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PFGE as a Component of FDA Compliance Efforts

2009 Annual PulseNet Meeting
September 25th 2009

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The U.S. Food & Drug Administration
Center for Food Safety and Applied Nutrition
Office of Regulatory Science

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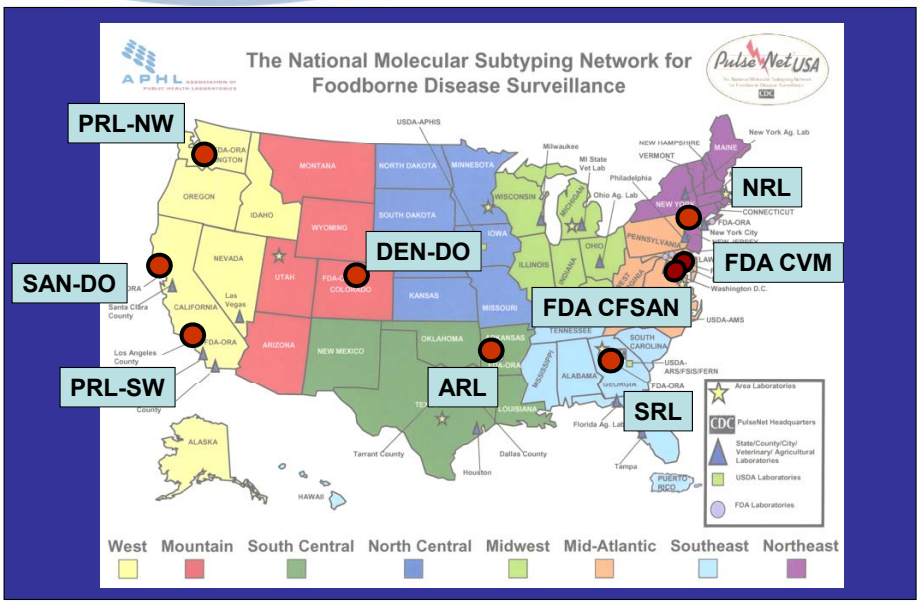
FDA PulseNet Certified Field Lab



Where our isolates come from:

- Domestic and Import Surveillance
- Special Assignments
  - Investigation efforts of food types
  - specific firms

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Some Specific Concerns to FDA

- Salmonella enterica:
  - raw seafood, egg products, cake mixes, unpasturized milk and salad dressings,
  - Many types of produce (peppers, leafy greens, sprouts, etc.)
- Listeria monocytogenes:
  - ready to eat (RTE)
    - deli meats, raw milk, soft cheeses

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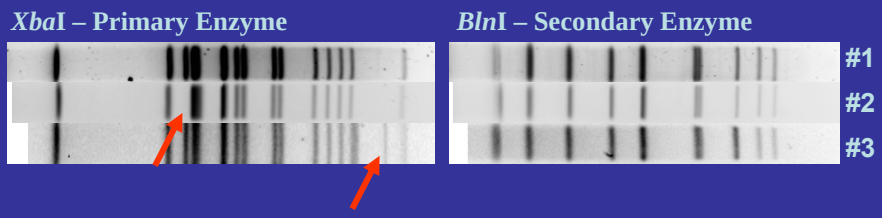
Peanut Butter – 2006/7
(3 concurrent clusters eventually linked)



Patient A → **#1**, **#2**

Patient B → **#1**, **#3**

Peanut Butter → **#1**, **#3**

(With confirmed history of peanut butter consumption)

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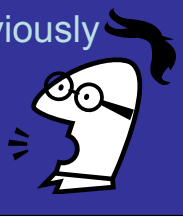
Changes in 2007

- Increased focus on determining whether or not microorganisms are present in food processing environment
- Field instructed to gather **5 to 10 fold** the number of environmental samples previously collected prior to 2007.



New Strategy Started in 2007

- Doubled the inspectional and analytical resources for Environmental sampling
- Implemented new training module in the Certification training regimen given field investigators
- Anticipated that FDA will continue to increase resources for environmental sampling
  - Importance of analytical results

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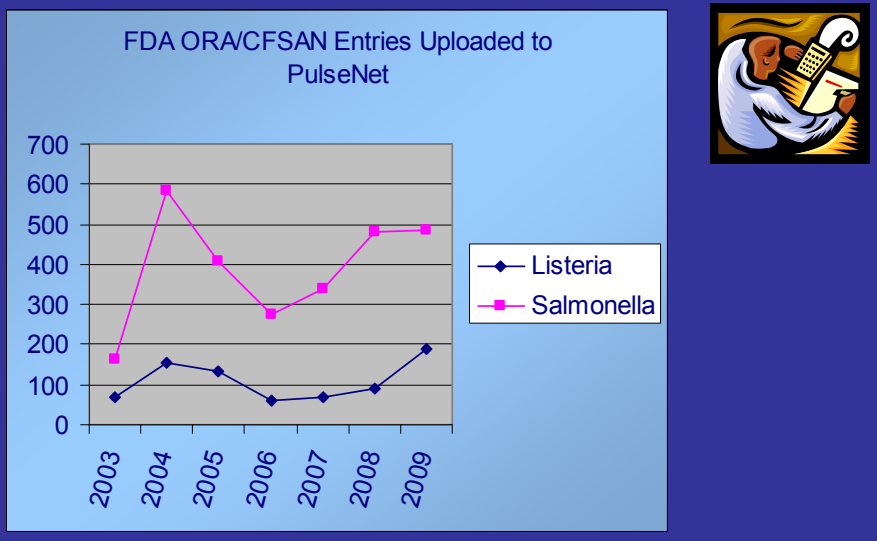

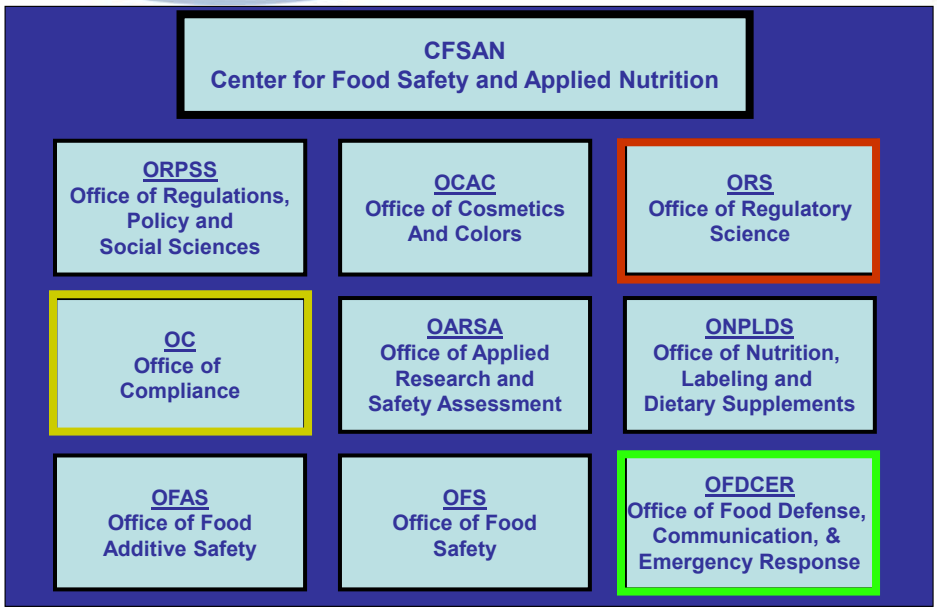

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Compliance Objectives

- To Keep the Public safe from unadulterated products
- To aid industry in having unadulterated product
  - Ensure that industries system is working
- To collect evidence that will hold up in court

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Case Development:

Collect Product and/or Environmental Samples

Samples sent to FDA lab for analysis and PFGE.

Review of EIR and/or violative sample findings
Prepares recommendations to CFSAN O.C.

CFSAN Compliance officer evaluates case
Case forwarded to Office of Chief Counsel

FDA/OCC attorney reviews case
Referal package → DOJ

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Adulteration Charges

FDA's Legal Authority:

- Federal Food, Drug and Cosmetic Act
  - Section 402 (a)(1)
    - "A food shall be deemed to be adulterated within the meaning of 402(a)(1) if it "bears or contains any **poisonous or deleterious substance** which may render it **injurious to health**." For example, this charge is cited when there is FDA laboratory confirmation of a human pathogen in product."
  - Section 402 (a)(4)
    - "A food shall be deemed to be adulterated within the meaning of 402(a)(4) if it has "been **prepared, packed, or held** under insanitary conditions whereby it may have become contaminated with filth, or whereby it may have been rendered **injurious to health**."

Interpretations of PFGE

- Environmental samples that match product contamination
  - Can help show a firm is responsible for contamination of product
- PFGE is persistent in a processing facility over months or years
  - Permanent Resident Pathogen

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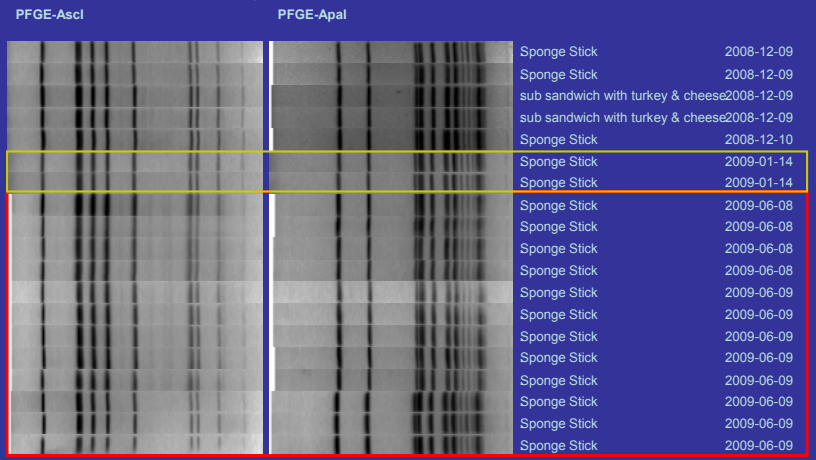
Listeria monocytogenes
(in ready to thaw and eat sandwiches)



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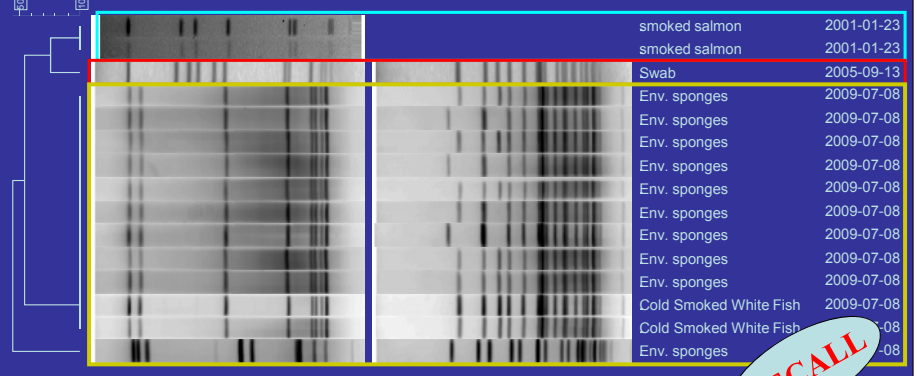
Listeria monocytogenes (in ready-to-eat hot & cold smoked fish)



RECALL

Sporadic L. mono contamination and lapses in sanitation?

Voluntary Recall was announced for products sold Dec. 7, 2008 through June 18th 2009

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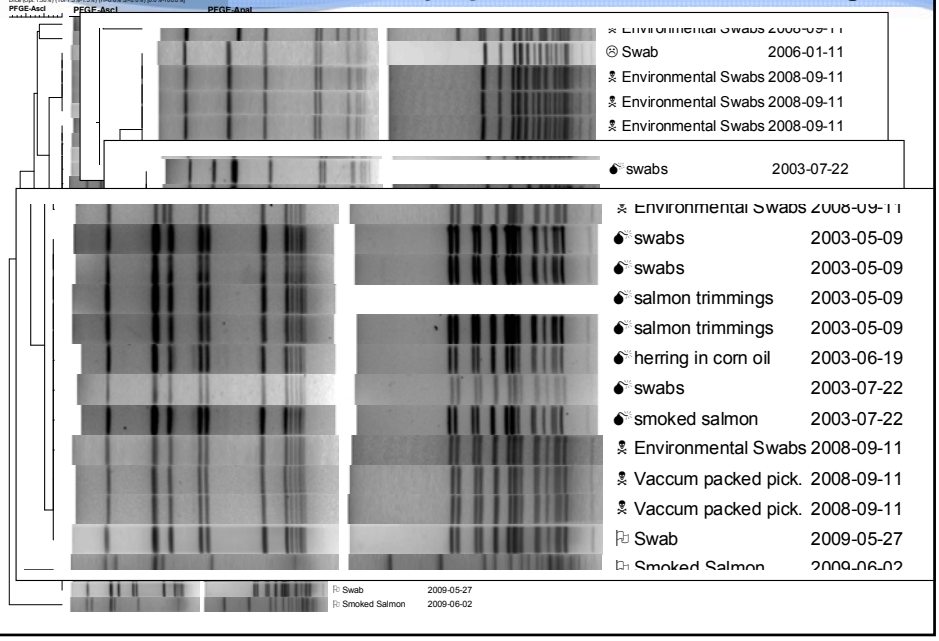
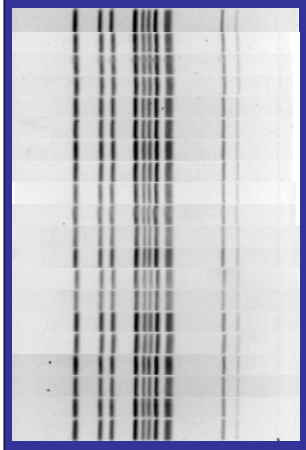
Listeria monocytogenes in a Seafood Processing Firm



Salmonella Agona (in cereal)

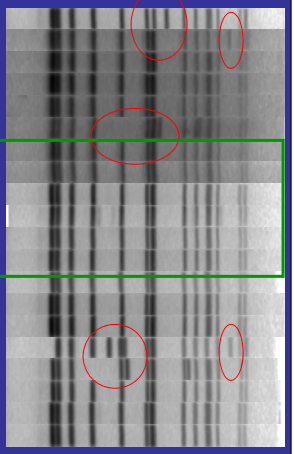
Interesting…

PFGE-XbaI



Cereal - Toasted Oats	1998-06-26
Swab	2008-04-09
Swab	2008-04-09
Swab	2008-04-09
Swab	2008-04-09
Swab	2008-04-09
Swab	2008-04-09
Cereal	2008-04-09
Swab	2008-04-10
Puffed Rice Cereal	2008-04-16
Puffed Rice Cereal	2008-04-16
Swab	2008-04-17
Swab	2008-04-17
Swab	2008-04-17
Swab	2008-04-17
Unsweeten Puffed Rice Cereal	2008-04-17
Unsweeten Puffed Rice Cereal	2008-04-17
Puffed Wheat Cereal	2008-04-18
Puffed Wheat Cereal	2008-04-18

PFGE-BlnI



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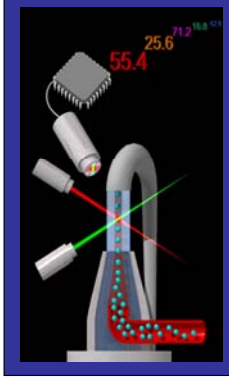
COLLABORATIVE SCIENCE WITH CDC



- **FDA-PULSENET**
  - in step with CDC innovations and new platforms (*i.e.*, MLVA)
  - continued populating of *Salmonella* national database with food and veterinary associated PFGE patterns
- **FACILE SEROTYPING METHODS**
  - CDC-developed 'BIOPLEX' assay for O-group antigen assignment
  - FDA scientists are training at CDC to adopt technology
  - validation and evaluation efforts underway

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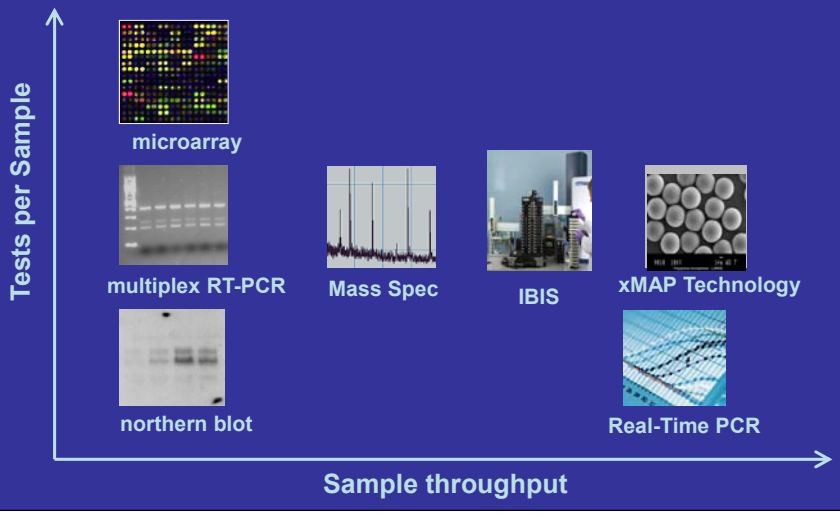
ONGOING PROJECTS



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ONGOING PROJECTS

Genome scanning strategies:

454 Technologies whole-genome sequencing systems:

- ► allows for multiple coverage of a complete bacterial pathogen's genome in 3 days
- ► provides raw data for SNP discovery
- ► allows for identification of rapidly changing genetic markers

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ONGOING PROJECTS

- **SPROUT SAFETY**
  - strategies for reducing *Salmonella* growth under commercial sprouting conditions
  - chemical and biological interventions being evaluated
  - part of FDA 'produce assignment'
- **SALMONELLA SURVIVAL STUDIES**
  - studies underway to assess survivability of *Salmonella* on fruit surfaces and in the food processing environment
  - studying resistances to desiccation stress and storage
- **TOMATO SAFETY…**

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TOMATO OUTBREAKS

- associated with specific geographical locations and growing seasons where there have been multiple outbreaks:

Eastern United States
- Late Spring – Florida
- Summer and Fall - Virginia
- Winter – Florida

- widely dispersed, individual patient-cases in many states
- trace backs are difficult due to complexity of the supply chain
- implicated produce is rarely still available, the crop is no longer in the field

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TOMATO OUTBREAKS

•	1998	*S.* Baildon	86 cases
•	2000	*S.* Thompson	29 cases
•	2002	*S.* Newport	512 cases
		S. Newport	12 cases
		S. Javiana	90 cases
•	2004	*S.* Javiana	471 cases
		S. Braenderup	123 cases
•	2005	*S.* Newport	71 cases
		S. Enteriditis	77 cases
		S. Braenderup	76 cases
•	2006	*S.* Newport	107 cases
		S. Typhimurium	186 cases

= *single PFGE type*

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TOMATO OUTBREAKS

- **Four Sources of Potential Contamination**
  - Soil
  - Domestic and Wild Animals
  - Water
  - Farm Workers




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PRELIMINARY TOMATO RESEARCH

partners: FDA-CDC-USDA/ARS-NCSU-UMD-VTECH

CURRENT SUBTYPING/MOLECULAR EPIDEMIOLOGY SUPPORT

►Genetic and molecular epidemiological analysis of outbreak and environmental strains of *S.* Newport from tomato farms located on the Virginia eastern shore -

- ●PFGE pattern comparisons with FDA foods and veterinary databases across several enzymes
- ●antibiotic resistance profiling
- ●MLST analysis

ECOLOGY STUDIES

►growth and survival characteristics on tomato fruit, pond water, other niches

►sampling of potential reservoirs associated with tomato farms (*i.e.*, ponds, native flora, migratory fowl)

►planned competition studies of *S.* Newport and other serovars on tomatoes

INTERVENTION

►in vitro and in vivo screening of tomato farm epiphytic microflora for antagonistic activity against *S.* Newport

►potential field application for biological control of *S.* Newport

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CURRENT METHOD DEVELOPMENTS IN STRAIN DIFFERENTIATION

PFGE-BASED

►exploration of novel restriction enzyme combinations and cluster methods
►enhanced PFGE schemes with up to six enzymes for retrospective analysis of closely related *Salmonella* strains

DNA SEQUENCE-BASED

►application of conventional multi-locus sequence typing (MLST) schemes to various homogeneous serovars
►currently exploring other 'hypervariable' gene targets for increased discriminatory power of highly homogeneous serovars (*i.e.*, *S.* Enteritidis)
►application of several novel SNP detection platforms for rapid subtyping of *Salmonella* strains

OTHER SUBTYPING METHODS BEING DEVELOPED

►LC-MS protein profiling
►Phenotype microarray

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The Risk