FDA FOOD SAFETY CHALLENGE

Team: Purdue University

Physical Method for Concentrating *Salmonella* to Detectable Methods Using Automated Microfiltration

July 7, 2015
Team Purdue
Microbiological Analytical Methods

Sample Preparation + Detection = SCREENING METHOD

Sample Preparation + Detection + Isolation Identification = CONFIRMATION METHOD

2 to 4 hours + 3 to 4 hours = 5 to 8 hours

24 to 48 hours + 2 to 4 hours + 24 to 48 hours = 2 to 4 days
Ask New Questions

1. What do I expect not to find? How could I attune to the unexpected?

2. What might I be discounting or explaining away a little too quickly?

3. What would happen if I shifted one of my core assumptions on an issue, just as an experiment?

Source: Delighting in the possible, McKinsey Quarterly, March, 2015
Trying to Shift a Core Assumption

Shaker Flask

Petri Dish

Hollow Fiber Module

Single Hollow Fibers

200 μM

Feed stream

Permeate
Microfiltration

Practiced for 70 years.

Fouling (short membrane life, long processing times, decreasing flux) is a consistent challenge.

Many interacting mechanisms cause reduced yield upon filtration or microfiltration.

Addition of enzyme results in high flux and membrane re-use

Characteristics of a biological material. Different types of samples require different processing conditions.
**Enzyme Treatment**

Proposed protocol:

- **Sample**
  - Enzyme incubation (*Salmonella enrichment*)
  - Pre-filtration
  - Microfiltration and centrifugation
  - *Salmonella* detection (BAX or conventional PCR)

USDA and FDA approved Molecular biology based method (PCR)

Detection (1 day later)

Non-selective Enrichment

**Days 0**

**Hours 0, 2, 4, 6, 8**
Start microfiltration of enzyme treated spinach extract
2 samples being run in parallel
4 minutes later – approaching end of run
At 6 minutes sample collected in plastic tube
Sample tube removed from instrument
Decant into centrifuge tube
Centrifuge for 10 min
What might we be discounting or dismissing?

How fast per sample?

How many samples per day?

At what cost?

Compatibility with Detection Techniques?

Ease of use?

Role of enrichment?

Scale-up (commercialization) pathway
Cleaning system and membranes between uses:

Reuse membrane: 15-20 times

<table>
<thead>
<tr>
<th>Step</th>
<th>Reagent used</th>
<th>Time (min)</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>200 mM NaOH</td>
<td>5</td>
</tr>
<tr>
<td>2</td>
<td>70% (v/v) ethanol</td>
<td>10</td>
</tr>
<tr>
<td>3</td>
<td>Sterile water</td>
<td>5</td>
</tr>
<tr>
<td></td>
<td>Total time for cleaning</td>
<td>20</td>
</tr>
</tbody>
</table>

After step 1: cleaning with 0.2 M NaOH

After step 2 and 3: cleaning with 70% ethanol
Large volumes for triaged samples

Step 1: Large volume (10 L to 55 mL)

Step 2: Small Volume (55 mL to 0.5 mL)

≈ 10³ CFU / mL
≈ 10⁴ CFU / mL

BBL™ brain heart infusion agar
PCR result for initial cell concentration of 1 CFU/G spinach

Initial volume of 500 mL with 3 hr enrichment (lactose then RV) Automated microfiltration followed by centrifugation = $10^3$ CFU/g in final volume of 1 mL for samples S1, S2, and S3.  
PC = positive control.  NC = negative control
## Current Cost of Goods

Membranes drive cost of assay. Cost reduction being pursued.

<table>
<thead>
<tr>
<th>Item</th>
<th>Unit Cost</th>
<th>Cost/Sample</th>
</tr>
</thead>
<tbody>
<tr>
<td>Hollow Fiber (20 uses)</td>
<td>$175 / module</td>
<td>$8.75</td>
</tr>
<tr>
<td>Pre-filtration Membrane (5 uses)</td>
<td>4. / membrane</td>
<td>0.80</td>
</tr>
<tr>
<td>Enzyme</td>
<td>8. / kg</td>
<td>0.04</td>
</tr>
<tr>
<td>Aseptic Cleaning Solution</td>
<td>8. / L NaoH</td>
<td>0.48</td>
</tr>
<tr>
<td></td>
<td>1. / L Ethanol</td>
<td>1.00</td>
</tr>
<tr>
<td>Plastic ware, microfuge tubes</td>
<td></td>
<td>1.00</td>
</tr>
<tr>
<td><strong>Total / sample</strong></td>
<td></td>
<td>≈ $12.</td>
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</tbody>
</table>