

**Antimicrobial Efficacies of 2.3 PPM Aqueous Ozone  
Against *E.coli* and General Aerobic Bacteria  
(Based On Tests Performed Using Porcine Intestines)**

**Author:**

George Kraft, Kraft Science Consulting, 821 Main Street, Boyden, IA 51234

**Abstract:**

Porcine intestines harvested, cleaned, boxed and ready for commerce using industry standard procedures were tested for the presence of general aerobic bacteria and generic *E.coli*. Half of the samples collected were subjected to aqueous ozone at a concentration of about 2.3 ppm and the other half of the samples were left untreated. All samples were analyzed and it was found that the treated samples had an average 1.9 log reduction of general aerobic bacteria and a 1.7 log reduction of *E.coli* when compared to the untreated samples. In general, any antimicrobial that can achieve greater than a 1 log pathogen reduction is considered to be significantly efficacious.

**Materials:**

Sterile “Whirl-Pak” sampling bags (10)  
25 ml Sterile Butterfield’s Buffer (10)  
1 ml micropipette  
3M Petrifilm for APC (10)  
3M Petrifilm for Generic *E.coli* (10)  
Sharp Knife  
Incubator at 36 degrees C  
SIG’s Aqueous Ozone Application System  
Ozone Concentration Test Kit

## **Methods:**

10 separate hog intestines (bung/large intestine) were removed from their box where they had been placed ready for commerce (all processes complete). An approximate 5 cm section was cut off from each of the 10 samples and then incisions were made to open them. 5 of these sample sections were placed into individual Whirl-Pak sample bags and placed aside. The other 5 sections were laid flat upon a sanitary surface in the Hot Box and both sides were sprayed with aqueous ozone from our application system. The concentration of ozone was tested to be approximately 2.3 ppm (based on color comparisons to standards). Each of the treated samples was then placed into individual Whirl-Pak sample bags. 25 ml of sterile buffer was added to each of the 10 sample bags in preparation for plating. After stomaching all samples were plated onto individual 3M Petrifilms for APC and for generic *E.coli*, then incubated at 36 degrees C for 24 hours. All resulting plates were then counted.

**Results:**

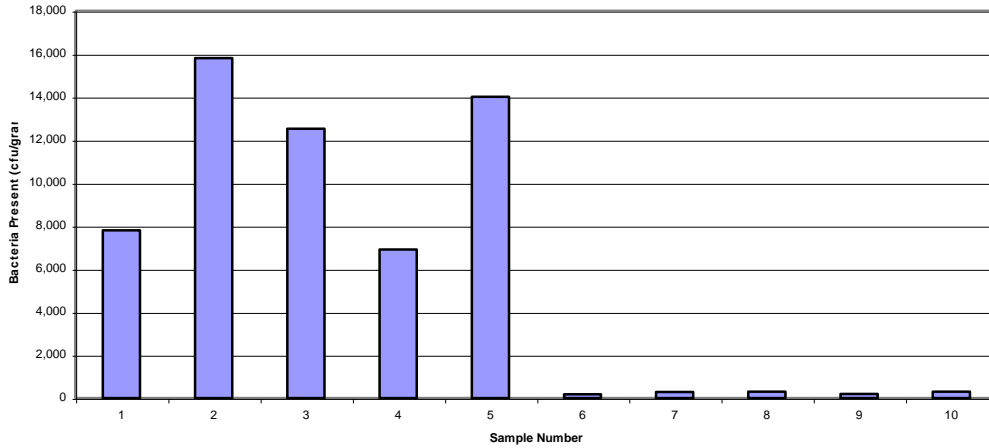
**Untreated Samples**

Sample #	APC (cfu/g)	E.coli (cfu/g)
1	7,800	2,200
2	15,806	4,274
3	12,525	2,828
4	6,905	3,393
5	14,000	3,688

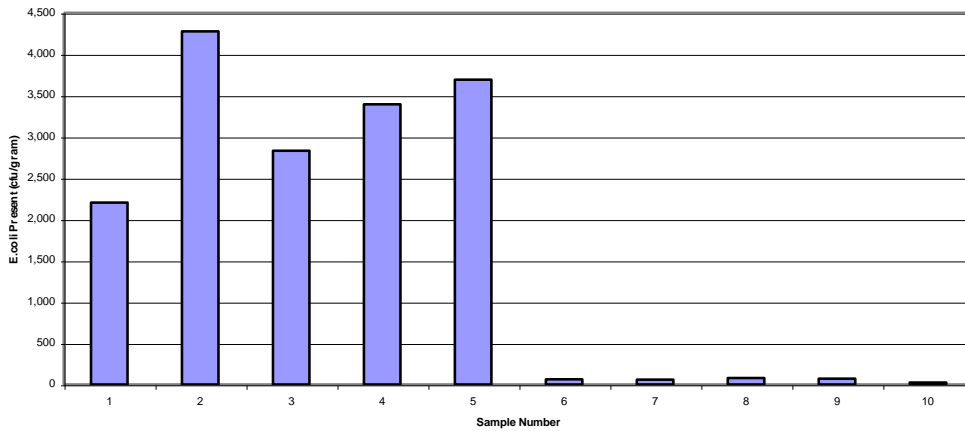
**Treated Samples**

Sample #	APC (cfu/g)	E.coli (cfu/g)
6	176	61
7	278	57
8	296	79
9	196	71
10	295	23

Aerobic Plate Count Comparisons Of Untreated (1-5) and Treated (6-10) Samples



E.coli Plate Count Comparisons Of Untreated (1-5) And Treated (6-10) Samples



## **Conclusions:**

This was the first time I had ever checked the microbial status of our bung product after all washing processes were complete and although I would hypothesize that they would still have large microbial loads I was surprised by the amounts I actually found.

Application of 2.3 ppm aqueous ozone to these products clearly had a significant effect relative to decreasing total microbial loads.

Ozone is consumed when it destroys a bacterial cell, therefore it can be stated that the extremely high microbial loads on these products consumed all ozone applied and by increasing the concentration of ozone a greater log reduction can be achieved.