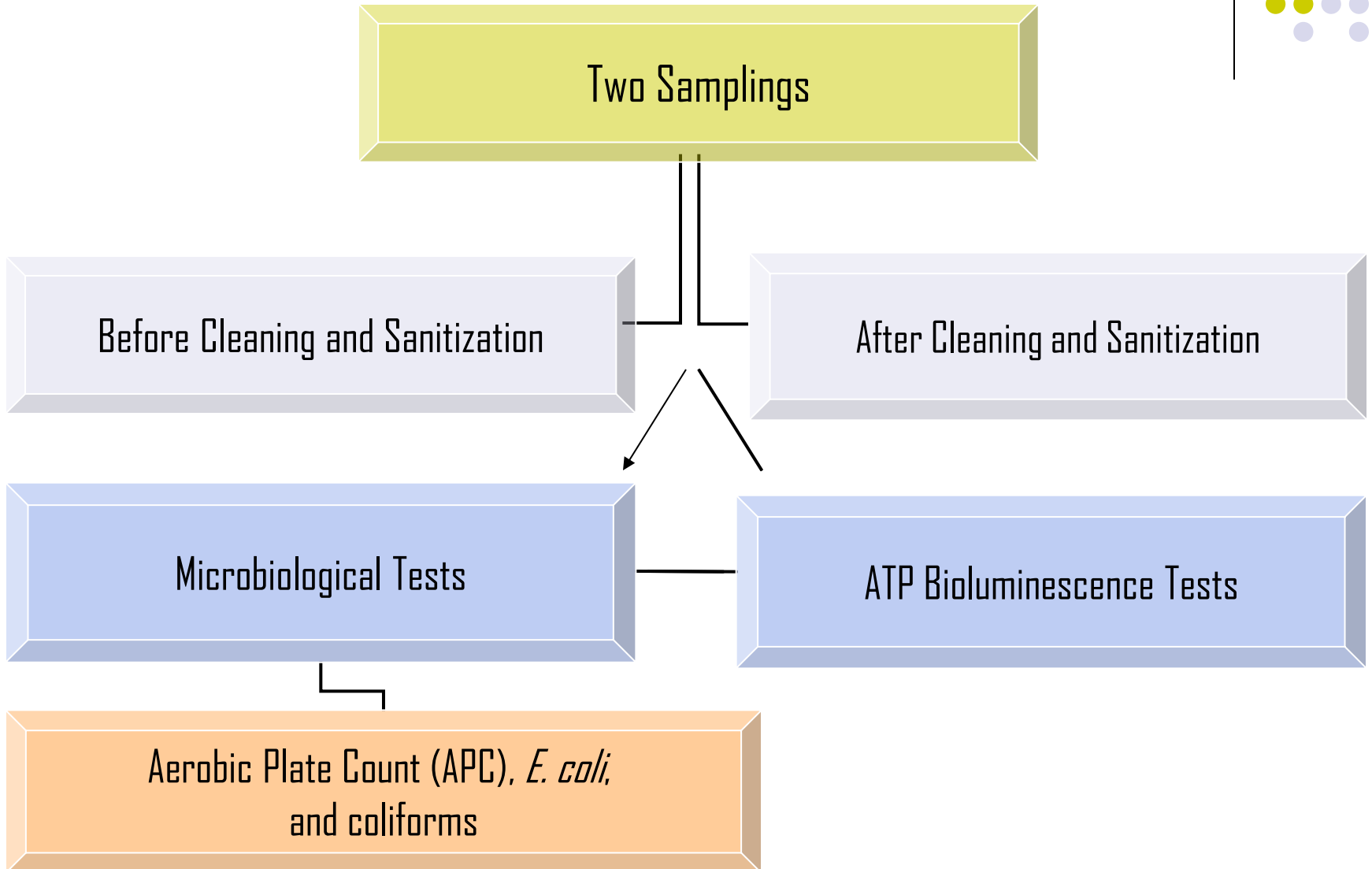
- **PROJECT:** FSIS Project Number FSIS-C-16-2005
  - FSIS Project Manager & Phone: Autumn Canaday  
(202) 690-6520
  - Cooperator: University of Puerto Rico at Mayagüez
  - Cooperator Contact: Edna Negrón, Ph.D.
  - Amount of Award: \$50,000
  - Term of Agreement: Sep 15, 2005 to Sep 15, 2006
  - Project Description: This project is to develop a training program to promote the reduction of *Listeria monocytogenes* in retail food establishments where Spanish is the main language spoken, and to provide training and outreach materials in Spanish on food animal production, food safety, and food defense to food protection officials.
  - [http://www.fsis.usda.gov/About\\_FSIS/Cooperative\\_Agreements/index.asp](http://www.fsis.usda.gov/About_FSIS/Cooperative_Agreements/index.asp)

# Project Objectives



- Use of rapid methods to determine the efficiency of cleaning and sanitization techniques performing microbiological tests, such as APC, *E. coli*, and coliforms and compare the results with a rapid method as cleaning indicator (ATP bioluminescence tests).
- Evaluate if the use of this rapid method can be used as a tool for the Inspector, Delegated Personnel, Supervisors, and/or Managers.

# Methodology



# Methodology



- Environmental Sampling



- Microbiological tests

- APC

- *Escherichia coli* and coliforms

- Petrifilm™ 3M™ Plates

- Validated and used by the USDA

- ATP bioluminescence

- Luminometer

- Portable
- Easy-to-use
- Results immediately

# Methodology



- Petrifilm™ 3M™ Plates
  - Sample collection
  - Sample Preparation
    - Sterile diluents, pH adjustment, mix, and homogenize
    - Dilution in series
  - Plate Inoculate
  - Incubation
  - Interpretation
  - Statistical Analysis







# Petrifilm 3M



# Methodology



- ATP Bioluminescence
  - Luminometer
    - This system detects microorganisms and food residues by measuring adenosin trifosfate (ATP) using luciferase bioluminescence.
      - ATP determination – energy molecule
        - Animals, vegetables, bacteria, yeasts and molds
    - ATP + reagents (luciferin y luciferase)
    - The higher the microbial load and/or product residue, the higher is the ATP amount as related to the light output.



# Methodology



- ATP Bioluminescence Tests
  - Advantages
    - Rapid Method
    - Easy to use
    - Detection in microbiological contamination and food residue
    - ATP measurements are correlated with laboratory samples
  - Disadvantages
    - Disinfectants may affect the ATP measurements
      - Enzyme (Luciferase)
    - Contamination of food organic matter

# Methodology



- Luminometer
  - Sampling - Swab
  - Activation-Agitation-reagents
  - Results readings
    - A swab is inserted in the luminometer and the results are read
  - Results Interpretation



# Luminometer SystemSURE II



# Main Points



1. Continuity

2. Controls

3. Sanitation GMP

4. Verification: **Bioluminescence, APC**



## Safe Food Depends on **You**

If **We** All Work Together **We** Can Provide Safe Food  
for **Our** Customers



# References



- Cutter, C. N. y W. R. Henning. 2003. Control de *Listeria monocytogenes* en Pequeñas Plantas Procesadoras de Carnes y Aves. Information and Communication Technologies, Colegio de Ciencias Agrícolas, Pennsylvania State University.
- Mead, P. S.; L. Slutsker; V. Dietz; L. F. McCaig; J. S. Bresee; C. Shapiro; P. M. Griffin and R. V. Tauxe. Food-related illness and death in the United States. *Emerg. Inf. Dis.* 5:607-625.
- "Guidelines to Prevent Post-Processing Contamination from *Listeria monocytogenes*," National Food Processors Association, submitted to Dairy, Food, and Environmental Sanitarian, April, 1999.
- "Interim Guidelines: Microbial Control During Production of Ready-to-Eat Meat and Poultry Products," Joint Industry Task Force on Control of Microbial Pathogens in Ready-to-Eat Meat and Poultry Products, Washington, DC, February 1999.  
[[http://meatami.org/Guidelines\\_Microbial\\_Pathogens\\_299.pdf](http://meatami.org/Guidelines_Microbial_Pathogens_299.pdf)]