White Paper

Evaluation of Assist™ as an Online Reprocessing Treatment in a Gen 3™ Reuse System and its impacts on Post Chill Whole Bird and Chicken Wing microbiological quality.

Data Analysis and Results

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**Full Technical Report is on file with Technical Services Department-Safe Foods Corporation.*

Introduction and Objective

An OLR application of Assist™ antimicrobial was tested in an FSIS inspected facility in the Southeast. The overall purpose of the trial conducted was to compare the microbiological quality (*Campylobacter, Enterobacteriaceae)* of poultry WOGs at pre–Online Reprocessing (OLR) (prior to treatment), post OLR (after treatment), post chill, pre and post wing dip. The facility would provide us with microbiological data (*Salmonella* spp., and *E. coli*) at the pre chill (post OLR) and post chill sampling locations, in accordance with their routine sampling program.

Materials and Methods

The OLR application was tested to highlight the microbial efficacy of Assist™ antimicrobial directly following OLR, post chill, and parts. The Assist was utilized in a Gen3™ recycle system and administered at OLR through double manifold drench nozzles. Assist is measured in pH value and the range for this trial was 1.20-1.30 pH. Sample locations include pre OLR with 10 samples per week for five weeks, post OLR with 10 samples per week for five weeks, post chill with 10 samples per week for five weeks, pre wing dip with 5 samples per week for five weeks, and post wing dip with 5 samples per week for five weeks. This in total is 40 samples per week with a trial total of 200 samples for five weeks.

Whole Bird and parts were sampled in accordance with FSIS Directive 10,250.1. utilizing Neutralized Buffered Peptone (nBPW), Edge Biologicals. Samples were shipped to a Third-Party Laboratory, Food Safety Net Services in Springdale, AR. The microbiological quality of poultry carcasses and parts by evaluating (*Campylobacter, Enterobacteriaceae* (EB)). 3M Petrifilm (ENTERO) was utilized in the analysis of *Enterobacteriaceae* levels within the poultry samples. *Campylobacter* analysis was measured utilizing a *Campylobacter* Cefex-USDA quantitative method. The facility provided internal data (*Salmonella* spp., *E. coli*). Internal data was collected using Buffered Peptone Water (BPW).

Baseline data was collected by Safe Foods on one sampling day prior to Assist™ install. Treatment data was collected on one sampling day a week for four weeks. The baseline collection from in plant data and Safe Foods data was collected across three flocks. Treatment weeks 1, 2, 3, and 4 consisted of four flocks, two flocks, four flocks, and two flocks, respectively. Average head slaughtered numbers per day ranged from 161,518 to 167,356, across a total of 5 sampling weeks. All data for the trial was sent to Smart Data Science Solutions for analysis and reporting.

Figure 1: Antimicrobial intervention and sampling location flow chart.

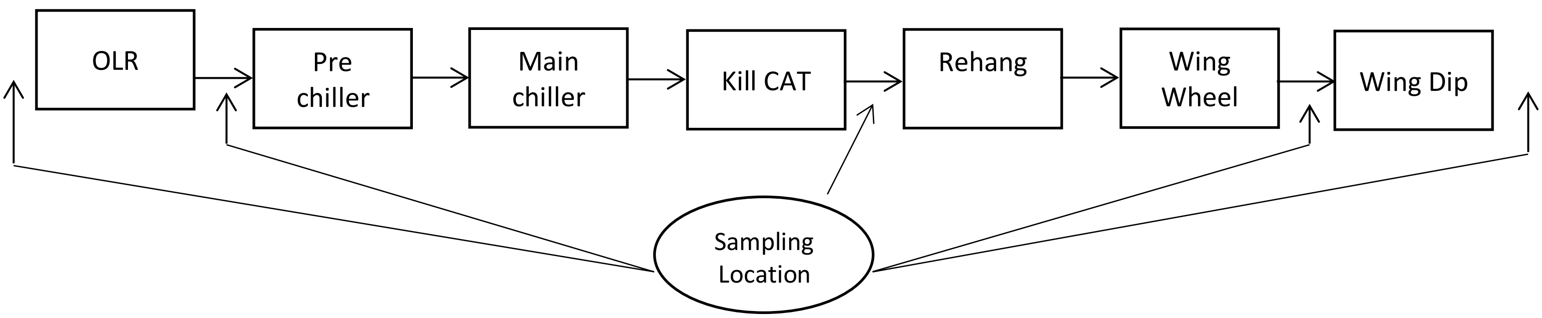

Table 1: Antimicrobial intervention monitoring log for time of sampling, measured in either parts per million (ppm) or pH value as measured and reported by the establishment, or Safe Foods

Concentrations/pH Values	Baseline	Treatment 1	Treatment 2	Treatment 3	Treatment 4
OLR (Baseline = PAA, Treatment = Assist pH)	150ppm	1.23pH	1.18pH	1.24pH	1.27pH
Pre chill 1 (PAA, Caustic pH)	35ppm 10.0pH	20ppm 9.5pH	25ppm 10.0pH	25ppm 10.0pH	40ppm 10.0pH
Pre chill 2 (PAA, Caustic pH)	35ppm 10.0pH	20ppm 9.0pH	25ppm 10.0pH	25ppm 9.5pH	35ppm 10.0pH
Main chill 1 (PAA, Caustic pH)	25ppm 8.5pH	20ppm 9.0pH	20ppm 8.5pH	20ppm 8.5pH	20ppm 9.0pH
Main chill 2 (PAA, Caustic pH)	30ppm 9.0pH	20ppm 9.0pH	20ppm 9.0pH	20ppm 9.0pH	20ppm 9.5pH
Kill cat 1 (PAA)	150ppm	150ppm	150ppm	150ppm	150ppm
Kill cat 2 (PAA)	150ppm	150ppm	150ppm	150ppm	150ppm
Citrilow dip (Citrilow pH)	1.09pH	1.39pH	1.43pH	1.36pH	1.26pH

**Please note that concentrations and pH values vary for the duration of the trial.*

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Executive Summary

Question 1: Was the use of Assist associated with improved Campylobacter results?

Yes. The use of Assist was associated with reduced Campylobacter loads in all *post-application* tested locations - post OLR, post chill, pre wing dip, and post wing dip. The results were consistent between both combined analyses (comparison of means and regression) and the weekly analysis and found reductions in load between 0.4-1.6 logs, depending on location, and reductions in prevalence between 25-70% depending on location.

Question 2: Did the use of Assist translate to improved Campylobacter results for post chill?

Yes. The use of Assist was associated with a 0.5 log reduction in load and a 75% reduction in prevalence at post chill.

Question 3: Did the use of Assist translate to better Campylobacter outcomes on wings?

Yes. The use of Assist was associated with a 0.4 log reduction in load and a 50% reduction in prevalence at post wing dip.

1. SFC Data & Results

Summary

Results - Campylobacter

- **The use of Assist was statistically significantly associated with decreases in both the load and prevalence of Campylobacter in all tested locations**
    - **These differences were consistent between both combined analyses and the weekly analyses**
- **Decreases in load were between 0.4-1.6 logs depending on location**
    - **The largest differences in load were at post OLR (-1.3 log) and pre wing dip (-1.6 log)**
- **Decreases in prevalence were 25-70% depending on location**
    - **The largest differences in prevalence were at post chill (-70%) and post wing dip (-50%)**

Results - Enterobacteriaceae

- **The use of Assist was statistically significantly associated with decreases in the load of Enterobacteriaceae in two locations: pre wing dip and post wing dip**
    - **These differences were consistent between both combined and weekly analyses**
- **Decreases in load were between 0.4-0.6 logs depending on location**

SFC Data

Samples were collected for five weeks: one "baseline" week, before Assist was installed, and four "treatment" weeks following installation. There are 200 total samples in the dataset, each tested for two organisms, giving 400 total results:

Table 2. Sample breakdown

	EB		Campylobacter	
Location	**Baseline**	**Treatment**	**Baseline**	**Treatment**
Pre OLR	10	40	10	40
Post OLR	10	40	10	40
Post Chill	10	40	10	40
Pre-Wing Dip	5	20	5	20
Post Wing Dip	5	20	5	20

SFC Results

I. Combined Analysis – Comparison of Means

The first analysis we performed was a combined analysis, grouping together the treatment results from all four weeks into one treatment group, and comparing those results to the single control (baseline) group[1]. Given the relatively small number of samples per week, grouping the treatment results increases the statistical power to detect a difference from the control group.

The analysis was performed individually for each of the five locations: pre OLR, post OLR, post chill, pre wing dip and post wing dip. We utilized an unadjusted Student's t-test to compare the one-week baseline results to the combined four-week treatment results. This method assumes that there were no other significant changes made in the processing plant during the study period other than the introduction of the treatment.

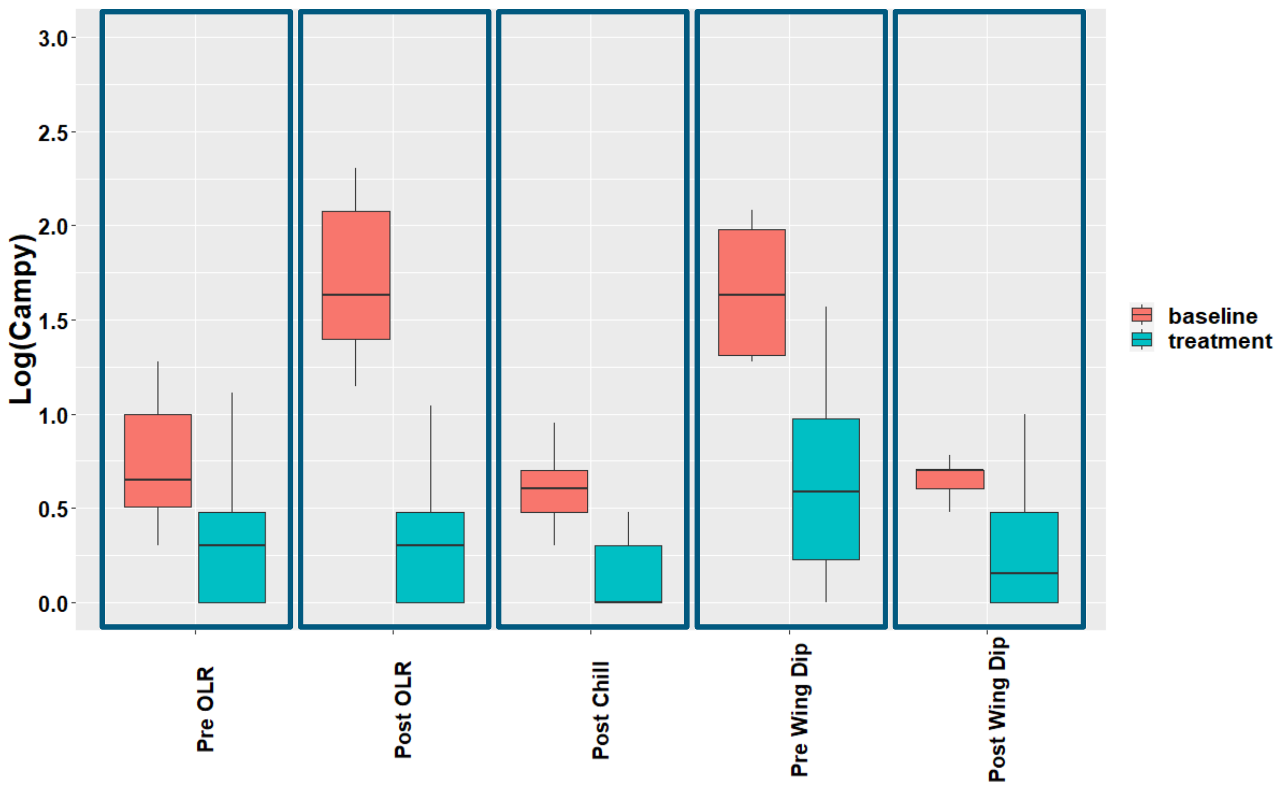
Campylobacter

We found statistically significant decreases in the amount of Campylobacter present during the treatment period compared to the baseline period for all locations[2]. These decreases were on the order of 0.4-1.6 logs, depending on the location.

Table 3 (and Figure 2). Differences in Campylobacter load among all locations

	Baseline	Treatment	p-value
Pre OLR	**0.76**	**0.38**	**p = 0.0098**
Post OLR	**1.71**	**0.37**	**p < 0.0001**
Post Chill	**0.61**	**0.10**	**p < 0.0001**
Pre-Wing Dip	**2.19**	**0.62**	**p = 0.0468**
Post Wing Dip	**0.65**	**0.26**	**p = 0.0002**

[1] For the remainder of this Report the terms "baseline" and "control" will be used interchangeably
[2] Campylobacter was measured in CFU/ml and log-transformed using $\log_{10}$(CFU/ml + 1) to eliminate infinite and NaN ("not a number") results


Log(Campy)
3.0
2.5
2.0
1.5
1.0
0.5
0.0
Pre OLR
Post OLR
Post Chill
Pre Wing Dip
Post Wing Dip
baseline
treatment

We also found statistically significant decreases in the prevalence of Campylobacter during the treatment period compared to the baseline period for all locations[3]. These decreases ranged from 25-70%, depending on the location.

Table 4. Differences in Campylobacter prevalence among all locations

	Baseline	Treatment	p-value
Pre OLR	**100%**	**55%**	**p < 0.0001**
Post OLR	**100%**	**55%**	**p < 0.0001**
Post Chill	**100%**	**30%**	**p < 0.0001**
Pre-Wing Dip	**100%**	**75%**	**p = 0.0210**
Post Wing Dip	**100%**	**50%**	**p = 0.0003**

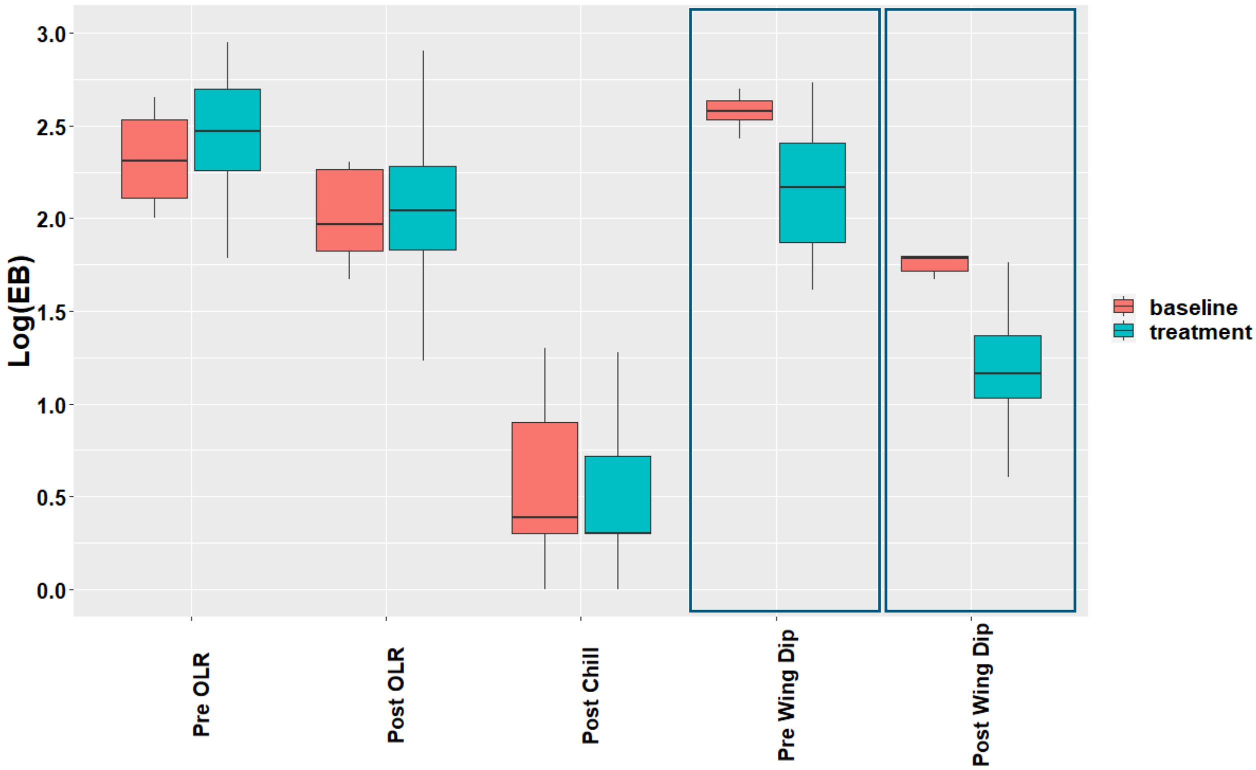
Enterobacteriaceae

We found statistically significant decreases in the amount of EB present during the treatment period compared to the baseline period for two of the five locations: pre wing dip and post wing dip[4]. These decreases were on the order of 0.4-0.6 logs, depending on the location.

Table 5 (and Figure 3). Differences in EB load among all locations

	Baseline	Treatment	p-value
Pre OLR	2.52	2.61	p = 0.6221
Post OLR	2.14	2.17	p = 0.8731
Post Chill	0.58	0.52	p = 0.7440
Pre-Wing Dip	**2.58**	**2.19**	**p = 0.0002**
Post Wing Dip	**1.80**	**1.22**	**p < 0.0001**

[3] Campylobacter prevalence is *positive* if the CFU/ml is >= 1 and *negative* if <1
[4] EB was measured in CFU/ml and log-transformed using log_{10}(CFU/ml + 1) to eliminate infinite and NaN ("not a number") results



2. Plant Data & Results

Summary

Results – E. Coli

- **There are no statistically significant differences between the E. Coli loads in the baseline or treatment weeks in either the combined analysis or the weekly analysis**

Results - Salmonella

- **There are no statistically significant differences between the *Salmonella* prevalence in the baseline or treatment weeks in either the combined analysis or the weekly analysis**

Plant Data

Samples were reported from approximately eight weeks: four "baseline" weeks[5], before Assist was installed, and four "treatment" weeks following installation[6].

Table 7. Sample breakdown

	E. Coli		Salmonella	
Location	**Baseline**	**Treatment**	**Baseline**	**Treatment**
Pre-Chill	171	180	105	112
Post Chill	131	139	105	112

Plant Results

I. Combined Analysis

The first analysis we performed was a combined analysis, grouping together the baseline results from all four weeks into one baseline group and the treatment results from all four weeks into one treatment group.

The analysis was performed individually for both locations: pre chill and post chill. We utilized an unadjusted Student's t-test to compare the combined four-week baseline results to the combined four-week treatment results. This method assumes that there were no other significant changes made in the processing plant during the study period other than the introduction of the treatment.

[5] These include 1 partial week (week of 5/31) and 3 full weeks (weeks of 6/7, 6/14, and 6/21)
[6] These include 4 full weeks (weeks of 6/28, 7/5, 7/12, and 7/19)

E. Coli

There were no statistically significant differences in the amount of E. Coli present between the baseline group and the treatment group in either location.

Table 8. Differences in EC load between locations

	Baseline	Treatment	p-value
Pre-Chill	1.91	1.91	p = 0.9902
Post Chill	0.14	0.13	p = 0.7861

Salmonella

There were no statistically significant differences in *Salmonella* prevalence between the combined baseline group and the combined treatment group at either location.

Table 9. Differences in Salmonella prevalence between locations

	Baseline	Treatment	p-value
Pre-Chill	**22.9%**	**28.6%**	**p = 0.3375**
Post Chill	**0.0%**	**0.9%**	**p = 0.3195**

3. Caveats and Other Notes

As stated above, all of the analyses and thus the validity of the conclusions rest on one assumption: that, other than the installation of Assist, there were no other changes made during this period.

There are two possible sources of changes: the plant and the live side.

If other systems in the plant were changed during the period, then the results are possibly biased because the observed differences could be due to those other changes and not the installation of Assist. This assumption is testable by following up with plant management to confirm that no other changes were made.

For the analyses which utilize t-tests to compare the raw means, we must assume that the incoming load from the live side is roughly the same. However, analysis of the pre OLR data, which is taken before Assist is applied, suggests that the incoming Campylobacter load in week 3 is statistically significantly different from the baseline week. To adjust for this difference, we utilized regression analysis to control for the incoming load. The assumption for the regression analysis is that the pre OLR measurement is an accurate representation of Campylobacter load coming into the plant before Assist is applied.

****Please note, a full technical report is available upon request from Safe Foods Technical Services Department.**