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

TEXAS TECH UNIVERSITY

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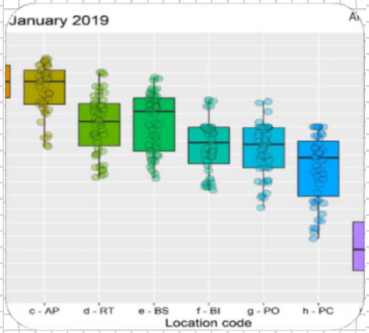
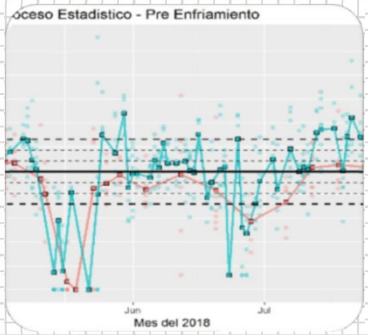
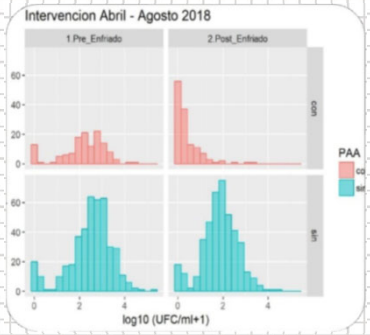
Outline







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?

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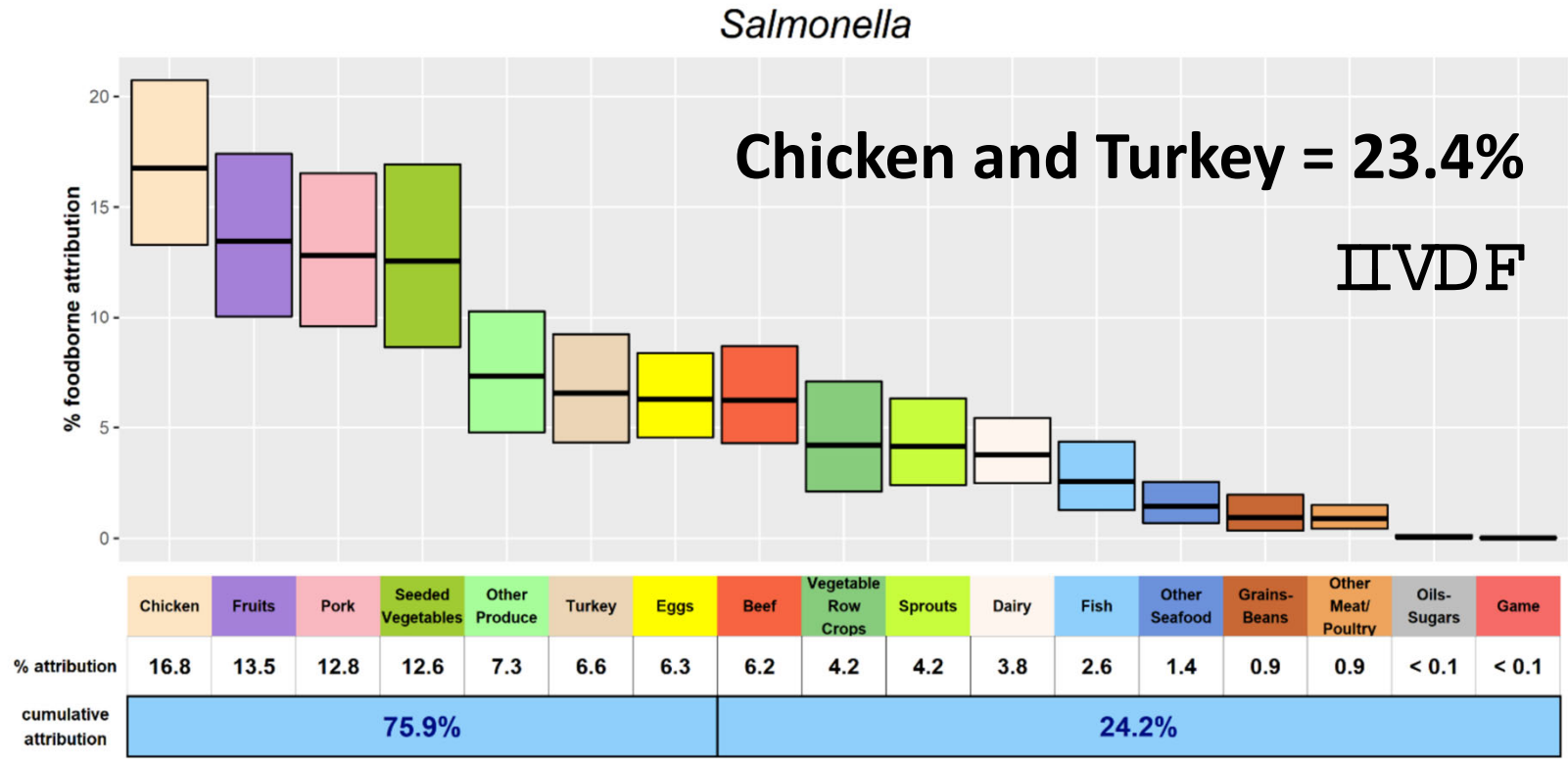
Interagency Food Safety Analytics Collaboration

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.



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Poultry Performance Standards, US

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

Product	Maximum of Acceptable Positives		Performance Standard	
	Salmonella (%)	*Campylobacter (%)*	*Salmonella*	*Campylobacter*
Whole chicken	9.8	15.7	5 of 51	8 of 51
Whole turkey	7.1	5.4	4 of 56	3 of 56
Ground chicken (325g)	25	1.9	13 of 52	1 of 52
Ground turkey (325g)	13.5	1.9	7 of 52	1 of 52
Chicken parts (4lb)	15.4	7.7	8 of 52	4 of 52

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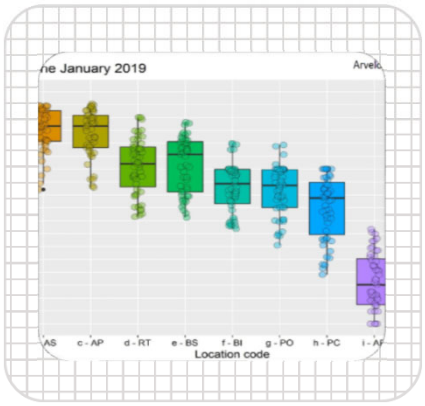
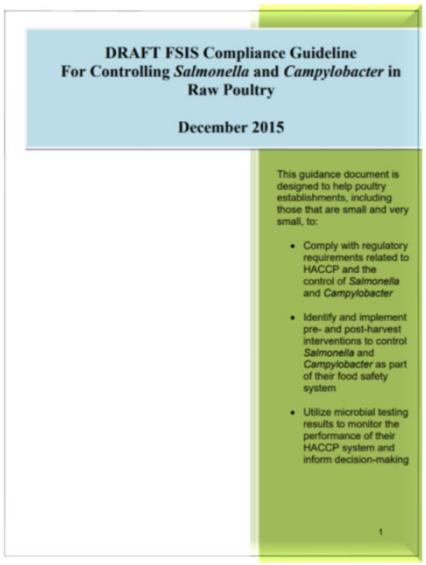
Why Biomapping?



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



DRAFT FSIS Compliance Guideline
For Controlling *Salmonella* and *Campylobacter* in Raw Poultry

December 2015

This guidance document is designed to help poultry establishments, including those that are small and very small, to:

- Comply with regulatory requirements related to HACCP and the control of *Salmonella* and *Campylobacter*
- Identify and implement pre- and post-harvest interventions to control *Salmonella* and *Campylobacter* as part of their food safety system
- Utilize microbial testing results to monitor the performance of their HACCP system and inform decision-making

1

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…*"

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Why Biomapping?

Table 1 - Indicator Organism Median Values for Chickens

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

* Excluding necks & giblets

Table 2 - Indicator Organism Median Values for Turkeys

	Median (CFU/mL)			
	Generic E. coli	APC	Enterobacteriaceae	Total Coliform
Carcass – *Rehang*	22	1,800	50	40
Carcass – *Post Chill*	<1.2	18	<1.2	<1.2
Skin-on Parts*	Not available from FSIS sources			
Skin-off Parts*				
Necks				
Giblets				
Comminuted				

* Excluding necks & giblets

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…".*

Vwdqgdug#HuuruB
Ordgv#ri#sdwkrjhqvBB

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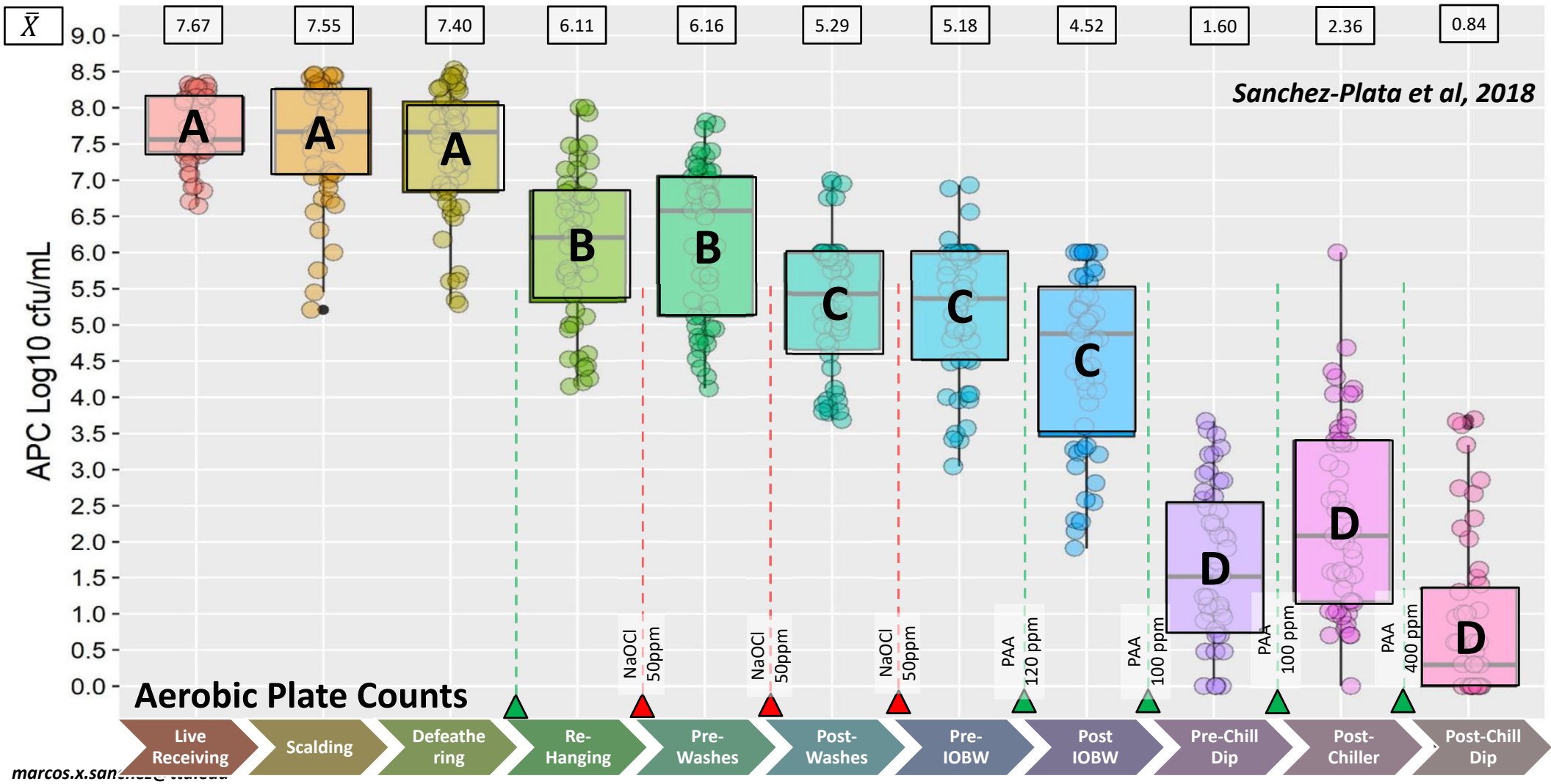
Biomapping for Statistical Process Control. SPC



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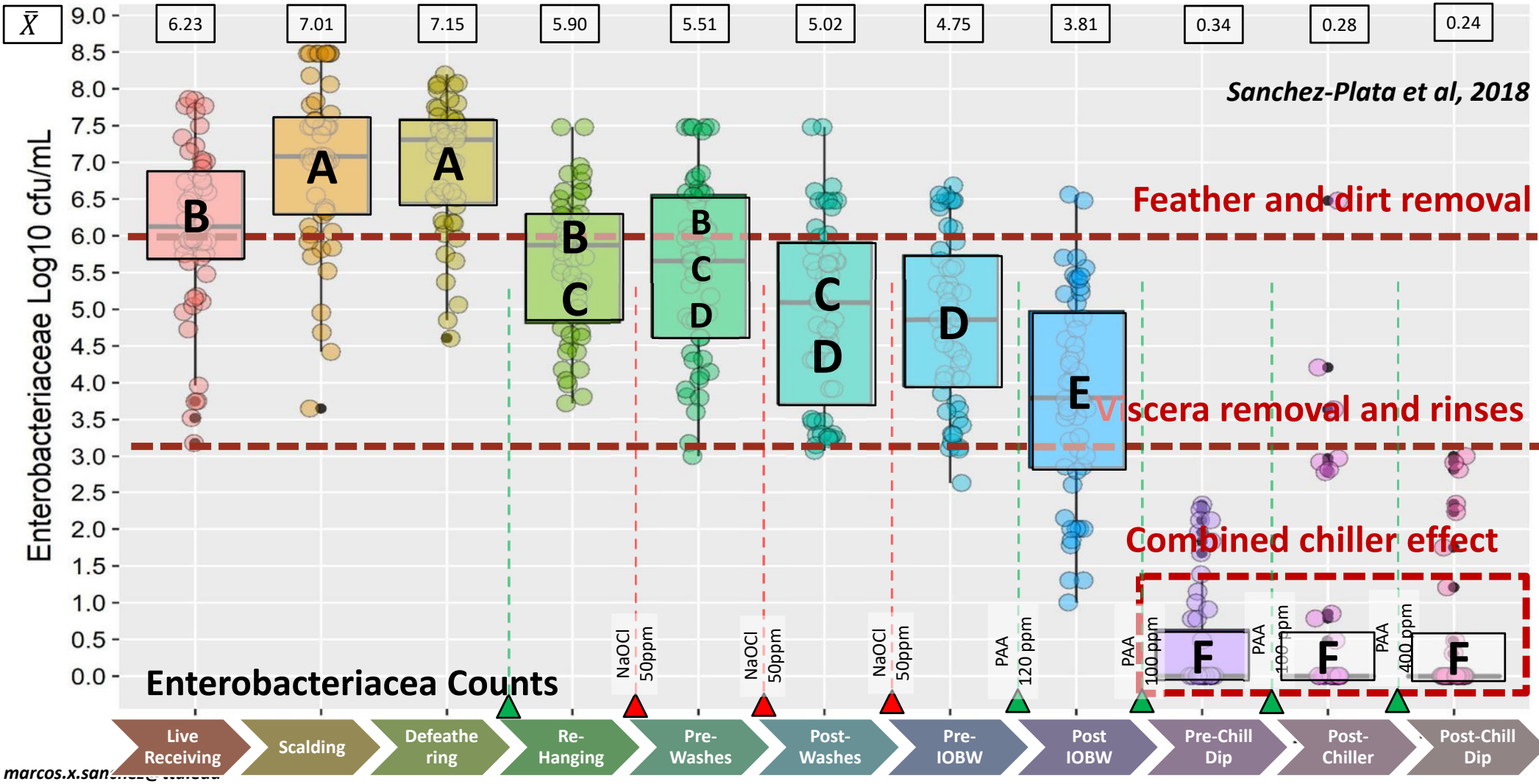
Biomapping for Statistical Process Control. SPC



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Experimental Design for Baselines

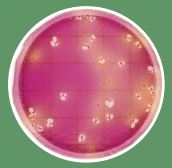
3. WHERE and WHEN should we Sample in a poultry processing operation?

5 different processing days (if weeks even better):
- flock and day effect. Modify intervention regime

2 shifts: morning and afternoon:
- time and accumulation effect

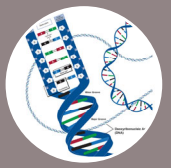
5 samples per process location/ shift:
- 10 samples per location x 5 days = 50-52 total samples

Total Viable Counts



Enterobacteria Counts, EB (COL or EC)

Campylobacter Counts & %



Salmonella Counts & %

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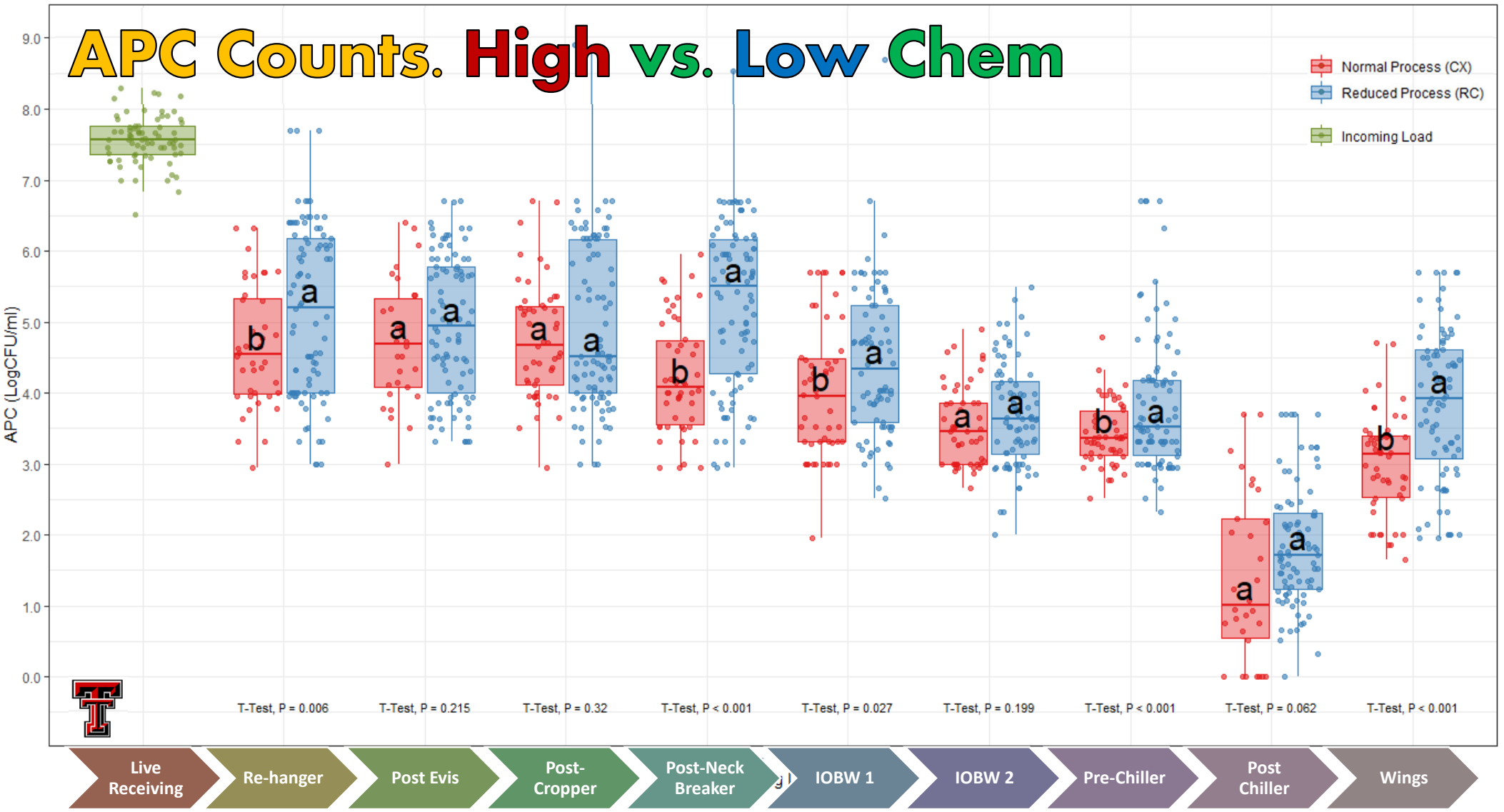
APC Counts. High vs. Low Chem



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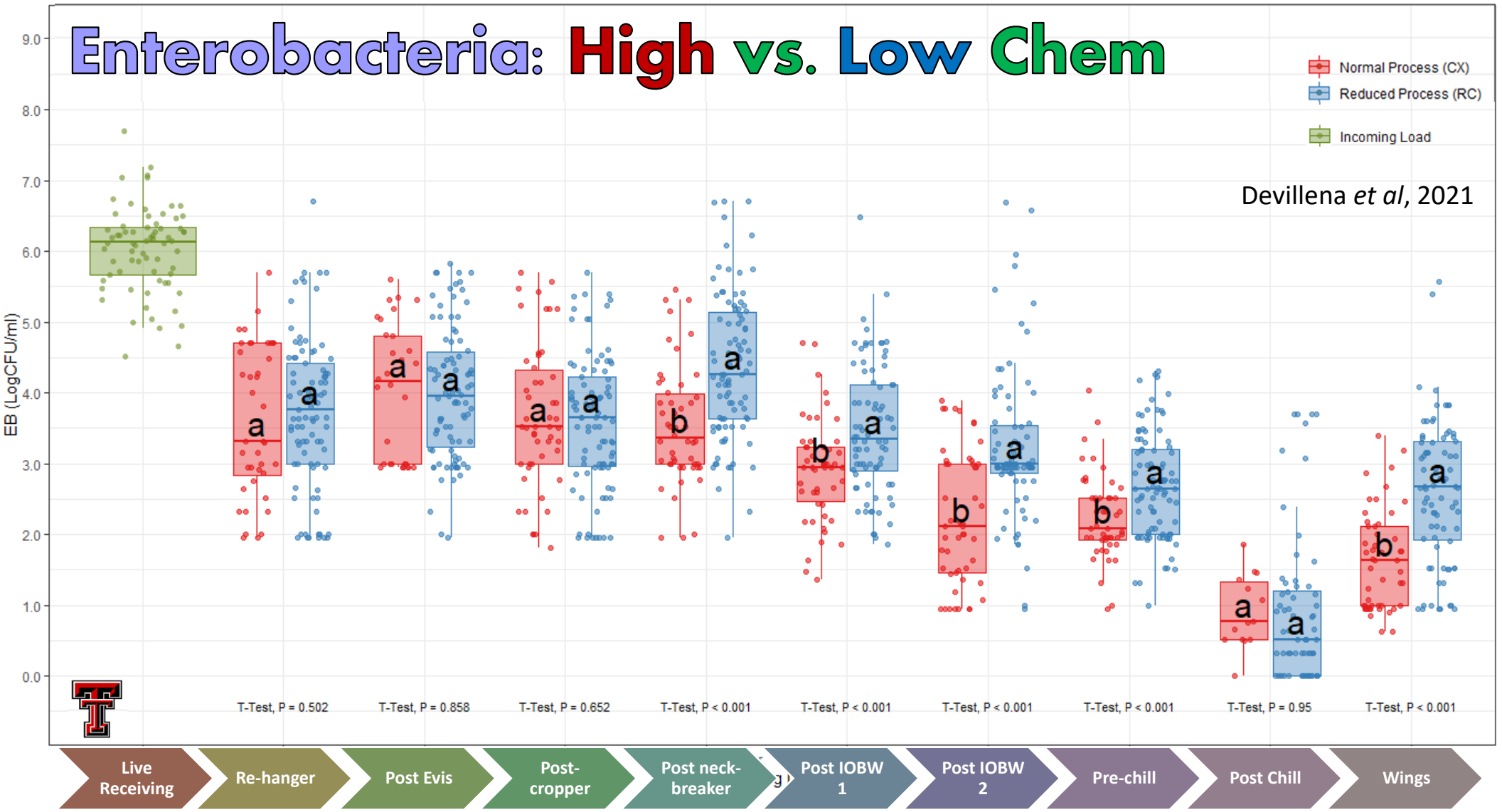
Enterobacteria: High vs. Low Chem



Devillena *et al*, 2021

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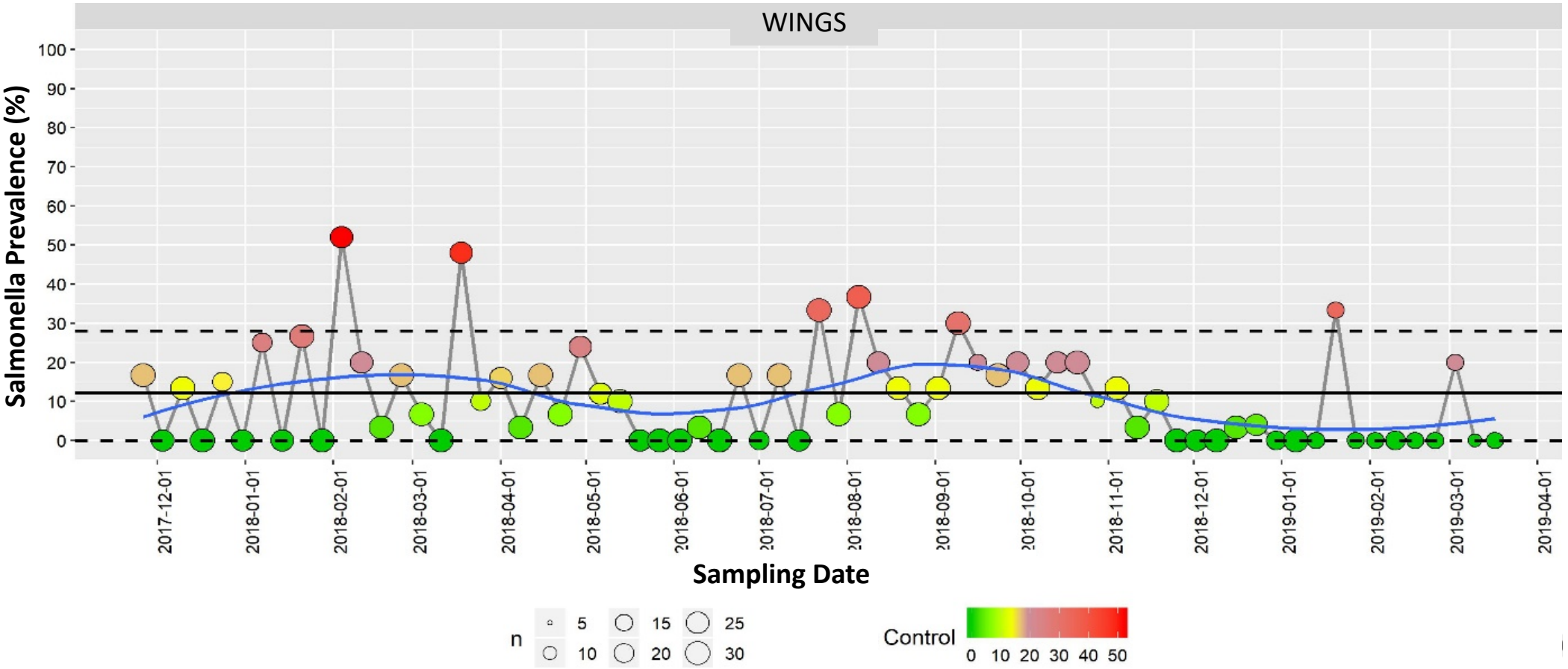
Statistical Process Control Salmonella Prevalence Parts

Intervention Prevalence and Control Limits

N: 25 and Detection ONE YEAR= 12.2%



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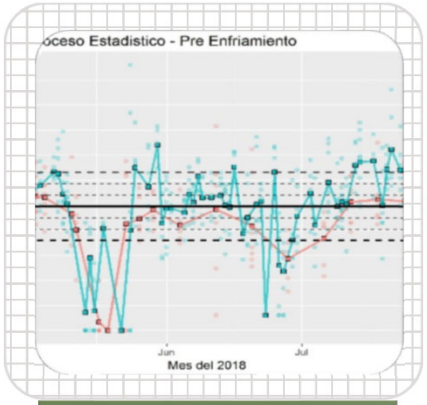
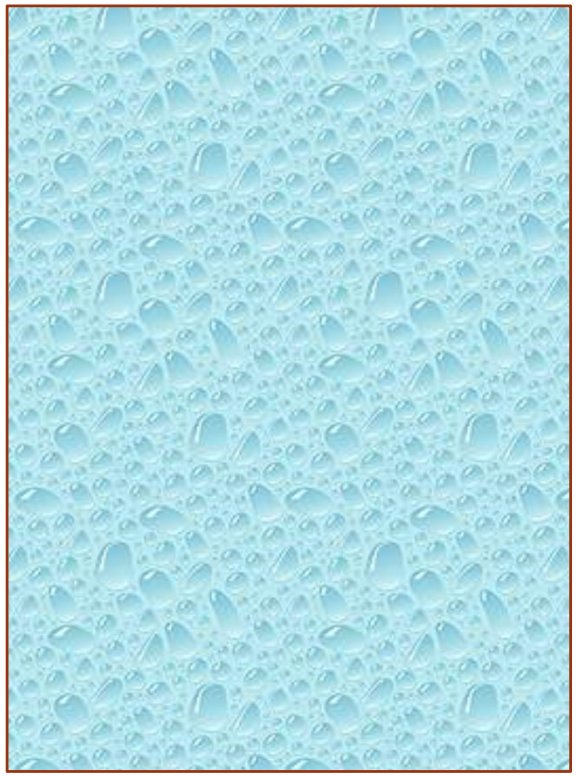
Sanchez-Plata et al, 2019

Detection vs. Enumeration?...



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



Detection: ✚

Concentration: ? But low

Detection: ✚

Concentration: ? But high

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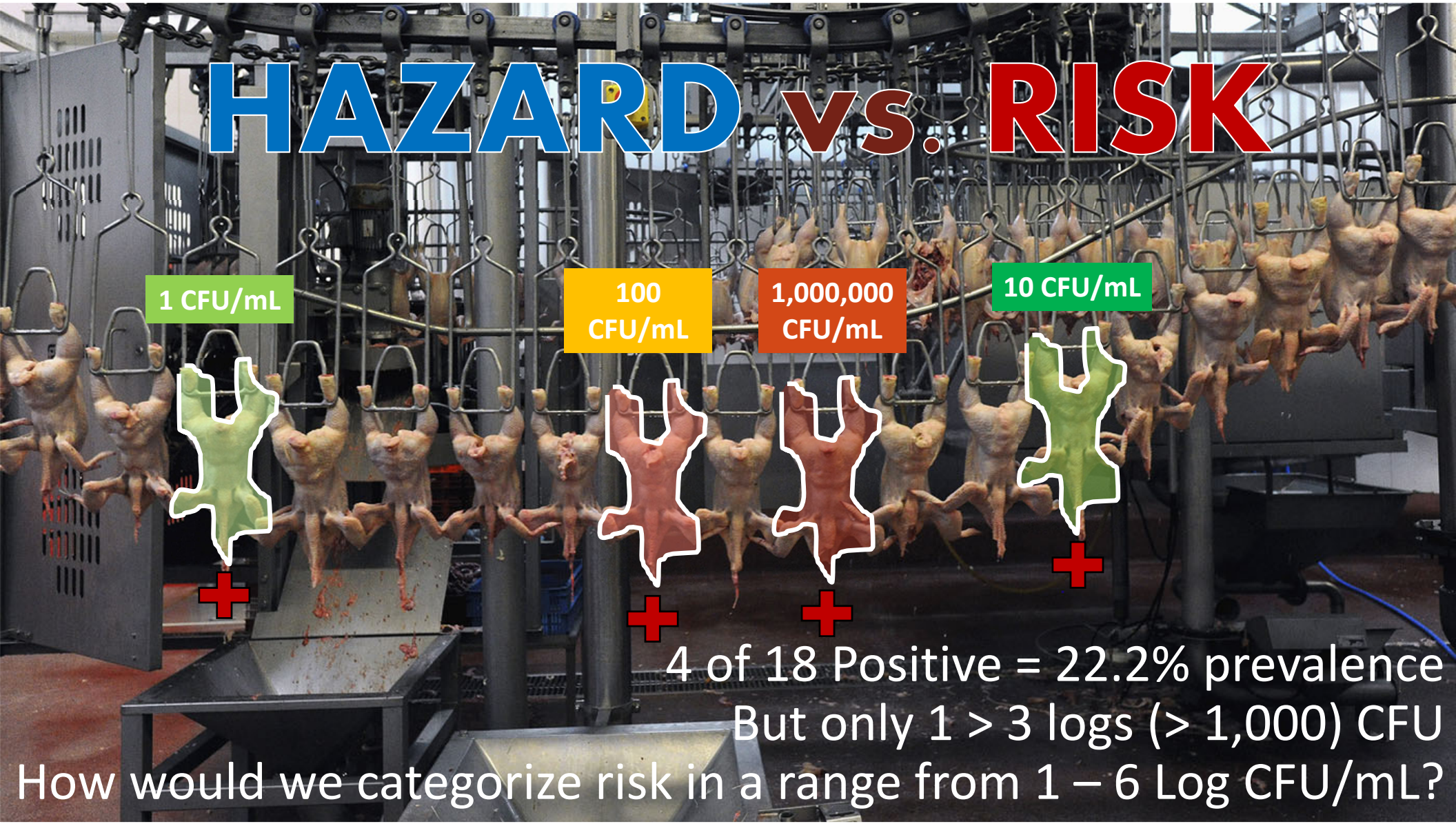
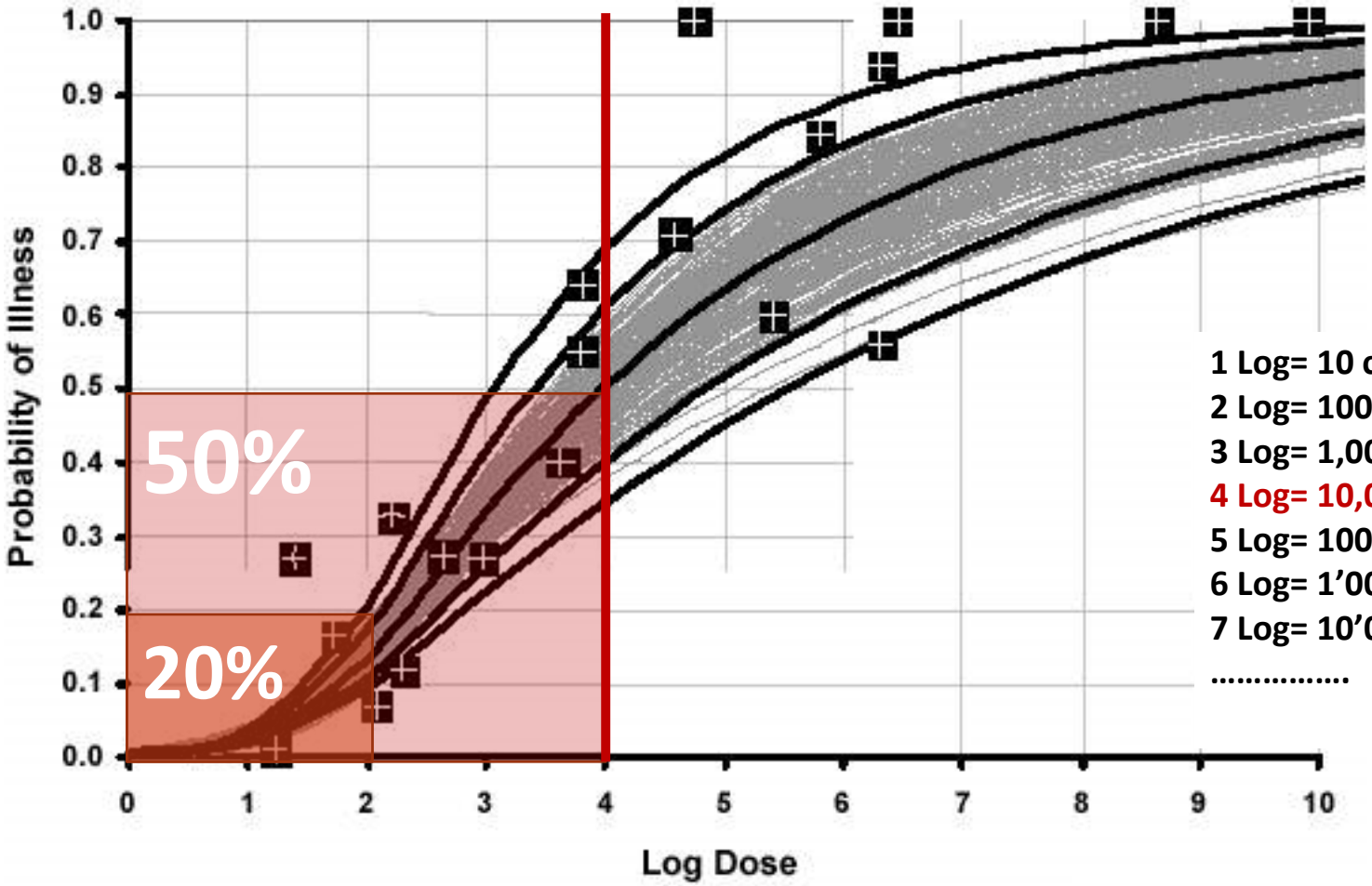

HAZARD vs. RISK
1 CFU/mL
100 CFU/mL
1,000,000 CFU/mL
10 CFU/mL
4 of 18 Positive = 22.2% prevalence
But only 1 > 3 logs (> 1,000) CFU
How would we categorize risk in a range from 1 – 6 Log CFU/mL?

Salmonella. Probability of Illness vs. Log Dose



1 Log= 10 cfu/ g
2 Log= 100 cfu/ g
3 Log= 1,000 cfu/ g
4 Log= 10,000 cfu/ g
5 Log= 100,000 cfu/ g
6 Log= 1'000,000 cfu/ g
7 Log= 10'000,000 cfu/ g
…….

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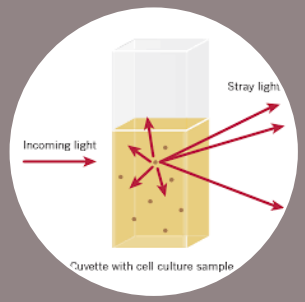
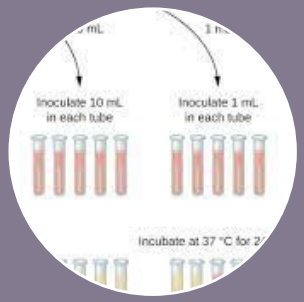
FAO/WHO Risk Assessment, 2002

Quantifying Pathogens



Direct counting (optical microscopy)

- Limited application
- Interference

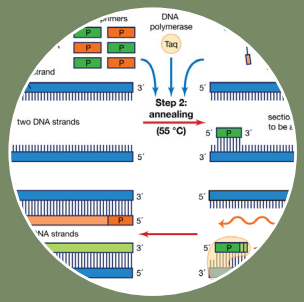


Most Probable Number (MPN)

- 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

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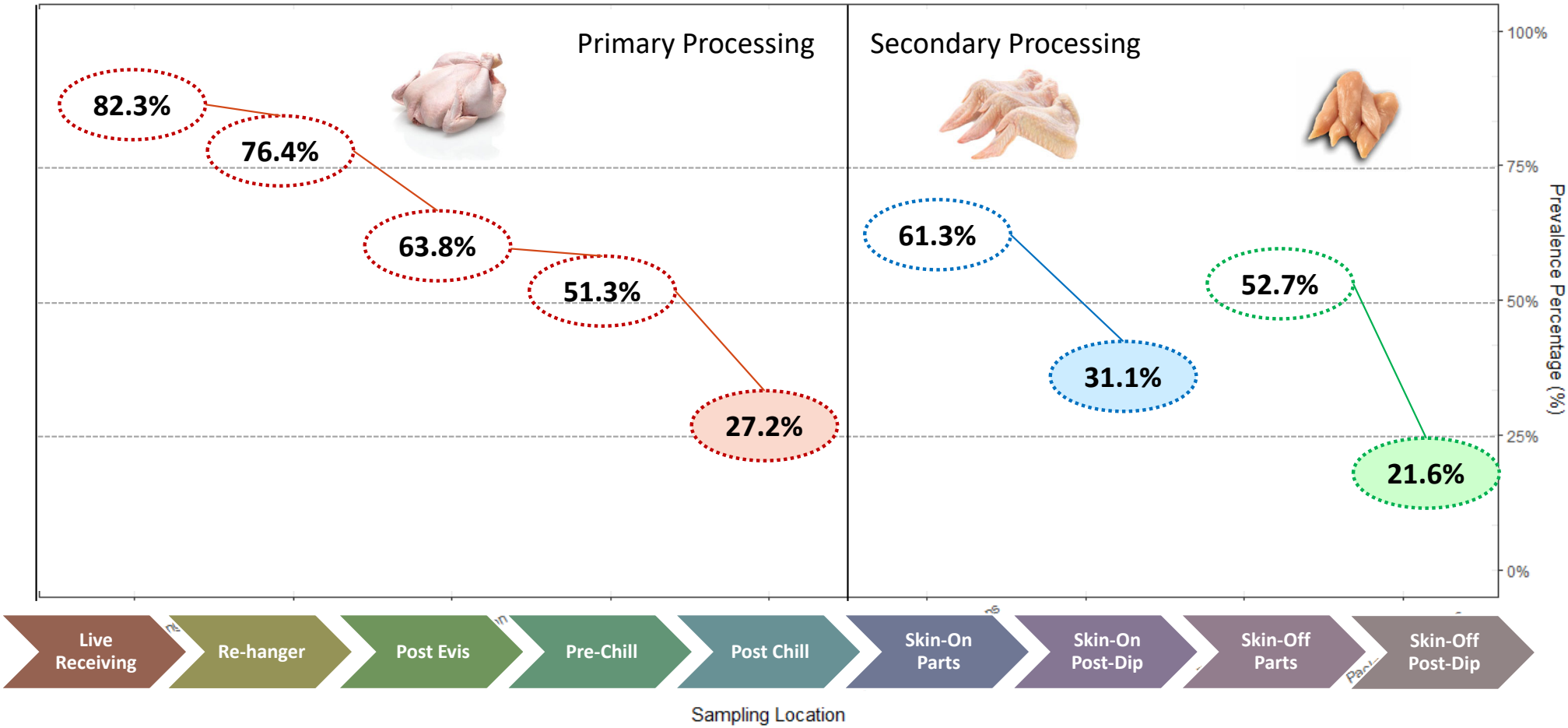
Salmonella Prevalence vs Counts



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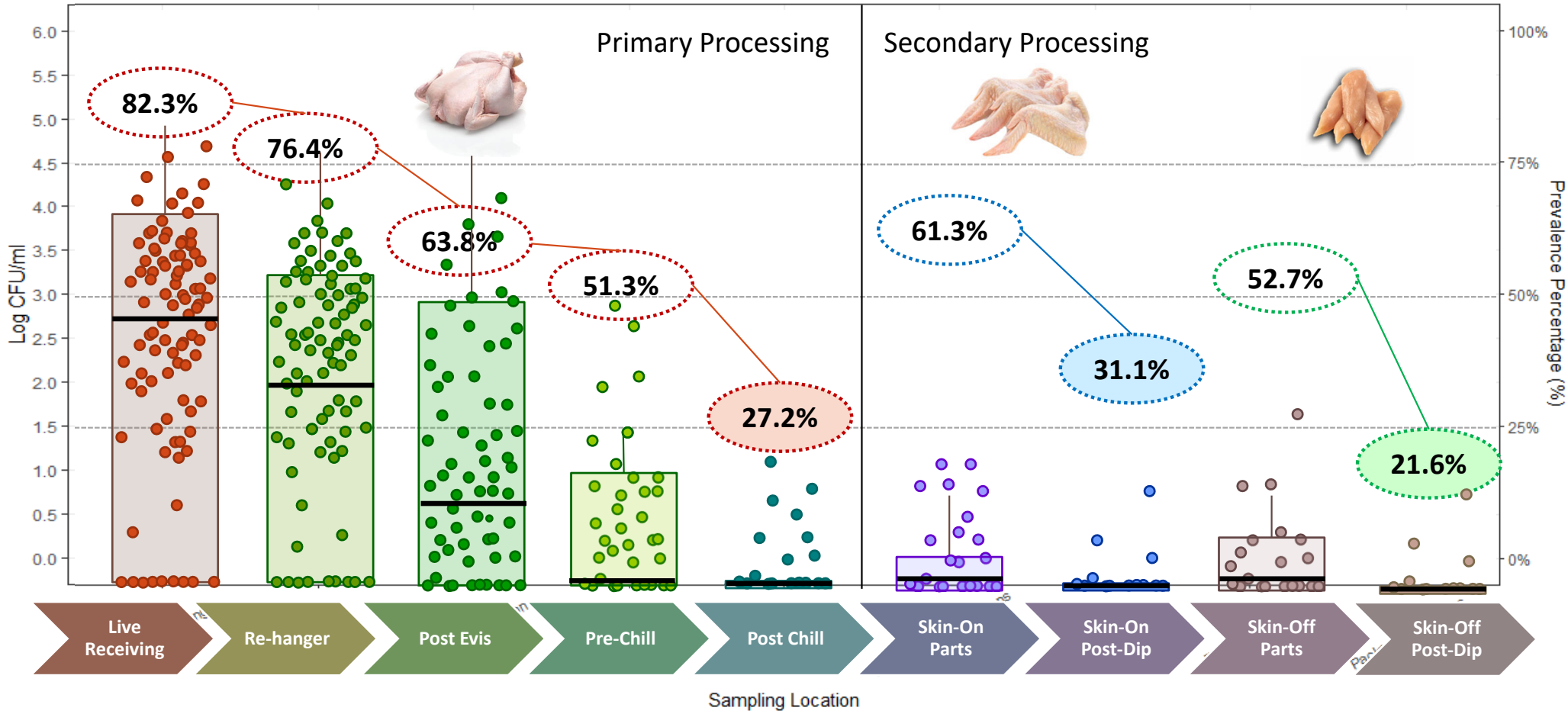
Sanchez-Plata et al, 2021

Salmonella Prevalence vs Counts



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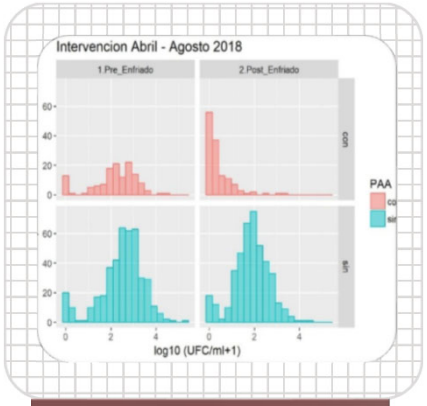
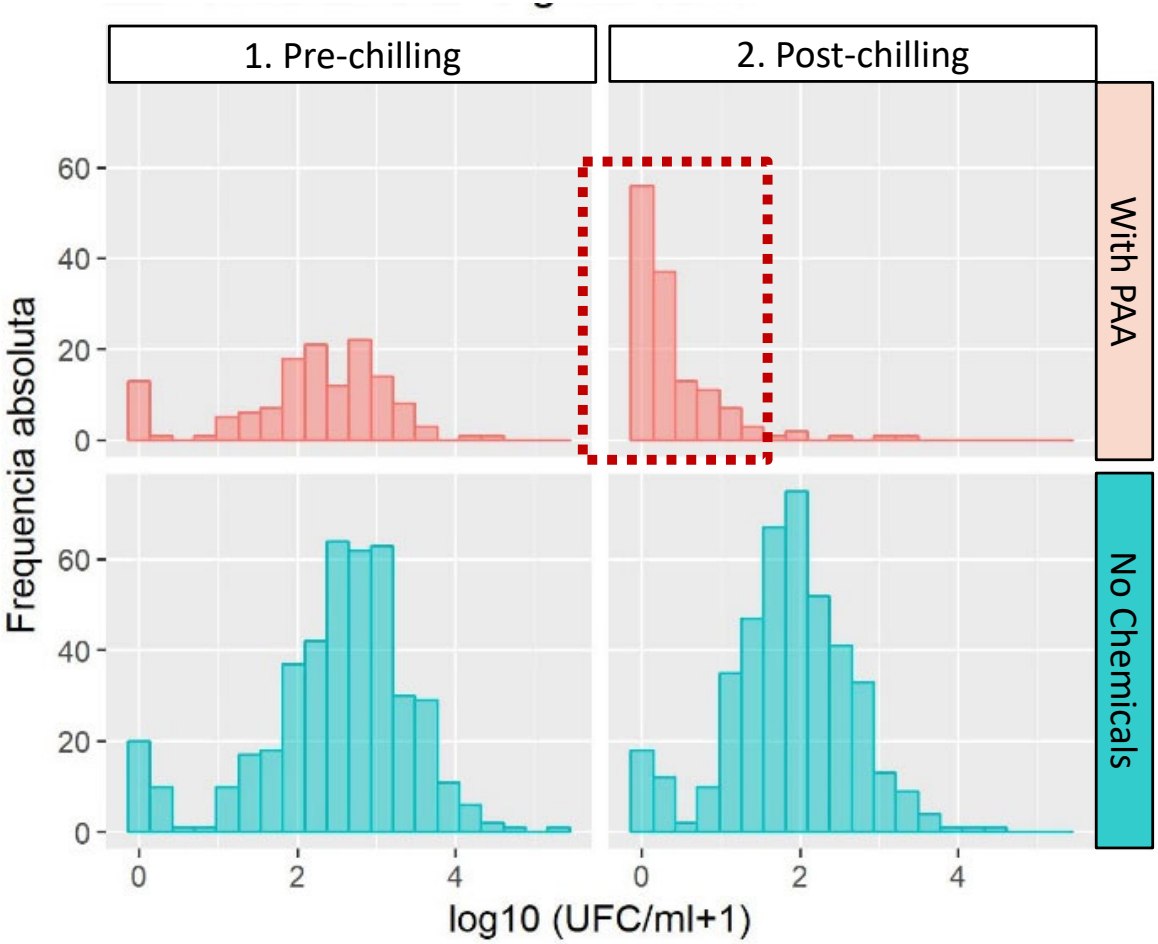
How to interpret YOUR data...?



5. HOW should these surveillance activities be conducted and HOW is the Data Analyzed?

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.



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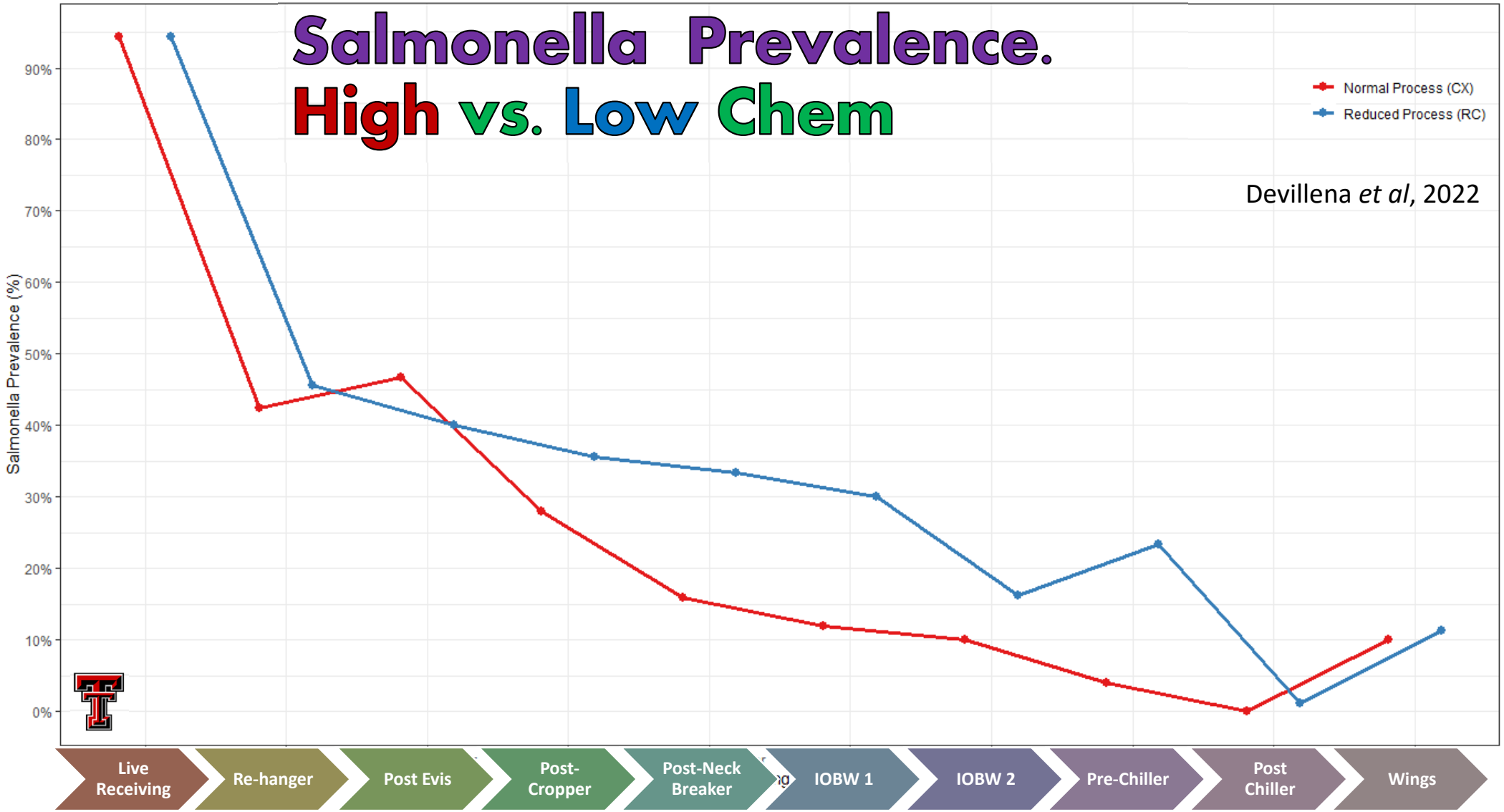
Salmonella Prevalence.
High vs. Low Chem



Devillena *et al*, 2022

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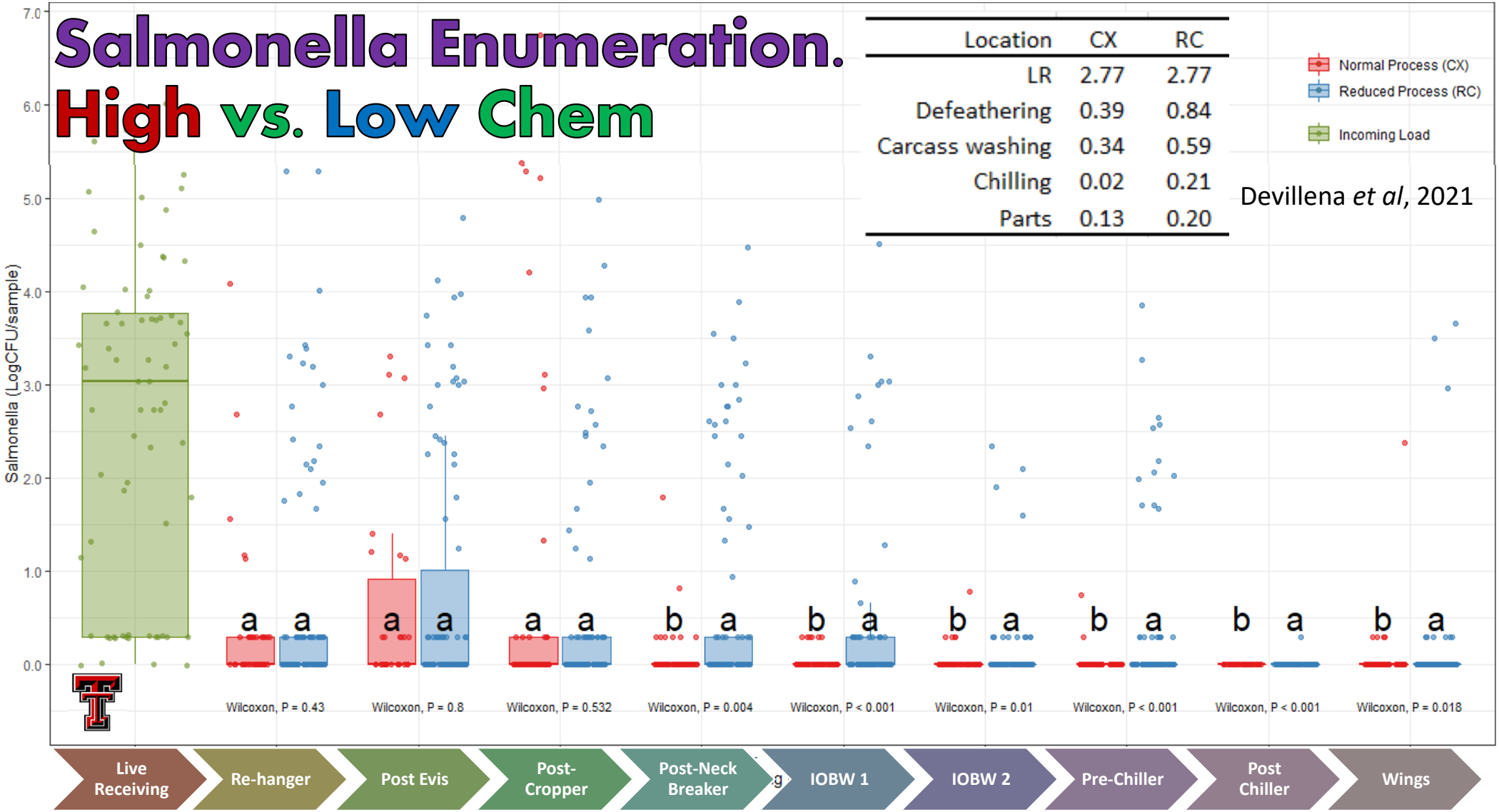
Salmonella Enumeration.
High vs. Low Chem

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

Devillena *et al*, 2021



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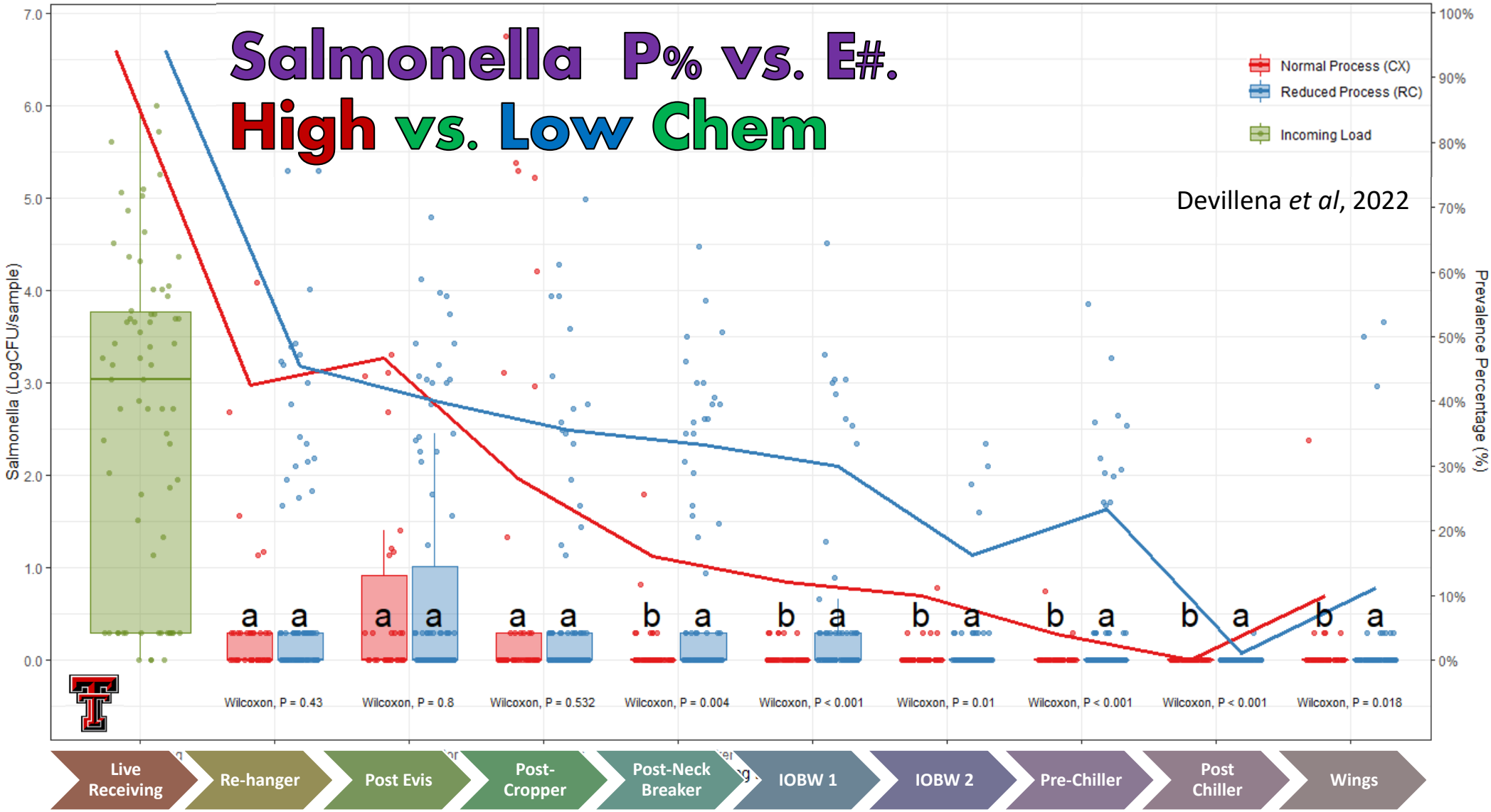
Salmonella P% vs. E#.
High vs. Low Chem



Devillena *et al*, 2022

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

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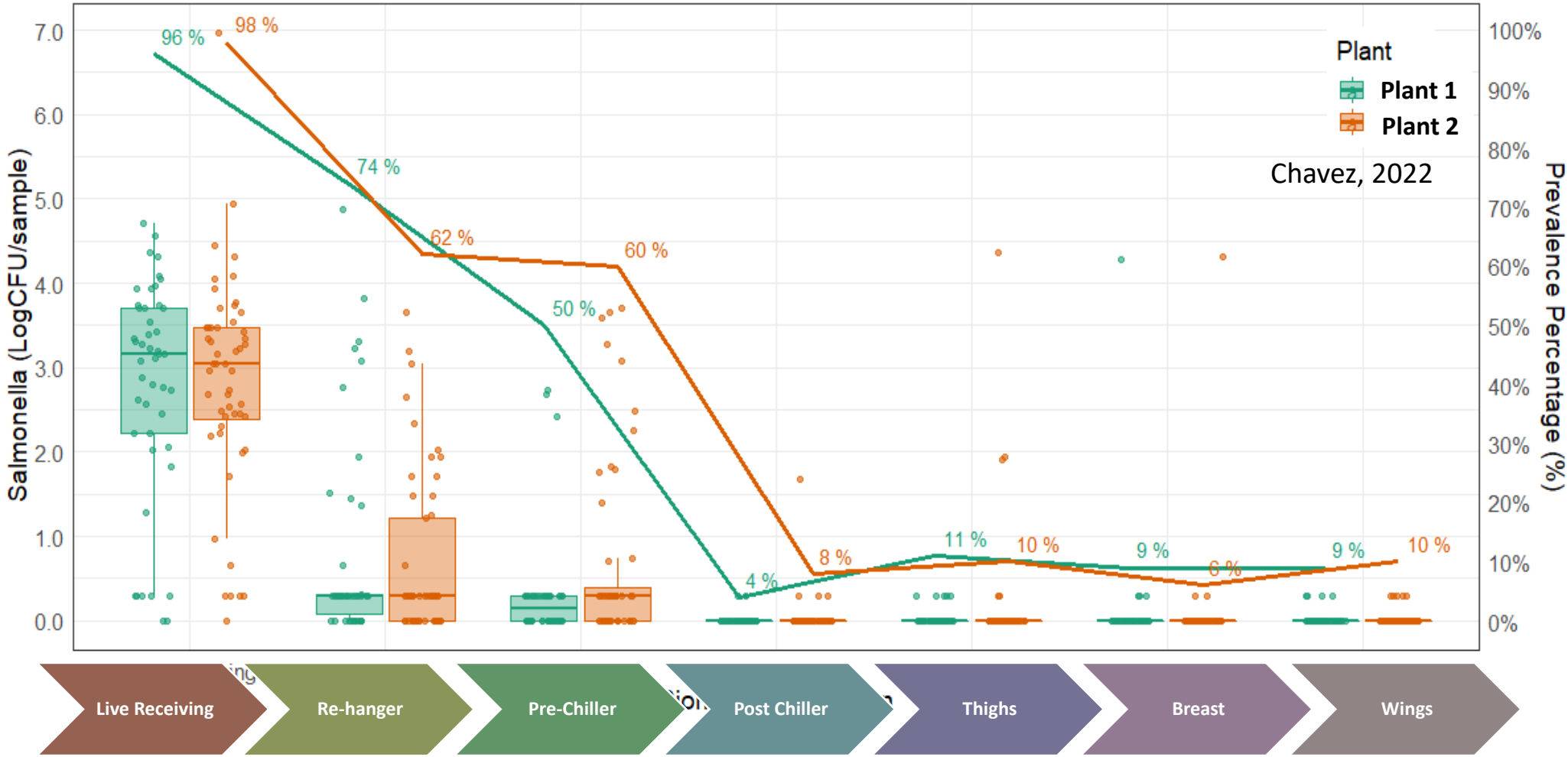
Salmonella P% vs. E#. Plant Comparison



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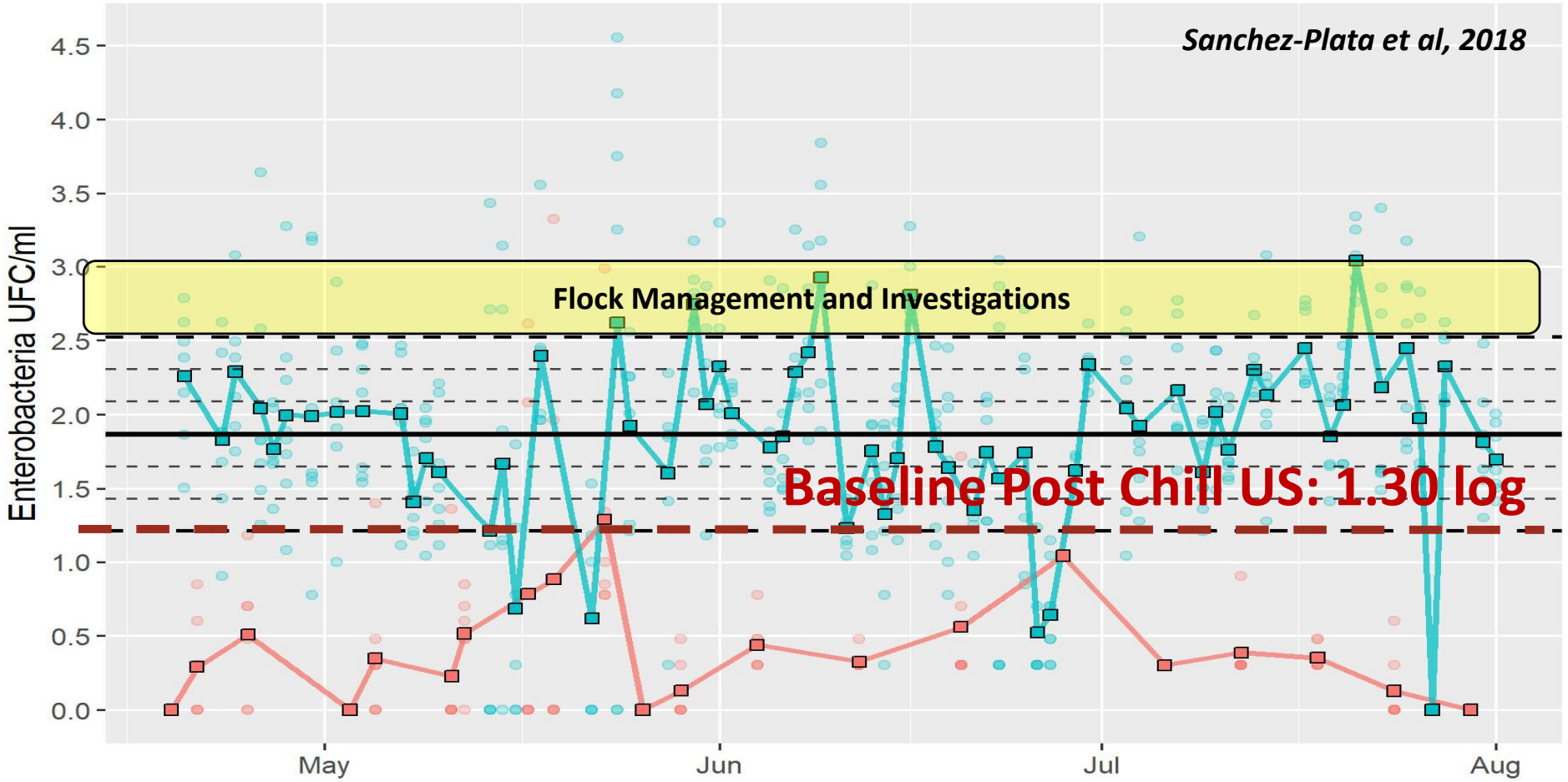
SPC with and without chemical interventions..
SA vs US: Post-Chill



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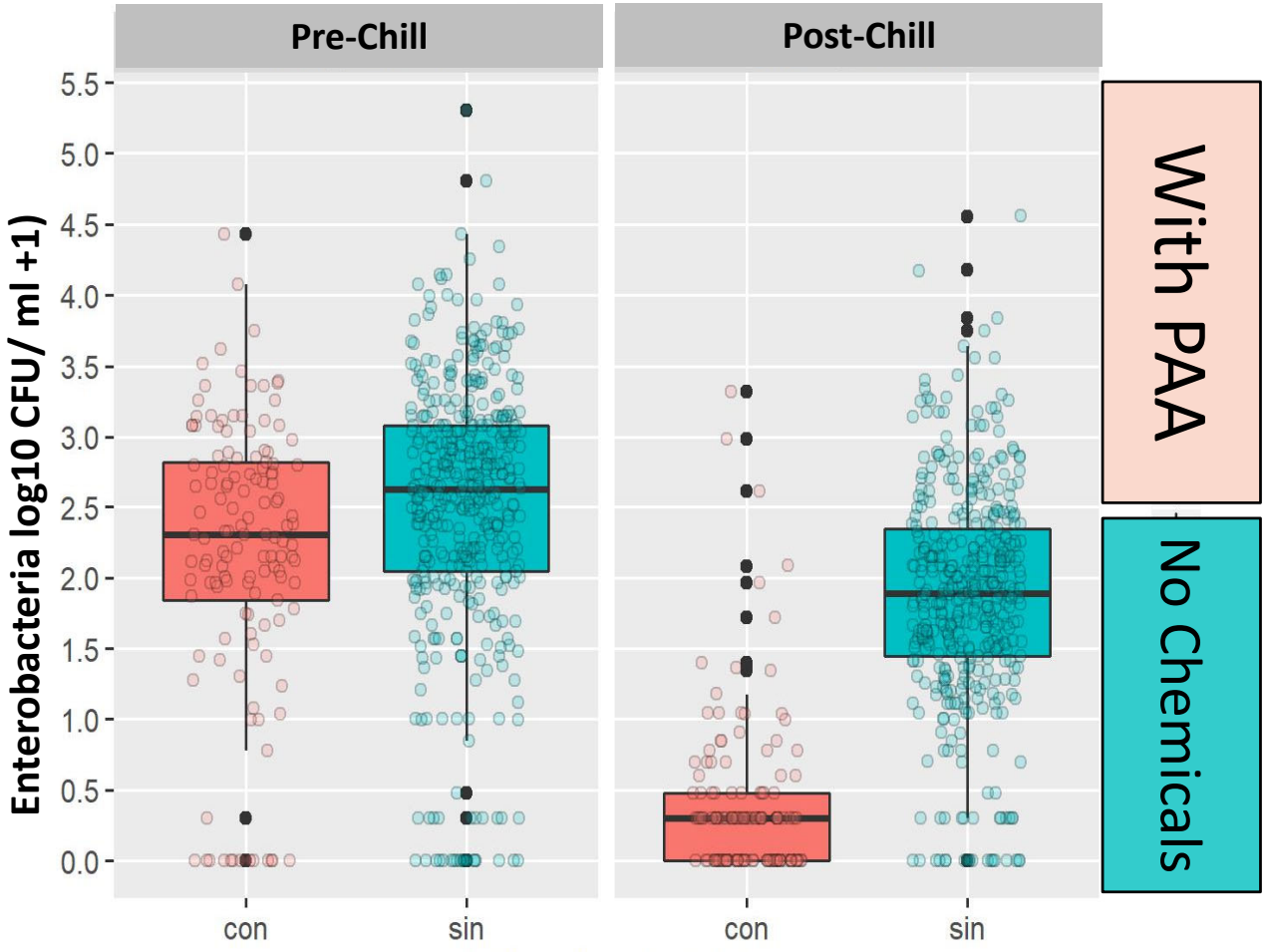
Validation of Interventions. Monitoring Data



Location	N	Log_{10}(CFU/mL +1)			
		Average	SD.	Min	Max
Pre-Chilling	133	2.18	0.98	0.00	4.43
	425	2.49	0.96	0.00	5.30
Post-Chilling	133	0.39	0.58	0.00	3.32
	421	1.86	0.79	0.00	4.56

Sanchez-Plata et al, 2018

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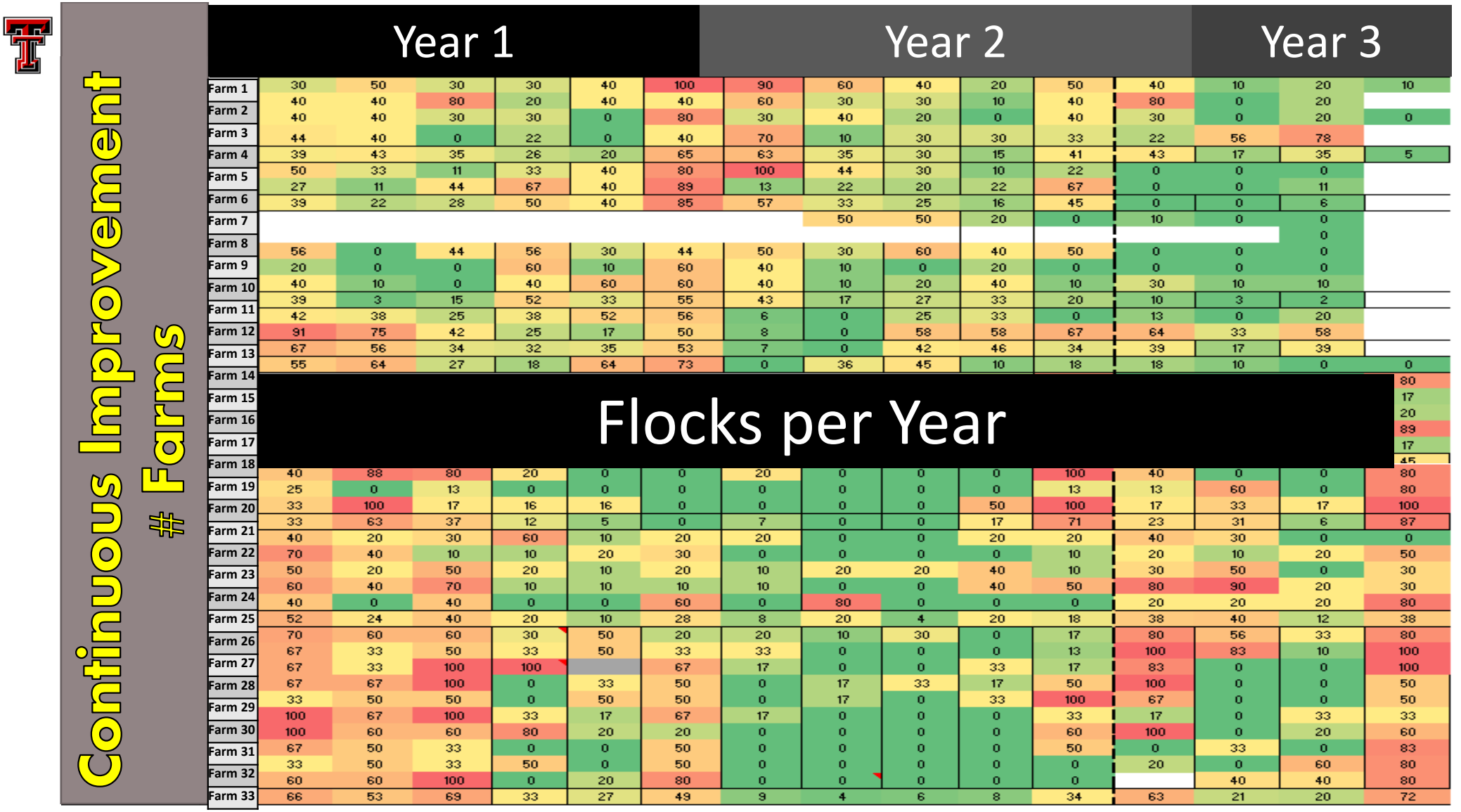

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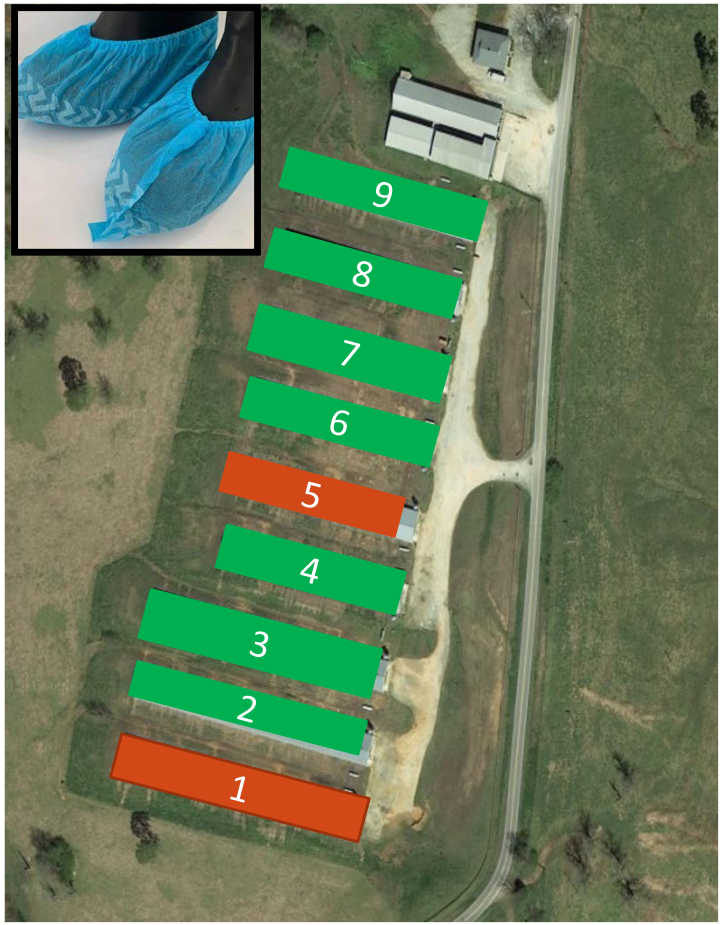
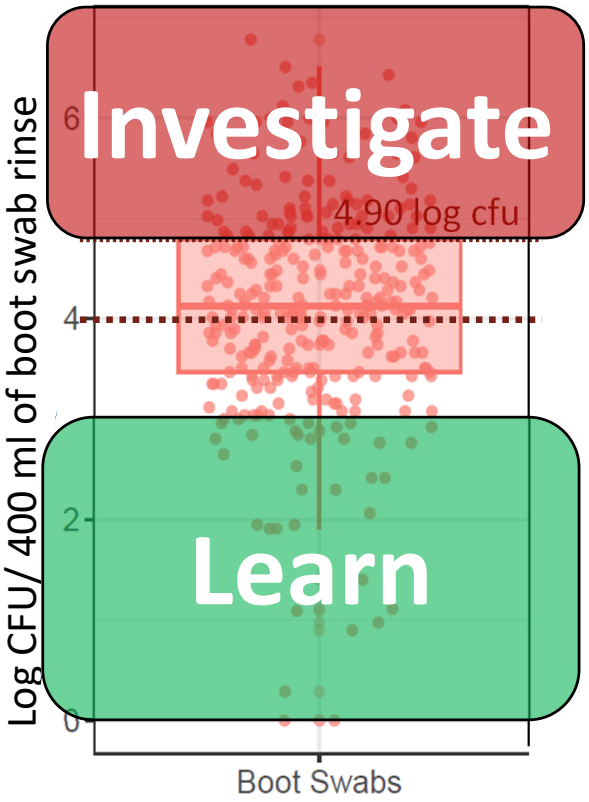
Sanchez-Plata et al, 2018

Biomapping. Pre-harvest (boot swabs)





H	Load day 21-28	Ranking	Schedule
1	6.42	3	Last
2	4.40	1	First
3	4.32	1	First
4	4.44	1	First
5	6.00	3	Last
6	2.96	1	First
7	4.32	1	First
8	3.93	1	First
9	4.32	1	First
A	4.58 ± 0.95		

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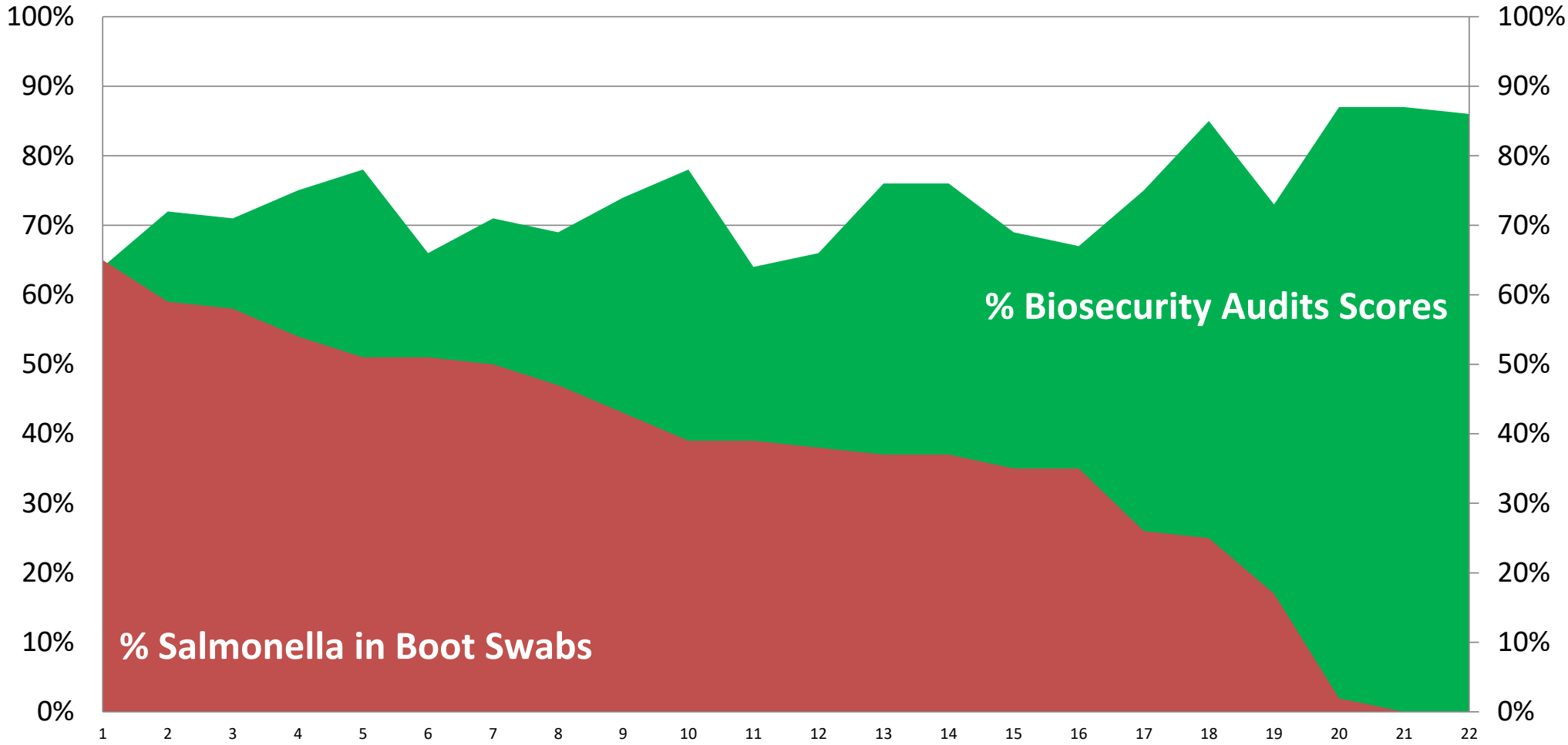
Salmonella in Farms vs. Biosecurity Audits



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Contamination Level Reduction vs. Risk Reduction

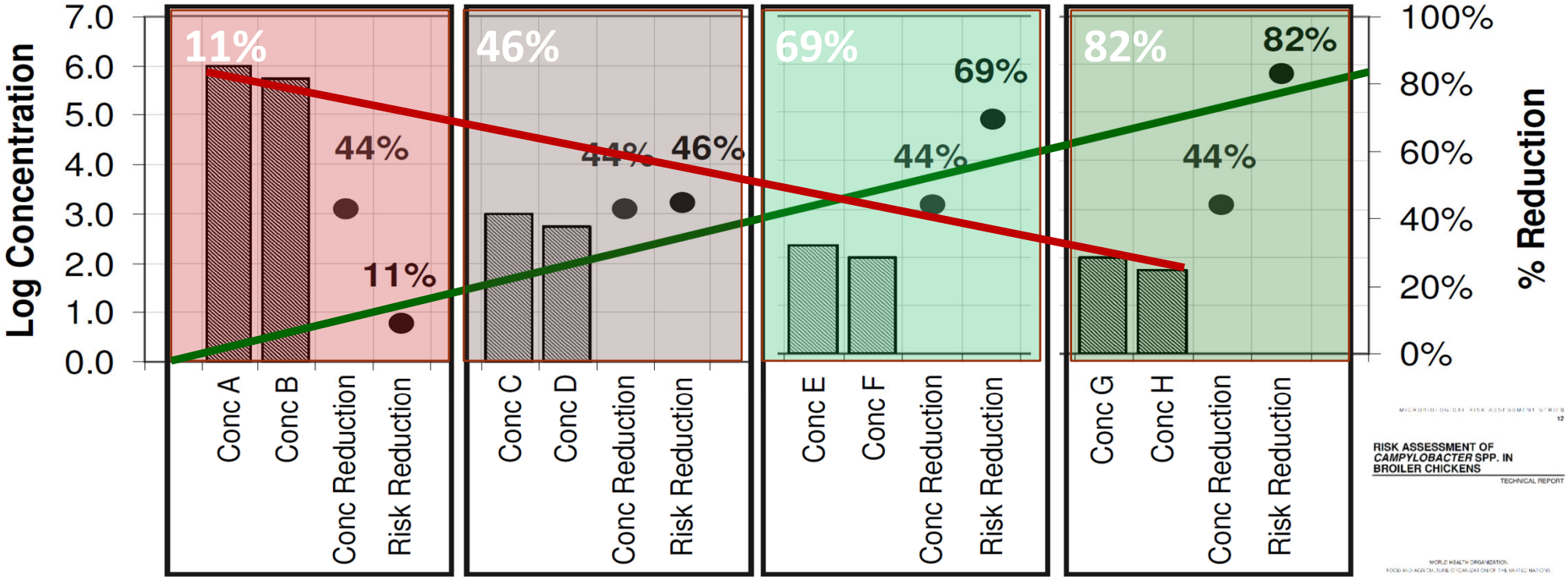

Figure 5.7 Four representative illustrations of the effect on the reduction in risk of reducing the contamination level.

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FAO/WHO Risk Assessment, 2009

Risk-based Categorization. P & C

1. **Category 1. Consistent Process Control:**
   50% or less of the 52-week moving window in the last 6 months
2. **Category 2. Variable Process Control:**
   50% or more of 52-week moving window in the last 6 months
3. **Category 3. Highly Variable Process:**
   Exceeds the performance standard for the 52 moving window cycle in the last 6 months

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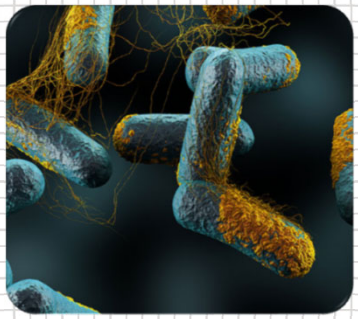
What other projects can be done?



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

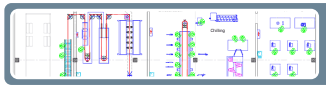
Farm and flock risk-ranking

Pre-harvest intervention decision-making

Flock management continuous improvement

Customized processing, lot separation/ scheduling trials

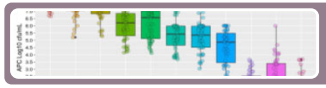
Validation of interventions: physical, chemical, etc.



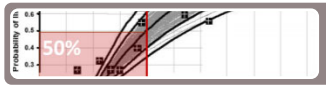
Microbial baselines for plant-to-plant comparison

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

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Questions?

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