Challenges in Working with Food
Detection of Toxins and Bacterial Pathogens in Food for Human Consumption

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What is FERN?

• Network developed in 2004 to enhance nation’s ability to respond to threats to the food supply.
• Partnership among federal, state and local labs.
• Joint oversight provided by HHS-FDA and USDA-FSIS
Mission of FERN

Integrate the nation’s multilevel food-testing laboratories to detect, identify, respond to and recover from a bioterrorism or public health emergency/outbreaks involving the food supply.
Network provides support and guidance for:

- Food sampling procedures/sample integrity/sample handling
- Analytical detection methods: microbiology, chemistry, rapid assays and radiological methods
- Support Program Development & Implementation
FERN Objectives

• Detect and identify biological, chemical, and radiological threat agents
• Prevent threats: operate targeted federal/state surveillance sampling programs
• Prepare for threats: strengthen lab capacities and capabilities
• Respond to threats: contribute surge capacity
• Recovery: assure consumer safety via food testing
Laboratory Outreach

- 168 Laboratories representing 50 US States and Puerto Rico have satisfactorily completed FERN Laboratory Qualification Checklists
  - Affiliations:
    - 36 Federal (FDA, USDA/FSIS, USDA/AMS, DHS, CBP)
    - 117 State
    - 15 Local
  - Disciplines:
    - 132 Microbiological
    - 106 Chemistry
    - 33 Radiological
Recent FERN Activities

• Gulf Oil Spill
• Salmonella in peanut butter outbreak
• EHEC in spinach outbreak - EHEC
  – Method development/validation
  – Storeroom participation
• Melamine in pet food contamination
  – Chemistry labs involved
  – Method development/validation
• Food defense assignments
  – FDSA and PSA
The Food

• Infinite possibilities---Anything can potentially be contaminated with anything
  – Risk assessment
    • Cookie dough
• Food interferes with assays in ways we do understand
  – pH, color, change in texture
• Food interferes with assays in ways we do not understand
• Background flora
Background Flora

• Present to some extent in most foods
• Most undefined and changes
• Confound culturing and assays
• Big Problem!!
Stomacher

- Change matrix characteristics affecting pathogen detection and recovery
  - Release assay inhibitors
  - Release natural flora
  - Change pH
    - Fruits and vegetables
    - Kill the pathogen of interest
Surface Contamination

- **Diluent**
  - Standard amount
  - Based on weight
  - Based on surface area

- **Process**
  - Rinse
  - Scrub
  - Soak

- **Time**
- **Remove samples**
  - Where?
  - How much?
  - Process
Salmonella and tomatoes

- *Salmonella* serotype Saintpaul--2008
  - Rare serotype
- Entry into the tomato
  - Wounds in the stem
  - Breaks in the outer skin
  - Through the roots into the flower and fruit???
- Pathogen inside the tomato
  - Acid tolerant
- Method for internal contamination
Karl Matthews and colleagues at Rutgers University investigated whether bacteria were getting inside the lettuce, rather than just sitting on the leaves.

Small gaps in growing roots are a known port of entry for plant pathogens, and may allow *E. coli* to get in.

New project to examine pathogen-produce interaction
Traditional Enrichment

• Increase the concentration of organism of interest while reducing background flora

• Culture Medium
  – Antibiotics and antifungal agents
  – Inhibitors
    • Sodium azide, tellurite, bile salts
  – Field isolates sensitive to inhibitors
    • Literature
Enrichment Plating

• In the best case scenario can culture onto non-selective or minimally selective media from enrichment culture
  – Highly selective enrichment medium
  – “Clean” foods

• Plate onto selective media
  – Consider loss due to selective media
  – Mixed culture
Results

The Goal

Overgrowth

Reality

No Growth
Ice Cream
Rapid Methods

• Food interferes with most if not all rapid methods
• Most methods do not even take food into consideration
  – Water or Milk
• Culture still the most reliable, although food can also make culture a challenge
Select Agents

- Bacillus anthracis
- Yersinia pestis
- Francisella tularensis
- Brucella spp.
- Burkholderia spp.
FUN FACTS

OR…..Food is So much Fun
Potato Salad

- Deli food
- Purchased from local grocery store
- Complex matrix
  - Potatoes, carrots, onions, celery, mayonnaise, eggs
  - No artificial preservatives
- Background flora
- Fat
- STERILE and EASY TO WORK WITH!
Juice--Category

- All juices are not equal
- Hawaiian Punch
  - pH ≤ 2.0
  - Death within minutes
- V8
  - Pathogen death after 24 h
  - pH = 4.13
- Apple Juice
  - Pathogens survive just fine
Oregano

• Smells great and makes the autoclave smell good
• Sticks to side of the bag when placed in diluent
• pH $\geq 8.0$
  – Buffered medium
• Kills pathogens
  – Antibacterial properties
• PCR positive
• Representative of all spices???
Baby Food

- Not all baby foods are equal
- Squash vs. Sweet Potatoes
  - Think they would be the same
  - Completely different in immunocapture
- Baby Lasagna
  - Comparable to baby squash
- Database of baby food and isolation of pathogens using immunocapture
Conclusions

• Food makes things interesting

• Each food and each pathogen are unique making the combination an experience

• Expect the unexpected

• Have Fun!
Foodborne Disease

Classification of Foodborne Diseases

Poisonings
- Chemical Poisonings
  - Poisonous Plant Tissues
  - Poisonous Animal Tissues

Intoxications
- Microbial Intoxications
  - Algal Toxins
  - Mycotoxins
  - Bacterial Toxins
    - Enterotoxins
    - Neurotoxins
    - Carbohydrate Metabolism Interference
    - DNA Replication Interference
What are toxins?

• A poisonous substance produced by living cells or organisms

• Toxins can be:
  – Small molecules
  – Peptides
  – Proteins

• Interact with enzymes, cellular receptors

• Cleave essential protein residues in DNA replication
Toxin Characteristics

- Nonreplicative
- Noninfectious
- Noncommunicable
- Nontransmittable (human to human)
Toxin Characteristics

- Effects humans through manual contamination (ingestion, injection or contact)
  - Organism, tissue or pure toxin
  - Intentional or unintentional

- Variable in their physical characteristics and natural sources

- Most are proteinaceous

- Most are fairly stable at standard conditions
  - Ranges for pH and temperature tolerances vary from toxin to toxin

- Nonvolatile, colorless, odorless and tasteless
Toxin Gray Zone

• Biological toxins are NOT

1. Classic organic chemicals
   • biological toxins are inherently different from toxic chemicals
     – Biological toxins from same source can vary widely in quantity produced
     – Leads to the inability to develop universal guidelines for exposure

2. Infectious agents
   • Non-replicative non-living agents
   • Can not assign Bio-Safety Level (BSL 1 - 4)
     – Must perform risk assessment for each situation
     – Varies for each facility
Other Differences....

- **Biological Toxins**
  - Treatment
    - Prophylactic vaccines and anti-toxin treatment
  - Symptoms
    - Associated mostly with acute effects
  - Routes of Exposure
    - Primarily inhalation, ingestion and injection

- **Toxic Chemicals**
  - Treatment
    - Supportive treatment post-exposure
  - Symptoms
    - Associated with carcinogenic, mutagenic and chronic effects
  - Routes of Exposure
    - Vapor hazards and skin absorption
Toxins in food

- Biological agents/toxins
- Foods often implicated
- Problems with working with food
- Control
- Inactivation
- Prevention
  - Properly canning food (strict thermal processes)
  - Not eating from dented or bloated cans
  - Adding salt and nitrites to vacuum-packed food
This talk will be focusing on four main toxins:
- Botulism Toxin (neurotoxin)
- Staphylococcal Enterotoxin (enterotoxin)
- Ricin (plant tissue toxin)
- Aflatoxins (fungal toxins)

In addition we will touch on two other classes of toxins
- Shiga and Shiga-like toxins (enterotoxins)
- Marine Toxins (GI and neurotoxins)
Botulism Toxin

- Etiological agent *Clostridium botulinum*
  - Reservoir: soil, mud, water and intestinal tracts of animals
  - Toxins A-G; A, B, E and F cause human disease

- Toxicity – lethal dose = 1 ng/kg
  - 1 tsp could deliver 1.2 billion lethal doses

- Mechanism of Action
  - Blocks release of acetylcholine at neuromuscular junction in the peripheral and central nervous systems
Botulism

- Incubation period: 2 hours - 8 days (12-48 hrs)

- Symptoms
  - Early: nausea, vomiting, diarrhea and abdominal pain
  - Late: afebrile, headache, dizziness, lethargy, double vision, unresponsive pupils, dysphagia, ataxia, dry mouth, weakness, constipation, respiratory distress, symmetrical descending flaccid paralysis
  - Death usually caused by respiratory failure
  - Partial paralysis may persist for 6-8 months

- Fatality Rate
  - 35 to 65% in 3-10 days
Botulism

- Implicated Foods:
  - Improperly canned low acid foods
    - Green beans, corn, beets, asparagus
  - Smoked Fish
    - Salmon, trout
  - Fermented Foods
    - Fermented beaver tail (AK), sauerkraut, pickled vegetables (pickles, olives) (US)
  - Food Preserved in oil or vacuum packaging
    - Fish, potatoes, cheeses
  - Improperly home-cured hams
    - Slow diffusion of curing ingredients to deep tissue
    - Endemic areas with inadequate temperature conditions
    - Poland and Portugal outbreaks
Botulism

• Laboratory Diagnosis
  – Blood serum and stool specimens most routinely tested
    • Mouse neutralization assay
    • Recovery of *C. botulinum* from stool-culture
  – ELISA based methods
    • DIG-ELISA
    • M1M
  – Food History and Food Tested
    • Availability
    • Patient is positive
Cases of Botulism

- September 2006 – Bolthouse Farms Carrot Juice
  - 6 cases (3 GA, 1 FL, 2 Ontario)
- July 2007 – Castleberry’s Chili
  - 8 cases (2 IN, 3 TX, 3 OH)
- March 2009 – Bamboo Shoots
  - 152 cases (40 in need of respirators, 42 comatose)
Ricin Toxin

• Etiological agent *Ricinus communis*
  – castor bean plant
  – Native to Ethiopian region of East Africa
  – Becoming increasingly abundant in SW U.S. along stream banks and river beads

• Toxicity – Lethal Dose = 3-5 mg/kg (ingestion)
  – 2-4 seeds (beans) = severe adult poisoning (8 fatal = 1-20 mg/kg)
  – Inhalation is more lethal (21-42 μg/kg)

• Mechanism of Action
  – Interferes with protein chain elongation leading to cellular death (cytotoxin)
Ricin

- Incubation Period: 1 - 3 days

- Symptoms
  - Early: burning sensation in mouth, nausea, vomiting (blood), abdominal pain, diarrhea, stomach and gastrointestinal bleeding
  - Late: disorientation, weakness, stupor, excessive thirst, hematuria, multisystem organ failure, vascular collapse
  - Death due to hypokalmia (severe electrolyte imbalance and fluid loss)

- Fatality Rate
  - 2-6 %
Ricin

- By-product of castor oil production
- Most reported ricin intoxication cases have been intentional in nature
- Unintentional poisonings due to eating or chewing on castor beans
  - Jewelry makers use seeds as beads and may accidently ingest or inhale some ricin toxin.
  - Sometimes the seeds are boiled in order to facilitate the piercing of their hard seed coats
  - Plant increasingly being used as an ornamental garden and house plant leading to unintentional child poisoning
Ricin

• Laboratory Diagnosis
  – Time-resolved fluorescence immunoassay
    • Antibody that binds to ricin and fluoresces to enable detection in environmental and food samples
  – PCR
  – HPLC-ESI-MS (Clinical)
    • CDC developed method for measuring ricin, a marker for ricin exposure, in urine.

• Laboratory Precautions
  • Work in appropriate PPE for BSL-2 conditions in a Biosafety Class-2 cabinet

• Decontamination
  – 0.5% sodium hypochlorite solution with pH lowered to 6-8 by adding distilled white vinegar with a 30 minute contact time.
Cases of Ricin

• 1978 – Assassination by Ricin
  – Gregori Markov (Bulgarian effector) in London stuck in the leg with an umbrella with a platinum ball on the end containing ricin – Died 3 days later

• 1993 – Thomas Lavy (Neo Nazi)
  – arrested in Canada en-route to the US with enough ricin to kill 300,000 people, 4 guns and 20,000 rounds of ammo

• 1997 – Thomas Leahy
  – Arrested in a shooting. Raid of home finds Ricin lab in basement as well as evidence he was trying to grow *Clostridium botulinum*.

• 2003 – White Supremacy
  – An undisclosed amount of ricin was uncovered in a raid of a London apartment. Four men of unknown nationality suspected in white supremacy plot to attack ethnic groups

• 2009 – Homosexual Community Targeted
  – 11 Gay bars in Seattle WA received a letter indicating that the writer had in his possession 67 grams of ricin to which he would target 5 of their patrons at random
Staphyloccocal Enterotoxin

- Etiological agent *Staphylococcus aureus*
  - Reservoir: anterior nares of humans, hands and skin.
  - Toxins A-F

- Toxicity – less than 1 μg can cause illness
  - Can cause toxic shock syndrome in large doses
  - Inhalation dose much more potent (mortality rate of 60-80%)

- Mechanism of Action
  - When ingested, SET causes a dysregulation of intestinal fluid regulatory system (cyclic AMP)
Staphylococcal Enterotoxin

- Incubation period: 1-7 hours (usually 2-4 hrs)

- Symptoms
  - Sudden onset of nausea, excessive salivation, vomiting, retching, diarrhea, abdominal cramps, dehydration, sweating, weakness, prostration
  - Fever is not common
  - Short duration of symptoms (1-2 days)

- Fatality Rate
  - Classified as an incapacitating agent resulting in extreme morbidity following ingestion
  - Very high exposure can cause pulmonary edema and very rarely death
  - Inhalation LD$_{50}$ may be as low as 20 ng/kg
Staphylococcal Enterotoxin

- Implicated Foods
  - Cooked ham, meat products, poultry
  - Hand made sauces, dressings and gravy
  - Cream filled pastries
  - Potato salad
  - Milk, cheese, bread pudding

- Foods made in large batches that have been manipulated/made by hand and temperature abused

- Toxin is heat stable so reheating does not eliminate the preformed toxin in the food but will kill the organism producing the toxin.
Staphylococcal Enterotoxin

- **Laboratory Diagnosis**
  - Culture from vomitus or feces of ill, nasal swabs from infected food handlers, suspect food
  - ELISA methods (M1M, VIDAS, TECRA) for toxin detection
  - Enumeration (greater than $10^5$ MPN/gram)

- **Treatment**
  - Limited to supportive care
  - No vaccine or antitoxin has been developed
  - Artificial ventilation may be required in severe cases
Cases of Staphylococcal Enterotoxin Poisoning

- 2009 Virginia Case Study
  - Egg salad sandwiches prepared for adult daycare center by local church auxiliary.
  - Egg salad was reported to be made at home and not refrigerated.
  - 18 ill, 2 hospitalized
  - Incubation 3-4 hours
  - Symptoms: vomiting, sweating, diarrhea
Marine Toxins

Very fast acting GI and Neurological symptoms

- **Saxitoxin**
  - Paralytic Shellfish Poisoning - “red-tide” (misnomer)
  - Sodium channel blocker

- **Ciguatoxin**
  - Reef fish including grouper, snapper and mackerel
  - GI, itching, cardiovascular disorders, CNS dysfunction

- **Conotoxin**
  - Paralytic poison of Pacific cone snail
  - Blocks nerve impulse from nerve to muscle

- **Scombroid Poisoning (Histamine)**
  - Most common form of fish poisoning in U.S. – Tuna, mahi-mahi, sardines and anchovies
  - GI and neurological – flushing and rash

- **Tetrodotoxins**
  - Pufferfish (Fugu), starfish, parrotfish - produced by associated bacteria
  - Neurotoxin (sodium channel blocker)
Shigatoxin and Shiga-like toxin

- Structurally and functionally identical
  - Shigatoxin – *Shigella dysentariae*
  - Shiga-like toxins – *Escherichia coli* (STEC)
- Severe diarrhea, abdominal pain
- Hemolytic uremic syndrome – severe kidney complication
- Inhibit host cellular protein synthesis
- Common in ground beef and produce irrigated with infected runoff water
- Treatment is supportive
  - Certain antibiotics trigger toxin release by activating lytic phage
QUESTIONS?

questions anyone?
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Staphylococcal Enterotoxin

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