Quantitative Biomapping for Risk-Based Pre-and Post-harvest Food Safety Management using Statistical Process Control

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1. WHY Salmonella and Campylobacter + indicators in Biomapping studies to establish process Baselines??

2. WHAT parameters to measure Process Microbial Performance under high vs low chemical scheme process?

3. WHERE and WHEN should we Sample in a poultry processing operation?

4. HOW MANY samples, replications and repetitions are needed and HOW OFTEN should this be repeated?

5. HOW should these surveillance activities be conducted and HOW is the Data Analyzed?
1. WHY Salmonella and Campylobacter + indicators in Biomapping studies to establish process Baselines?

Figure 2: Estimated percentage of foodborne *Salmonella* illnesses (with 90% credibility intervals) for 2019, in descending order, attributed to each of 17 food categories, based on multi-year outbreak data, *United States. Click here to download relevant data.*

**Salmonella**

**Chicken and Turkey = 23.4%**

<table>
<thead>
<tr>
<th>% attribution</th>
<th>16.8</th>
<th>13.5</th>
<th>12.8</th>
<th>12.6</th>
<th>7.3</th>
<th>6.6</th>
<th>6.3</th>
<th>6.2</th>
<th>4.2</th>
<th>4.2</th>
<th>3.8</th>
<th>2.6</th>
<th>1.4</th>
<th>0.9</th>
<th>0.9</th>
<th>&lt;0.1</th>
<th>&lt;0.1</th>
</tr>
</thead>
<tbody>
<tr>
<td>Cumulative attribution</td>
<td>75.9%</td>
<td>24.2%</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
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</tr>
</tbody>
</table>

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Poultry Performance Standards, US

1. WHY Salmonella and Campylobacter + indicators in Biomapping studies to establish process Baselines?

<table>
<thead>
<tr>
<th>Product</th>
<th>Maximum of Acceptable Positives</th>
<th>Performance Standard</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td><strong>Salmonella (%)</strong></td>
<td><strong>Campylobacter (%)</strong></td>
</tr>
<tr>
<td>Whole chicken</td>
<td>9.8</td>
<td>15.7</td>
</tr>
<tr>
<td>Whole turkey</td>
<td>7.1</td>
<td>5.4</td>
</tr>
<tr>
<td>Ground chicken (325g)</td>
<td>25</td>
<td>1.9</td>
</tr>
<tr>
<td>Ground turkey (325g)</td>
<td>13.5</td>
<td>1.9</td>
</tr>
<tr>
<td>Chicken parts (4lb)</td>
<td>15.4</td>
<td>7.7</td>
</tr>
</tbody>
</table>
Why Biomapping?

1. **Process mapping.** “Collect samples for one or more indicator bacteria at one or more points in the process (rehang, post-chill, after cut-up, etc.) while also collecting samples for the pathogen of interest on incoming and final product... this... will give the establishment more ongoing information about process performance”.

2. **Correlate pathogen vs. indicator levels.** “Compare pathogen levels on incoming and final product to determine whether the process is achieving the desired level of reduction of microbial load (measured in log). If the process is functioning correctly, then the results for indicator species represent the process when it is functioning correctly. If the process is not functioning correctly, make adjustments...”
Why Biomapping?

3. **Establishing limits.** “If the results for pathogens demonstrate that the process is functioning correctly, use the sample results for indicator bacteria to establish a maximum acceptable limit for each indicator and collection point. Common statistical techniques include setting the limit 2 or 3 standard deviations above the mean.

4. **Define actions.** “...to take if results are above the limits set in step 3. This plan should include what the action will be, who will take the action, how it will be recorded, and how it will be verified...”.

---

**Table 1 - Indicator Organism Median Values for Chickens**

<table>
<thead>
<tr>
<th></th>
<th>Median (CFU/mL)</th>
<th></th>
<th></th>
<th></th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Generic E. coli</td>
<td>APC</td>
<td>Enterobacteriaceae</td>
<td>Total Coliform</td>
</tr>
<tr>
<td>Carcass - Rehang</td>
<td>540</td>
<td>28,000</td>
<td>1,600</td>
<td>940</td>
</tr>
<tr>
<td>Carcass - Post Chill</td>
<td>20</td>
<td>260</td>
<td>20</td>
<td>20</td>
</tr>
<tr>
<td>Skin-on Parts*</td>
<td>20</td>
<td>10,000</td>
<td>160</td>
<td>50</td>
</tr>
<tr>
<td>Skin-off Parts*</td>
<td>20</td>
<td>53,000</td>
<td>450</td>
<td>110</td>
</tr>
<tr>
<td>Necks</td>
<td>95</td>
<td>16,000</td>
<td>275</td>
<td>165</td>
</tr>
<tr>
<td>Giblets</td>
<td>20</td>
<td>1,900</td>
<td>60</td>
<td>40</td>
</tr>
<tr>
<td>Comminuted</td>
<td></td>
<td>Not available from FSIS sources</td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

* Excluding necks & giblets

**Table 2 - Indicator Organism Median Values for Turkeys**

<table>
<thead>
<tr>
<th></th>
<th>Median (CFU/mL)</th>
<th></th>
<th></th>
<th></th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Generic E. coli</td>
<td>APC</td>
<td>Enterobacteriaceae</td>
<td>Total Coliform</td>
</tr>
<tr>
<td>Carcass - Rehang</td>
<td>22</td>
<td>1,800</td>
<td>50</td>
<td>40</td>
</tr>
<tr>
<td>Carcass - Post Chill</td>
<td>&lt;1.2</td>
<td>18</td>
<td>&lt;1.2</td>
<td>&lt;1.2</td>
</tr>
<tr>
<td>Skin-on Parts*</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Skin-off Parts*</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Necks</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Giblets</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Comminuted</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

* Excluding necks & giblets
Biomapping for Statistical Process Control. SPC

Sanchez-Plata et al, 2018

Aerobic Plate Counts

Live Receiving  Scalding  Defeathe ring  Re-Hanging  Pre-Washes  Post-Washes  Pre-IOBW  Post IOBW  Pre-Chill Dip  Post-Chiller  Post-Chill Dip

A  A  A  B  B  C  C  C  D  D

NaOCl 50ppm  NaOCl 50ppm  NaOCl 50ppm  PAA 120 ppm  PAA 100 ppm  PAA 100 ppm  PAA 400 ppm

7.67  7.55  7.40  6.11  6.16  5.29  5.18  4.52  1.60  2.36  0.84
Biomapping for Statistical Process Control. SPC

Sanchez-Plata et al, 2018

Feather and dirt removal

Viscera removal and rinses

Combined chiller effect

Enterobacteriacea Counts

NaOCl 50ppm

NaOCl 50ppm

NaOCl 50ppm

PAA 100 ppm

PAA 100 ppm

PAA 400 ppm

NaOCl 50ppm

PAA 100 ppm

PAA 100 ppm

PAA 400 ppm

Enterobacteriacea Log10 cfu/mL

6.23 7.01 7.15 5.90 5.51 5.02 4.75 3.81 0.34 0.28 0.24
3. WHERE and WHEN should we Sample in a poultry processing operation?

Experimental Design for Baselines

5 different processing days (if weeks even better):
- flock and day effect. Modify intervention regime
2 shifts: morning and afternoon:
- time and accumulation effect

5 samples per process location/shift:
- 10 samples per location x 5 days = 50-52 total samples

- Total Viable Counts
- Enterobacteria Counts, EB (COL or EC)
- Campylobacter Counts & %
- Salmonella Counts & %
APC Counts. High vs. Low Chem

Normal Process (CX)  Reduced Process (RC)  Incoming Load

Live Receiving  Re-hanger  Post Evis  Post-Cropper  Post-Neck Breaker  IOBW 1  IOBW 2  Pre-Chiller  Post Chiller  Wings

T-Test, P = 0.006  T-Test, P = 0.215  T-Test, P = 0.32  T-Test, P < 0.001  T-Test, P = 0.027  T-Test, P = 0.159  T-Test, P < 0.001  T-Test, P < 0.001  T-Test, P < 0.001

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Statistical Process Control Salmonella Prevalence Parts

Intervention Prevalence and Control Limits
N: 25 and Detection ONE YEAR= 12.2%

Sanchez-Plata et al, 2019
4. HOW MANY samples, replications and repetitions are needed and HOW OFTEN should this be repeated?

Detection: +
Concentration: ? But low

Detection: +
Concentration: ? But high
4 of 18 Positive = 22.2% prevalence
But only 1 > 3 logs (> 1,000) CFU

How would we categorize risk in a range from 1 – 6 Log CFU/mL?
Salmonella. Probability of Illness vs. Log Dose

FAO/WHO Risk Assessment, 2002
Direct counting (optical microscopy)
- Limited application
- Interference

Most Probable Number (MPN)
- Cumbersome
- Very expensive

Direct Plating
- Not certain that is actually *Salmonella*
- Requires confirmation

Quantitative PCR (qPCR)
- With or without enrichment/recovery
- LOD vs. LOQ
- Matrix dependent

Digital PCR (dPCR)
- In development

**Quantifying Pathogens**

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Salmonella Prevalence vs Counts

Sanchez-Plata et al, 2021
Not a normal distribution after PAA intervention (Shapiro-Wilk, p-value <0.05).

**Non-parametric** test by Wilcoxon to compare distribution of the range Mann-Whitney test.
Salmonella Prevalence. High vs. Low Chem

Devillena et al, 2022
Salmonella Enumeration: High vs. Low Chem

Devillena et al., 2021

Location | CX | RC
--- | --- | ---
LR | 2.77 | 2.77
Defeathering | 0.39 | 0.84
Carcass washing | 0.34 | 0.59
Chilling | 0.02 | 0.21
Parts | 0.13 | 0.20

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Salmonella P% vs. E#. High vs. Low Chem

Devillena et al, 2022
SPC with and without chemical interventions. 

**SA vs US: Post-Chill**

Sanchez-Plata et al, 2018

Baseline Post Chill US: 1.30 log
Validation of Interventions. Monitoring Data

<table>
<thead>
<tr>
<th>Location</th>
<th>N</th>
<th>$\log_{10}(CFU/mL +1)$</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td>Average</td>
</tr>
<tr>
<td>Pre-Chilling</td>
<td>133</td>
<td>2.18</td>
</tr>
<tr>
<td>Post-Chilling</td>
<td>425</td>
<td>2.49</td>
</tr>
<tr>
<td>Pre-Chilling</td>
<td>133</td>
<td>0.39</td>
</tr>
<tr>
<td>Post-Chilling</td>
<td>421</td>
<td>1.86</td>
</tr>
</tbody>
</table>

Sanchez-Plata et al, 2018
<table>
<thead>
<tr>
<th>Year 1</th>
<th>Year 2</th>
<th>Year 3</th>
</tr>
</thead>
<tbody>
<tr>
<td>Farm 1</td>
<td>Farm 2</td>
<td>Farm 3</td>
</tr>
<tr>
<td>Farm 4</td>
<td>Farm 5</td>
<td>Farm 6</td>
</tr>
<tr>
<td>Farm 7</td>
<td>Farm 8</td>
<td>Farm 9</td>
</tr>
<tr>
<td>Farm 10</td>
<td>Farm 11</td>
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</tr>
<tr>
<td>Farm 31</td>
<td>Farm 32</td>
<td>Farm 33</td>
</tr>
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</table>

### Flocks per Year

Sanchez-Plata et al, 2018
Biomapping. **Pre-harvest (boot swabs)**

<table>
<thead>
<tr>
<th>H</th>
<th>Load day 21-28</th>
<th>Ranking</th>
<th>Schedule</th>
</tr>
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<tbody>
<tr>
<td>1</td>
<td>6.42</td>
<td>3</td>
<td>Last</td>
</tr>
<tr>
<td>2</td>
<td>4.40</td>
<td>1</td>
<td>First</td>
</tr>
<tr>
<td>3</td>
<td>4.32</td>
<td>1</td>
<td>First</td>
</tr>
<tr>
<td>4</td>
<td>4.44</td>
<td>1</td>
<td>First</td>
</tr>
<tr>
<td>5</td>
<td>6.00</td>
<td>3</td>
<td>Last</td>
</tr>
<tr>
<td>6</td>
<td>2.96</td>
<td>1</td>
<td>First</td>
</tr>
<tr>
<td>7</td>
<td>4.32</td>
<td>1</td>
<td>First</td>
</tr>
<tr>
<td>8</td>
<td>3.93</td>
<td>1</td>
<td>First</td>
</tr>
<tr>
<td>9</td>
<td>4.32</td>
<td>1</td>
<td>First</td>
</tr>
<tr>
<td>A</td>
<td>4.58 ± 0.95</td>
<td></td>
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</tr>
</tbody>
</table>

Investigate

Log CFU/400 ml of boot swab rinse

Learn

Log 4.90 log cfu

Boot Swabs

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Contamination Level Reduction vs. Risk Reduction

Figure 5.7 Four representative illustrations of the effect on the reduction in risk of reducing the contamination level.
Risk-based Categorization. P & C

Category 1. Consistent Process Control:
50% or less of the 52-week moving window in the last 6 months

Category 2. Variable Process Control:
50% or more of 52-week moving window in the last 6 months

Category 3. Highly Variable Process:
Exceeds the performance standard for the 52 moving window cycle in the last 6 months

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6. **WHAT** type of projects can you consider to estimate **Process Microbial Performance**?

- Farm and flock risk-ranking
- Pre-harvest intervention decision-making
- Flock management continuous improvement
- Customized processing, lot separation/ scheduling trials
- Validation of interventions: physical, chemical, etc.
- Microbial baselines for plant-to-plant comparison
- Baselines for sanitary dressing procedures optimization
- Baselines for equipment adjustment, size, uniformity performance
- Baselines to compare line speed modifications for NPIS
- Overall process continuous improvement and risk assessments
Questions?

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