Early Detection of Foodborne Pathogens

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Outline

- Overview of foodborne pathogen issue
  - Our focus: Biosensor detectability
- Testing process and sources of variation
- Preliminary stochastic model
  - Results
- Further studies and issues

Foodborne Pathogens

- The CDC estimates 76 million people suffer foodborne illnesses each year in the US with 325,000 hospitalizations and over 5,000 deaths.
- Yearly estimated cost of foodborne illnesses is 5 to 6 billion dollars in direct medical expenses and lost productivity.

- Known pathogens account for an estimated 14 million illnesses, 60,000 hospitalizations, and 1,800 deaths.
- The most severe cases tend to occur in the very old, the very young, those who have an illness already that reduces their immune system function.

Monitoring and Management

- Government agencies (FDA and USDA) and the food industry have taken many steps to reduce contamination of food by pathogens
- Processed food monitored/ tested regularly

  Food Safety and Inspection Service (FSIS)
- When a processed food is found to be contaminated, food monitoring and plant inspections are intensified, and if necessary, the implicated food is recalled.
- Goal: Discover contamination prior to food leaving the plant and take corrective action

Management

- Implementation of Hazard Analysis and Critical Control Point (HACCP) Systems (1996)
  1. Analyze hazards
  2. Identify critical control points
  3. Establish preventive measures (critical limits)
  4. Establish procedures to monitor
  5. Establish corrective actions
  6. Establish procedures to verify the system
  7. Establish effective recordkeeping
- First implemented by NASA for astronauts

Surveillance

- Surveillance complicated by several factors.
  - **Underreporting**: milder cases are often not detected through routine surveillance
  - **Cause**: Many pathogens transmitted through food are also spread through water or from person to person
  - **Identifiability**: Some foodborne illnesses caused by pathogens have not yet been identified and thus cannot be diagnosed.
    - Many of the pathogens of greatest concern today (e.g., *Listeria monocytogenes*) were not recognized as causes of foodborne illness just 20 years ago.

Testing

- Current testing procedures can take weeks to get conclusive result ↔ food already on the market
- Want devices that detect pathogen quickly and at very low numbers

- FDA and USDA – testing process / device should result in no false negatives

Risk Assessment

- FSIS Risk Assessment for Lysteria monocytogenes in Deli Meats (2003)
  - Monte Carlo in-plant model describing concentration of L. monocytogenes in deli meat at retail
  - Subsequent models describe retail-to-table assessment and dose-response relationship
- Will focus here on one component of model – in-plant detection of contamination

Listeria Monocytogenes

- Dangerous foodborne pathogen, especially to those with a weakened immune system
- Vegetables can become contaminated from the soil or from manure used as fertilizer.
- Animals can carry the bacterium without appearing ill and can contaminate foods of animal origin such as meats and dairy products.
- Killed by pasteurization and cooking; however, in certain ready-to-eat foods such as hot dogs and deli meats, contamination may occur after cooking but before packaging.
- Since it grows at low temperatures (i.e., during refrigeration), need to detect pathogen at very low concentrations prior to distribution to stores

Our Focus

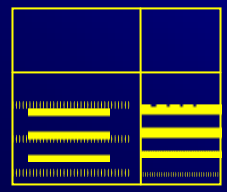
- Biosensor has been developed to rapidly detect pathogen
- Question: What is the probability of detecting target cell in contaminated processed food
  - Under different contamination concentrations?
  - Under different testing protocols?
- Strategy: Break process down into components we can study

General Procedure

Ziplock Bag

Hot dog (~50g) + PBST (0.05% Tween

Stomach machine (230 rpm) for 2 minutes



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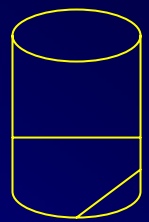
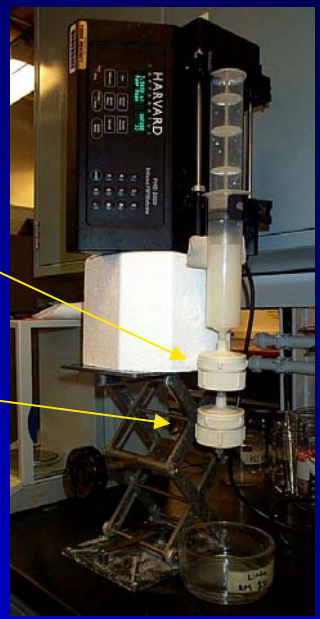


Resuspended in 250 mL PBS buffer

Run CCR Kit

2.7 µm glass filter paper

0.4 µm pc membrane



Portion of 500 µL sample applied to biosensor

General Procedure

Stage I

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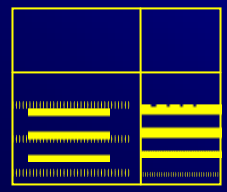

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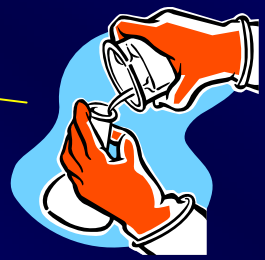

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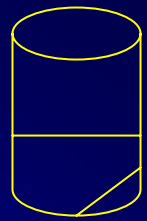

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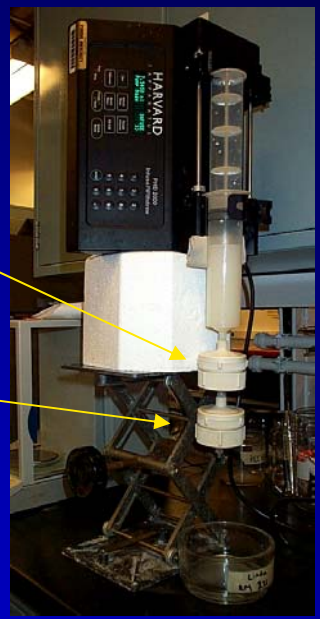

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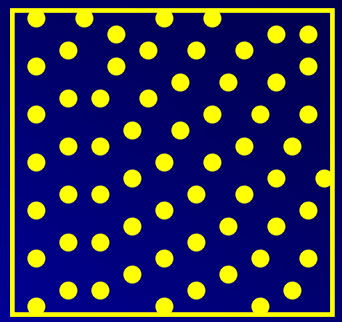
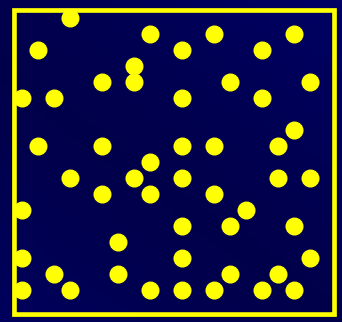
Stage 1 Procedures

- Processed food is sampled from plant and prepared for testing using best current practices
- Issues to consider
  - Spatial distribution of *L. monocytogenes* on food
  - Distribution of contamination in plant (hot spots?)
  - Spread and growth of contamination
- We focus on sampled product that has been injected with known number of pathogen cells

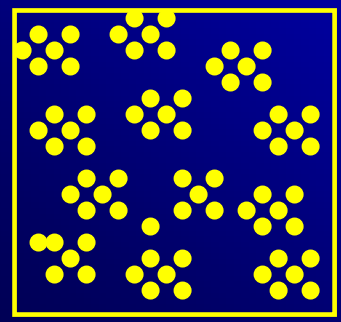
SPATIAL DISTRIBUTION



Regular distribution



Random distribution



Tendency to aggregate

Random distribution has been assumed but this has not been studied.

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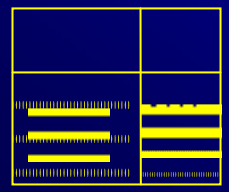

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Stage II

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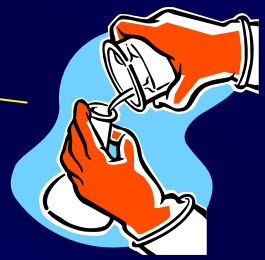

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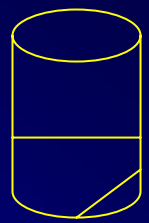

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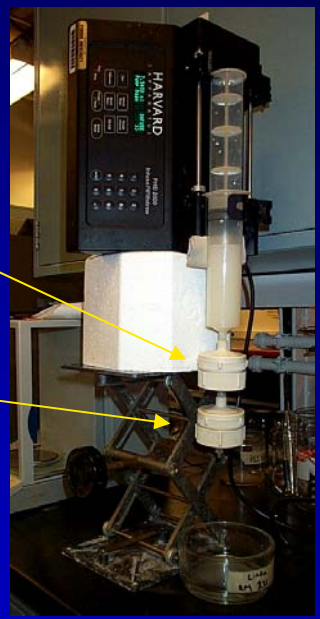

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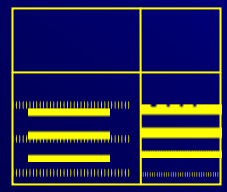

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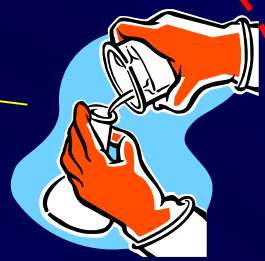

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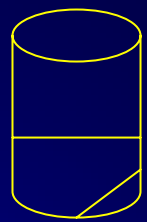

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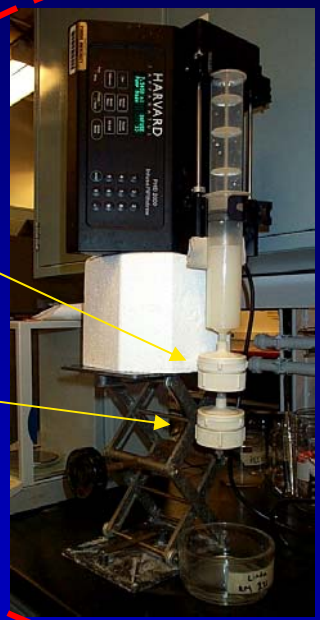

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Retention of Cells

- Will lose target cells during filtration processes
  - Question: How much meat should be sampled prior to filtration steps?
  - Question: How much volume must be processed in CCR filtration to guarantee retention of an adequate number of target cells?

Modeling the retention of cells

- A few experiments have been performed to investigate this
- System continually being upgraded

- Largest loss during CCR filtration
  - Depth and membrane filter
- Studies show this to be roughly 28%

- Less loss occurs in earlier filtration steps
- Previously modeled p_{ret}~Beta(50,50)

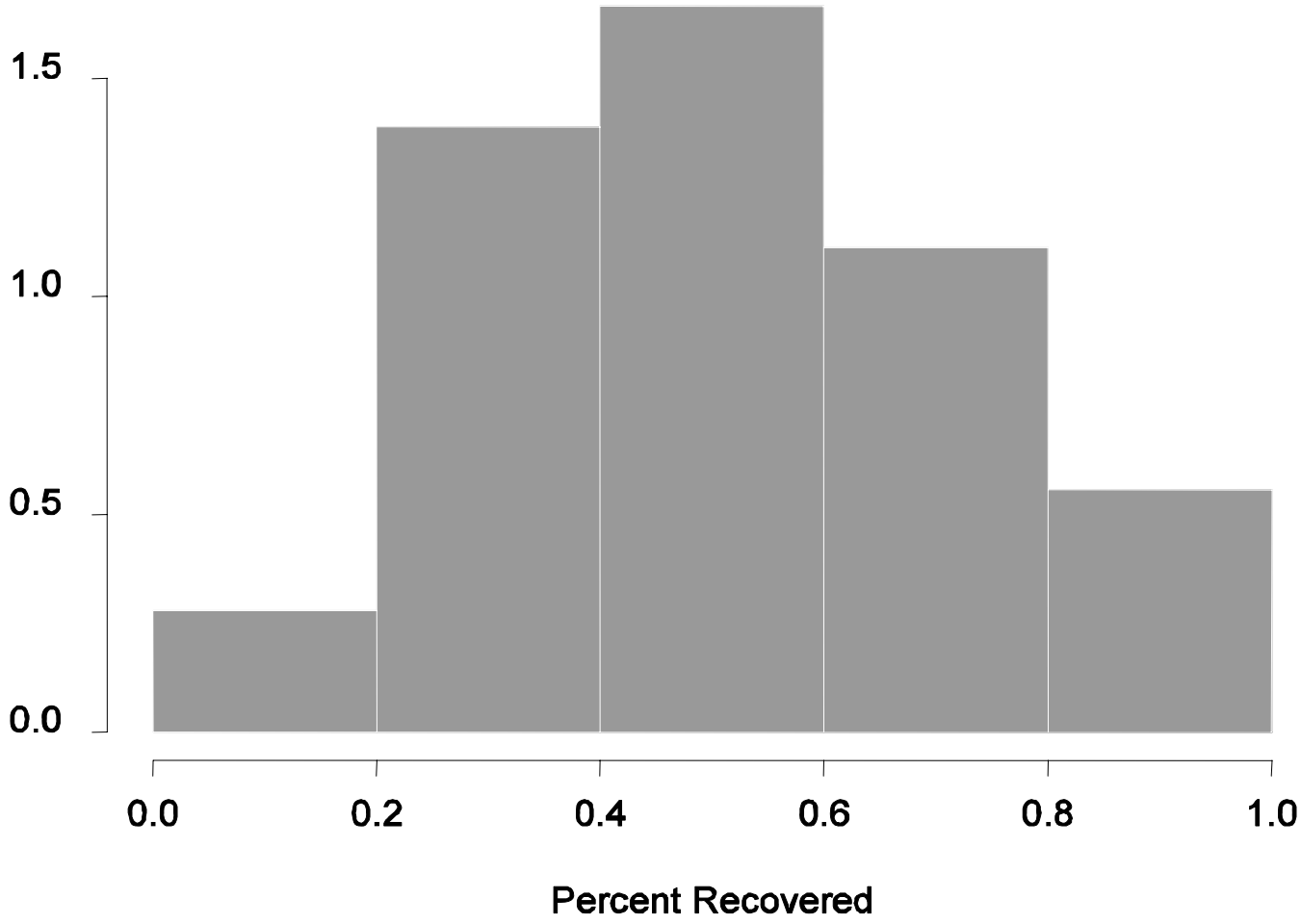
Retention through Depth Filter

- 6 runs
- Each run
  - Inject buffer with specific concentration
  - Divide buffer into 4 samples
  - Plate out one sample, run remaining three through the filter and plate out
  - After incubation, count the number of colonies

Comparison of Plates

Retention through Depth Filter

- Assume N_i live target cells (i=1,2,…,6)
- Retain X_{ij} live target cells (j=1,2,3)
- Investigated both
  - Binomial
  - BetaBinomial
- BetaBinomial provided better fit
  - $\hat{p} = 0.53, \hat{\alpha} = 3.345, \hat{\beta} = 2.969$


1.5
1.0
0.5
0.0
0.0
0.2
0.4
0.6
0.8
1.0
Percent Recovered

Retention through CCR

- Depth filter one component of CCR
- Limited data on overall retention
  - Average of 28% retention
  - Range 15% to 58%

- Will assume CCR is the same as two independent depth filters

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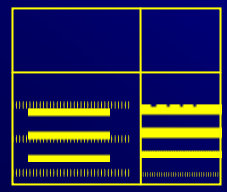

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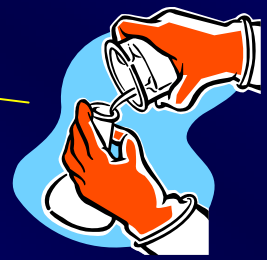

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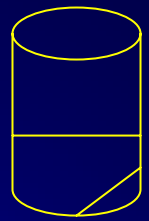

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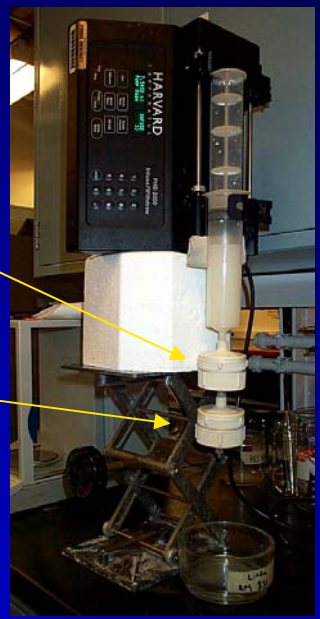

Stage IV

Portion of 500 µL sample applied to biosensor

Capture of Target Cells

- Biosensor might not capture target cells in biosensor
  - How many target cells are needed in a sample for the biosensor to guarantee capture of cells?
  - How large a volume should be placed on biosensor? Is 50 µL enough? Should multiple samples be studied?

Modeling the Probability of Capture

- Currently no empirical results to help describe this probability
- Have instead implemented detection limit
  - 1 cell : Biosensor will always detect pathogen if at least one cell is present in the sample
  - 5 cells: Biosensor will always detect pathogen if at least 5 cells are present
  - Could build model to describe probability over a range of cell numbers

Monte Carlo Example

- Assume N living target cells in food source
- Simulate filtration process (2 filter steps)
  - $p_1 \sim \text{Beta}(\alpha,\beta)$ and $p_2 \sim \text{Beta}(\alpha,\beta)$
  - $N_1 \sim \text{Bin}(N,p_1)$ and $N_2 \sim \text{Bin}(N_1,p_2)$
- Sampling:
  - Random distribution of remaining cells
  - p = sample volume / 500 µL
  - Cells on biosensor ~ $\text{Bin}(N_2,p)$

Detection Probabilities

N	Volume	P(Detect1)	P(Detect5)
10	250	83.8	13.6
10	500	100.0	30.9
15	250	88.2	16.2
15	500	100.0	48.2
20	250	91.5	23.1
20	500	100.0	60.5
25	250	96.5	51.1
25	500	100.0	71.1

Future Collaboration

- Capture of target cells in biosensor
  - Utilize fluorescent dye to count cells
- Better quantify recovery distribution
- Target cell distribution
  - Is it random in 500 µL buffer?
  - Less likely in food product?
- False positives?