# Far-UV Proof of Concept II

**August 7, 2014**

**Purpose:** To determine the effect of flipping the egg, water spray, and hydrogen peroxide spray on the ability of HEO3's Pathogen Reduction Box (PRB) to reduce microbial load on hatching eggs.

**Background:** We completed initial trials with HEO3's PRB and MicrobeBusterBox (MBB) the week of 6/30/14. After viewing the results of those trials on 7/21/14 we decided to continue our evaluation with eggs.

**Methods:** The overall experimental design is a 2X3 full factorial. So we have 6 conditions or factors X level combinations. The first factor is turning the egg after one minute exposure or not, labeled Yes or No. The second factor is spray. That will be no spray, water only, or 3% hydrogen peroxide (common pharmaceutical strength). Labeled none, water, peroxide. The total eggs required will be 2X3X4 = 24.

The overall procedure for execution will be as follows:

1. We set up the PRB on the counter in egg receiving as done on 6/23/14.
2. We will haphazardly select eggs from the closest rack without sorting out visible contamination.
. We will expose 4 eggs to each or the 6 possible factor/level combinations for a total of 24 rinses (see step four). Eggs will be exposed for 2 minutes total with no spray, water, or hydrogen peroxide spray prior to loading them in the PRB. Eggs will be turned or not depending upon treatment after 1 minute of exposure. We will use quartz glass shards to prevent rolling.
4. Once completed we will place the egg in a whirl pack, dump in diluent labeled 1 -24 (see data collection sheet), rinsed for 30 seconds, and then the diluent returned to the labeled container.
5. The rinses labeled 1 – 24 will be returned to the lab.
6. We will screen four new surfaces in the hatchery using 10 X 10 cm templates. These will be determined the day we do this trial.

**Response Variables:** $\log_{10}$ APC and EB.

From 6/25 the sample standard deviation for log APC eggs rinses was 0.454830721.

# Contents

- All egg rinse results treated for eggs treated for 120 seconds with Pathogen Reduction Box (PRB).

- The study was a 2 X 3 factorial with 6 factor /level combinations. Eggs were turned at 60 seconds, or not and then received no spray, water spray, or 3% hydrogen peroxide spray. Sprays were maintained at 110$^o$F.

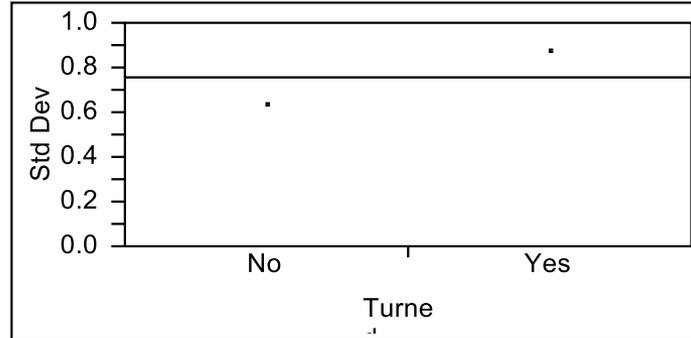- I compared the mean and then variation by treatment.

# Main Effect Turning
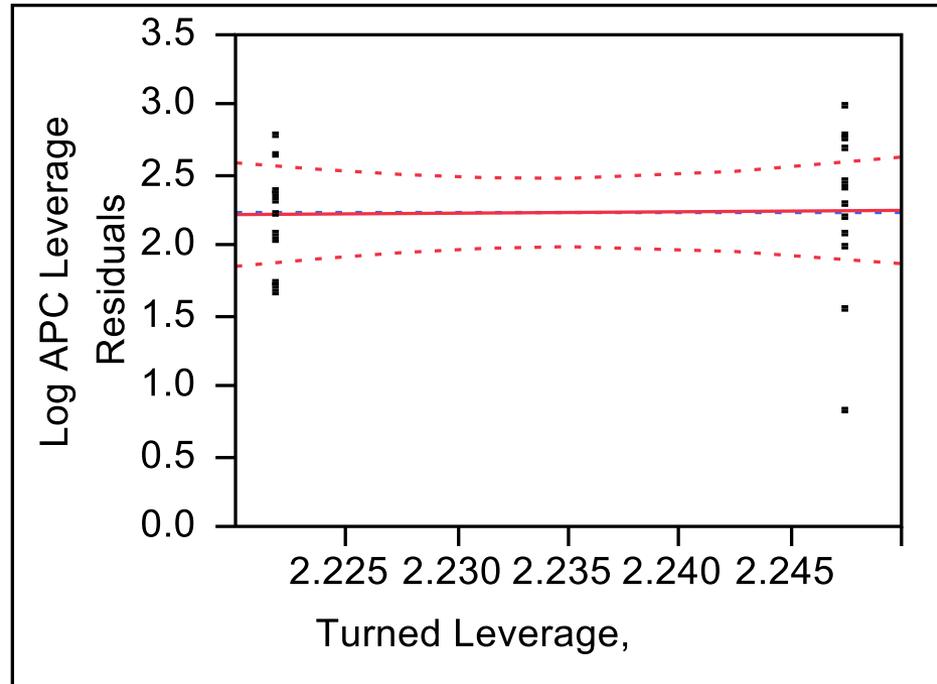


## Least Squares Means Table

| Level | Least Sq Mean | Std Error | Mean |
|-------|---------------|-----------|------|
| No | 2.2217981 | 0.16357918 | 2.22180 |
| Yes | 2.2474455 | 0.16357918 | 2.24745 |

# Main Effect Turning

| Level | Count | Std Dev | MeanAbsDif to Mean | MeanAbsDif to Median |
|---|---|---|---|---|
| No | 12 | 0.6263509 | 0.4766972 | 0.4723347 |
| Yes | 12 | 0.8662828 | 0.5537497 | 0.5489137 |

| Test | F Ratio | DFNum | DFDen | p-Value |
|---|---|---|---|---|
| O'Brien[.5] | 0.5172 | 1 | 22 | 0.4796 |
| Brown-Forsythe | 0.1212 | 1 | 22 | 0.7310 |
| Levene | 0.1271 | 1 | 22 | 0.7248 |
| Bartlett | 1.0877 | 1 | . | 0.2970 |
| F Test 2-sided | 1.9129 | 11 | 11 | 0.2971 |

## Welch's Test

Welch Anova testing Means Equal, allowing Std Devs Not Equal

| F Ratio | DFNum | DFDen | Prob > F |
|---|---|---|---|
| 0.0069 | 1 | 20.033 | 0.9346 |

**t Test**

0.0831

# Main Effect Spray
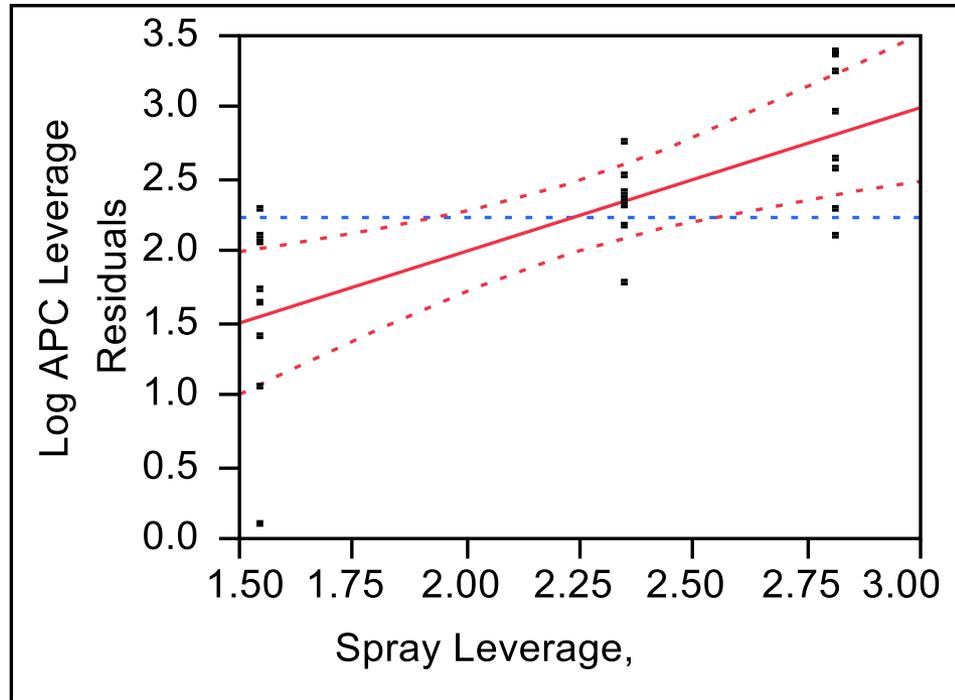


## Least Squares Means Table

| Level | Least Sq Mean | Std Error | Mean |
|---|---|---|---|
| None | 2.8155172 | 0.20034276 | 2.81552 |
| Peroxide | 1.5431638 | 0.20034276 | 1.54316 |
| Water | 2.3451844 | 0.20034276 | 2.34518 |

# Main Effect Spray
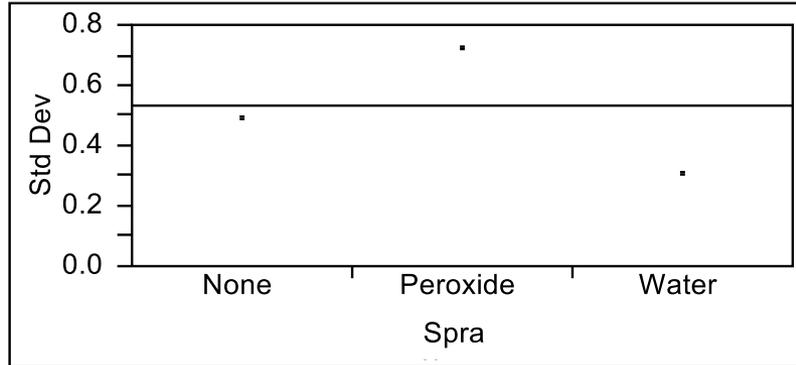
| Level | Count | Std Dev | MeanAbsDif to Mean | MeanAbsDif to Median |
|---|---|---|---|---|
| None | 8 | 0.4917251 | 0.4166148 | 0.4166148 |
| Peroxide | 8 | 0.7171010 | 0.4980004 | 0.4754243 |
| Water | 8 | 0.3070381 | 0.2097551 | 0.1965662 |

| Test | F Ratio | DFNum | DFDen | Prob > F |
|---|---|---|---|---|
| O'Brien[.5] | 1.0351 | 2 | 21 | 0.3726 |
| Brown-Forsythe | 1.3654 | 2 | 21 | 0.2770 |
| Levene | 1.6655 | 2 | 21 | 0.2131 |
| Bartlett | 2.1852 | 2 | . | 0.1125 |

## Welch's Test

Welch Anova testing Means Equal, allowing Std Devs Not Equal

| F Ratio | DFNum | DFDen | Prob > F |
|---|---|---|---|
| 8.1945 | 2 | 12.621 | 0.0052 * |

# Interactions
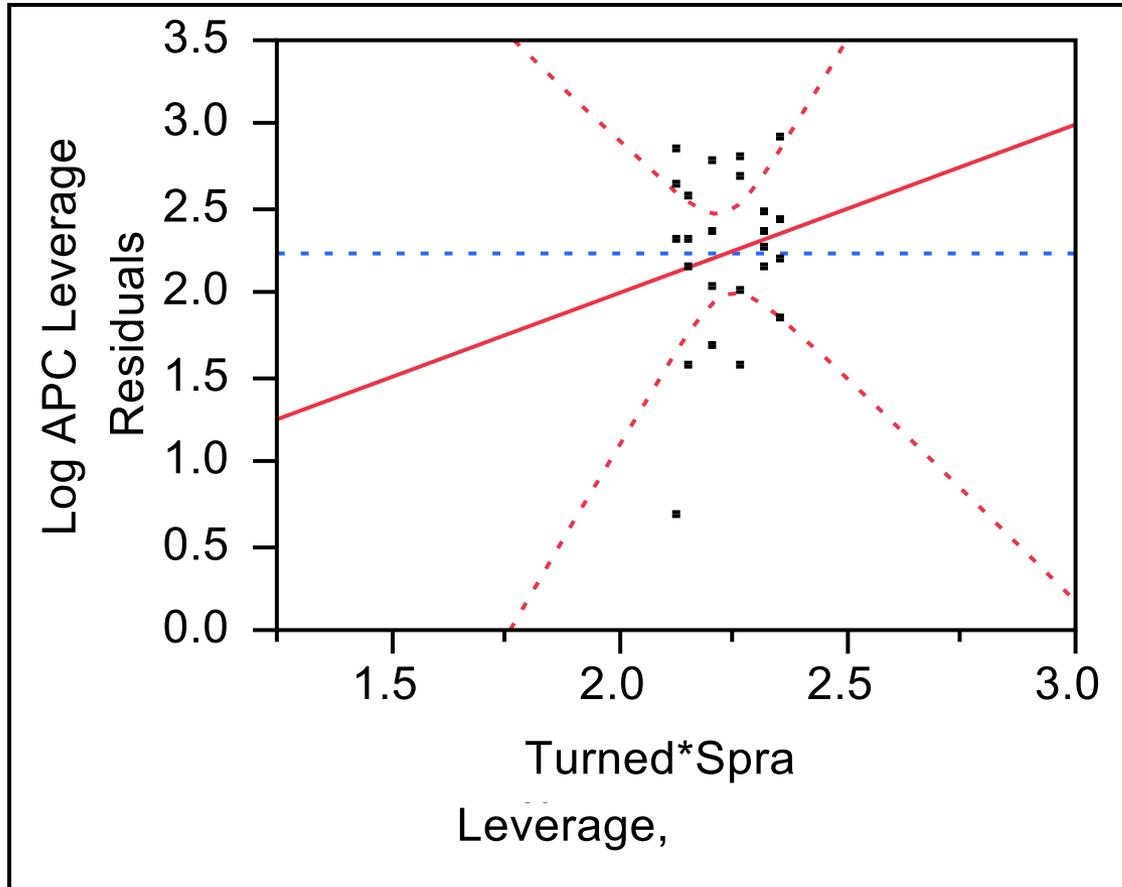


Interactions are NOT significant

# Conclusions

- Turning the eggs at 60 seconds in the PRB did not affect the log numbers of surviving bacteria.

- The spray used significantly affected the number of surviving bacteria. Water decreased the mean surviving by 0.48 log below no spray. Three percent hydrogen peroxide reduced surviving bacteria 1.28 log.

# Far-UV Proof of Concept III

## September 15, 2014

**Purpose:** To determine the effectiveness of HEO3's Pathogen Reduction Box (PRB) to reduce microbial load on hatching eggs for short (20 seconds or less) time periods. We will also evaluate the MicrobeBusterBox (MBB) on the egg receiving room floor.

**Background:** We completed initial trials with HEO3's PRB and (MBB) the week of 6/30/14. After viewing the results of those trials and the 8/14/14 trials we decided to continue our evaluation with eggs. We evaluated the effect of water and hydrogen peroxide sprays and turning the eggs on 8/14/14. We now would like to explore how quickly the PRB and MBB can reduce microbial loads at their current power settings.

**Methods:** The overall experimental design for eggs and surface will be a Completely Random Design (CRD), which is essentially a single factor experiment. For this experiment we will put 6 eggs into the PRB without turning for one of five different times. The times will be 0, 5, 10, 15, or 20 seconds. For the surface study we will lay out four grids of 10X10cm templates for treatment. The areas will be treated with the MBB for 5, 15, or 25 seconds.

The overall procedure for execution will be as follows:

1. We set up the PRB on the counter in egg receiving as done on 8/14/14.
2. We will haphazardly select eggs from the closest rack without sorting out visible contamination.
3. We will expose 6 eggs to each or the 5 times listed in the methods section, followed by microbial rinsing for enumeration as in step 4.
4. Once completed we will place the egg in a whirl pack, dump in diluent labeled 1 -30 (see data collection sheet), rinsed for 30 seconds, and then the diluent returned to the labeled container.
5. The rinses labeled 1 – 30 will be returned to the lab.
6. We will screen three new surfaces on the egg room floor in the hatchery using 10 X 10 cm templates. We will place four each templates close enough to be treated simultaneously with the MBB. We will measure two squares before UV treatment and two after treatment. Each of the templates will be sampled with sponges and labeled 31 – 42.

**Response Variables:** $\log_{10}$ APC and EB.

From 6/25 the sample standard deviation for log APC eggs rinses was
0.454830721.

Testing if there are differences among k means:

Alpha = 0.05
Std Dev = 0.4548
Extra Parameters = 0


Enter up to 10 Prospective Means showing separation across groups

0
0.75
1.5
2.25
3

Enter Power or Sample Size to get the other.
Enter neither to get a plot of Power vs. Sample Size


Sample Size  10
Power 0.9


Sample Size is the total sample size; per group would be n/k

# Contents

- Experiment was conducted on 9/29/14.
- For this experiment we put 6 eggs into the PRB (Pathogen Reduction Box) without turning or spraying for one of five different times. The times were be 0, 5, 10, 15, or 20 seconds. Total eggs treated was 30.
- The second part of the study was a surface study. For the surface study we layed out four grids of 10X10cm templates for treatment. The areas weretreated with the MBB (Microbe Buster Box) for 5, 15, or 25 seconds.
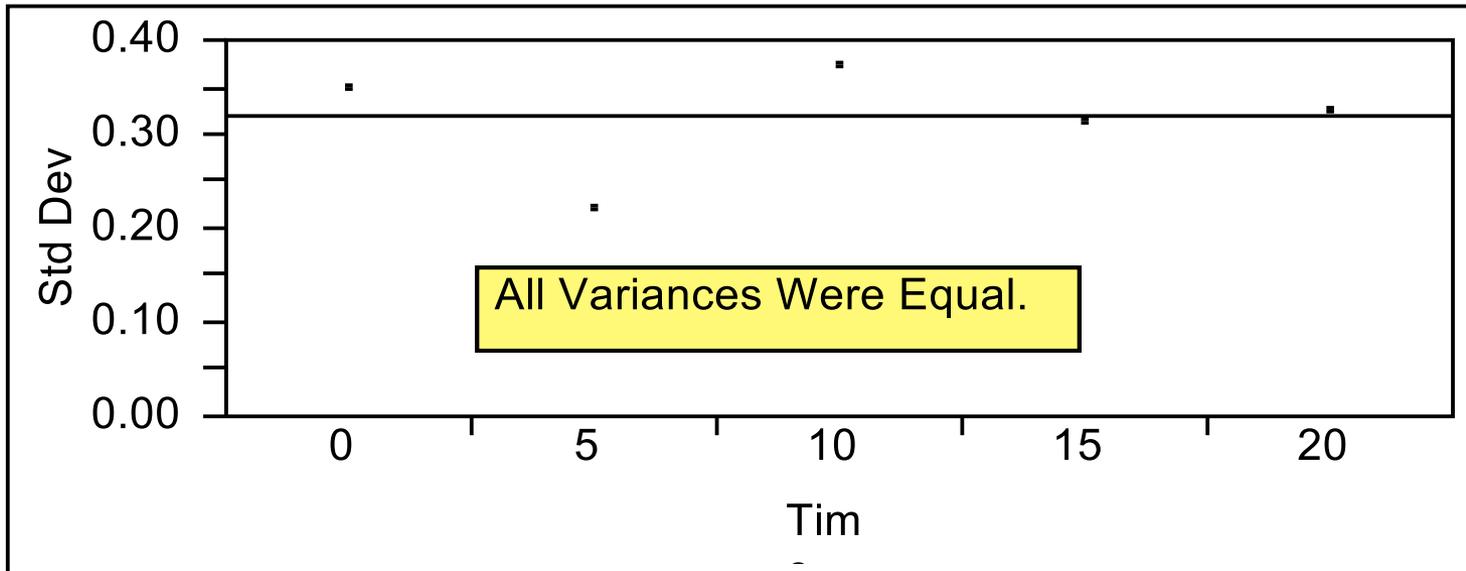- I compared the mean and variation by treatment.
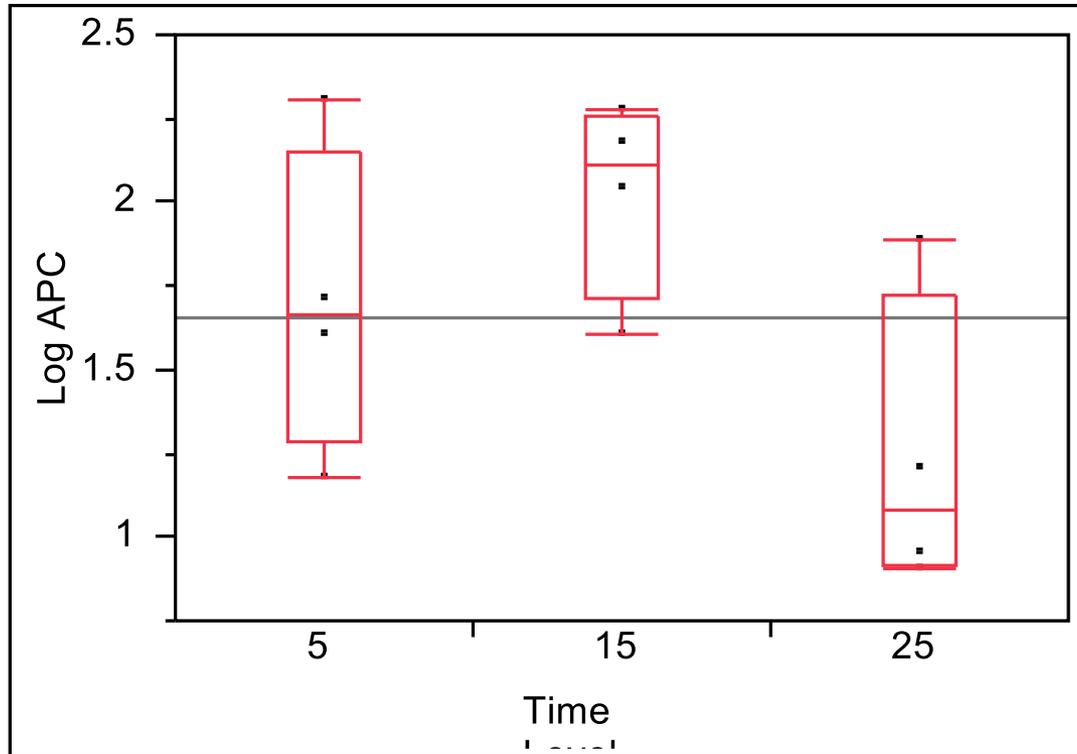
# PRB

# Effect of Time on Eggs
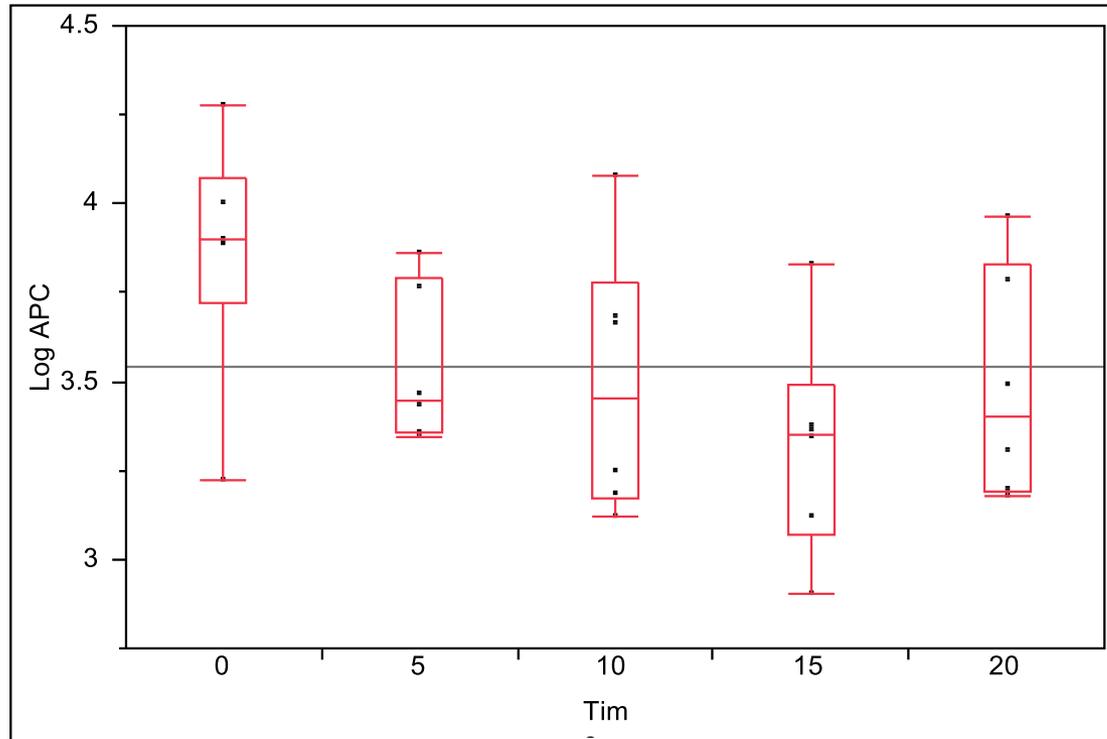


## Analysis of Variance

| Source | DF | Sum of Squares | Mean Square | F Ratio | Prob > F |
|---|---|---|---|---|---|
| Time | 4 | 0.9415924 | 0.235398 | 2.3090 | 0.0859 |
| Error | 25 | 2.5487155 | 0.101949 | | |
| C. Total | 29 | 3.4903079 | | | |

# Effect of Time on Eggs

# Effect of Time on Surfaces



## Analysis of Variance

| Source | DF | Sum of Squares | Mean Square | F Ratio | Prob > F |
|---|---|---|---|---|---|
| Time Level | 2 | 1.2554591 | 0.627730 | 3.6793 | 0.0680 |
| Error | 9 | 1.5354962 | 0.170611 | | |
| C. Total | 11 | 2.7909553 | | | |

# Effect of Time on Surfaces
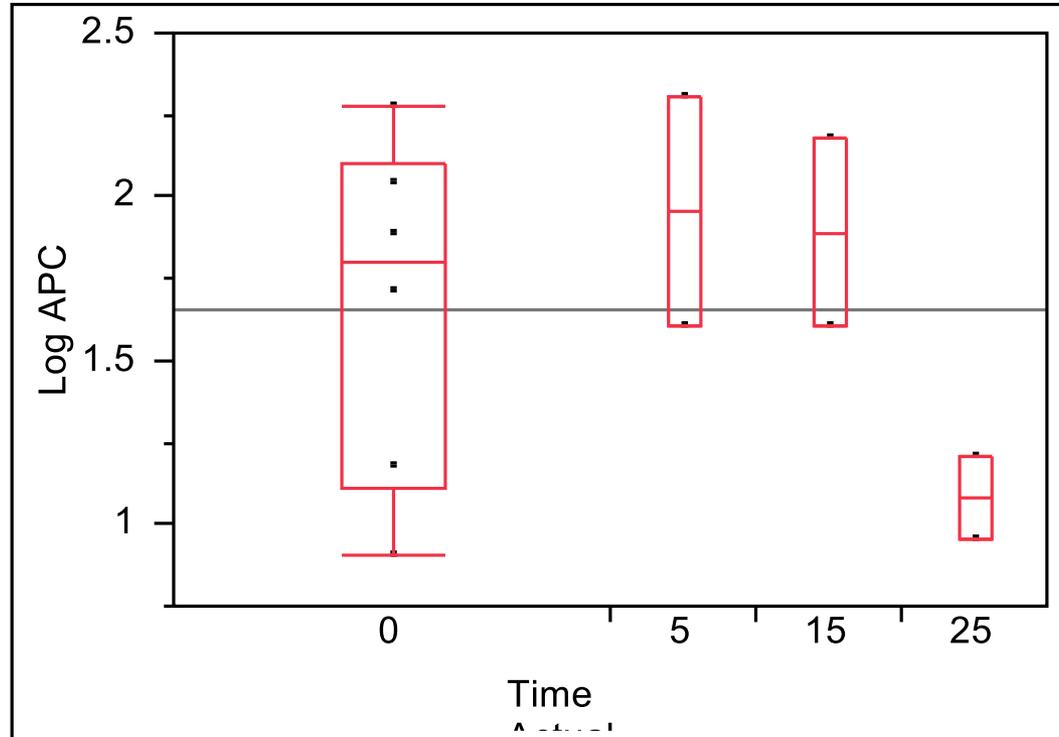


## Analysis of Variance

| Source | DF | Sum of Squares | Mean Square | F Ratio | Prob > F |
|---|---|---|---|---|---|
| Time Actual | 3 | 0.9524069 | 0.317469 | 1.3814 | 0.3168 |
| Error | 8 | 1.8385484 | 0.229819 | | |
| C. Total | 11 | 2.7909553 | | | |

# Results

- Based on the egg data we are reaching our max effectiveness by 15 seconds, but it may be as early as 5 seconds. Though more data would be needed to substantiate the exact time. I used too small a sample size.

- We had very few data points for surface treatment, but it appears the egg room floor reaches max kill in under 25 seconds.

# Conclusions

- Looks like the ideal egg system would wash the eggs, treat with Far-UV wet, and then dry.

- The MBB could be an effective surface or belt treatment. Key design parameters would include, time, power, surface cleanliness, etc.

- All of our data suggest the Far-UV technology is effective. Any application would need to be properly designed to be effective.