

## **Efficacy of Hot Water vs. Various Chemical Treatments on *E. coli* O157:H7 Inoculated Knives**

### Background:

There are a variety of intervention steps that meat and poultry processing facilities employ in order to assure that a safe and reliable food supply is delivered to the consumer. One intervention step that occurs during the processing of the meat or poultry carcasses involves disinfecting the knives, blades or other cutting tools. This step is significant because use of a microbiologically contaminated cutting tool can actually inoculate potentially harmful microbes into the deep tissue of the meat or poultry. These cutting tools are disinfected by either submerging them in hot water (180° F) between 11 and 15 seconds or by using sodium hypochlorite bleach (NaOCl) at 100 ppm as Cl<sub>2</sub> for at least 30 sec. These options are effective but both have their down falls. It can get quite costly for these plants to maintain a water temperature of 180° F for hours at a time in order to disinfect the cutting tools. NaOCl is extremely corrosive to the metal cutting tools and if they are not high grade stainless steel the corrosion occurs quickly, damaging the instruments.

The purpose of this study is to compare the efficacy (equivalency) of various sterilization methods, and contact times.

### Methods:

#### *Preparation of Test Microorganisms:*

One dehydrated pellet, containing *E. coli* O157:H7 (ATCC 35150), was rehydrated in nutrient broth and incubated for 24 hours at 37°C. The inoculum was transferred to fresh nutrient broth for two consecutive days to ensure a sufficient concentration of *E. coli* O157:H7 was available for the study. The broth and bacteria mixture was then centrifuged leaving the *E. coli* O157:H7 to be re-suspended in approximately 500 mL Butterfield's Buffer. The *E. coli* buffer solution was serially diluted and plated on 3M Petrifilm *E. coli* Plates, incubated at 35 degrees C for 24 hours where it was determined that the *E. coli* O157:H7 population was  $9.8 \times 10^7$  CFU/ml or log<sub>10</sub> 7.99.

#### *Preparation of Test and Control Solutions:*

1. Control: A water control, which consisted of approximately 4000 g of Modesto City water at 72° F, was used to determine the number of *E. coli* O157:H7 remaining after a given contact time.
2. Hot water: 4000 g of Modesto City water was heated on a hot plate until the temperature reached 180° F. This was used as a test solution at given contact times.
3. 100 ppm Cl<sub>2</sub>: A 100 ppm as Cl<sub>2</sub> solution of sodium hypochlorite bleach (NaOCl) was prepared for use as another test solution at given contact times.

4. 200 ppm PAA: Perasan MP-2 was used to prepare a 200 ppm PAA test solution. Perasan MP-2 is a product that contains 15% peroxyacetic acid, 5.5% hydrogen peroxide, 35% acetic acid, and 0.7% HEDP and if used less than 220 ppm as peroxyacetic acid complies in all respects to 21 CFR 173.370 and specifically with Food Contact Notification #887, for use on meat and poultry.
5. 200 ppm Br<sub>2</sub>: A hypobromous acid test solution used in this study was 200 ppm (mg/L) as bromine (0.02%) at given contact times.
6. 300 ppm Br<sub>2</sub>: A hypobromous acid test solution used in this study was 200 ppm (mg/L) as bromine (0.03%) at given contact times.

In Summary:

- a) Control- 15 seconds (2 replicates)
- b) Control- 20 seconds (2 replicates)
- c) Control- 30 seconds (2 replicates)
- d) Hot water- 5 seconds (2 replicates)
- e) Hot water- 10 seconds (2 replicates)
- f) Hot water- 15 seconds (2 replicates)
- g) 100 ppm Cl<sub>2</sub>- 5 seconds (2 replicates)
- h) 100 ppm Cl<sub>2</sub>- 30 seconds (2 replicates)
- i) 100 ppm Cl<sub>2</sub>- 45 seconds (2 replicates)
- j) 200 ppm PAA- 5 seconds (2 replicates)
- k) 200 ppm PAA- 10 seconds (2 replicates)
- l) 200 ppm PAA- 20 seconds (2 replicates)
- m) 200 ppm Br<sub>2</sub>- 5 seconds (2 replicates)
- n) 200 ppm Br<sub>2</sub>- 10 seconds (2 replicates)
- o) 200 ppm Br<sub>2</sub>- 20 seconds (2 replicates)
- p) 300 ppm Br<sub>2</sub>- 5 seconds (2 replicates)
- q) 300 ppm Br<sub>2</sub>- 10 seconds (2 replicates)
- r) 300 ppm Br<sub>2</sub>- 20 seconds (2 replicates)

The test solutions and controls were tested in the same manner by the following method.

*Test:*

1. Eight knives were purchased for use in this study, see [Image 1](#). Holding the spray bottle about 6 inches from the knives, one side of each knife blade was doused with a fine spray of the *E. coli* O157:H7 suspension. Care was taken to ensure that the knives were covered evenly, see [Images 2 and 3](#).
2. After the *E. coli* Butterfield's Buffer solution was completely dry, two knives (one per replicate) were submerged into the test solution or control for the appropriate contact time period, see [Image 4](#).
3. Immediately after removing the knives from the test solution or control, a technician swabbed half of the blade of the still-wet knife with a Q-tip-like swab known as a Quickswab. No area of the surface knife was contacted more than once with the swab, which was rotated slightly between swabbing strokes for uniform distribution of bacteria onto the absorbent pad, see [Image 5](#). This was

- followed by vortexing the swab into the 1 ml of nutrient broth that accompanied the Quickswab in order to dislodge the bacteria from the swab and into the aqueous phase. Another swab was then used to swab the other half of the knife in identical fashion. Thus, two fresh Quickswabs were used for each knife. The nutrient broth containing viable bacteria swabbed from the knives were subsequently serially diluted ( $10^0$ ,  $10^2$  and  $10^4$ ) using Butterfield's buffer, and plated onto 3M E. coli petrifilms for *E. coli* O157:H7.
4. The knives were disinfected after each use to be used in the next set of testing.

Image 1:



Images 2 and 3:



Image 4:



Image 5:



Results and Discussion:

The initial population of *E. coli* O157:H7 was determined by plating on 3M Petrifilm *E. coli* Plates. The count obtained and subsequently used in the study was  $9.8 \times 10^7$  CFU/ml or  $\log_{10} 7.99$ .

Table 1 demonstrates the efficacy of the test solutions at different contact times. The table shows that most test solutions are efficacious at disinfecting the *E. coli* contaminated knives at 15 seconds. There was no visible *E. coli* O157:H7 bacteria present on the 3M *E. coli* Petrifilms that contained the swab sample from the knives had been treated with 200 ppm PAA, 200 ppm Br<sub>2</sub>, and 300 ppm Br<sub>2</sub> at five seconds. On the other hand, the use of water at 180° F requires a 15 second contact time and the use of Cl<sub>2</sub> at a contact time between 10 and 30 seconds was necessary to disinfect the knives.

Table 1: Equivalency Test against *E. coli* O157:H7 (ATCC 35150)

**Equivalency Test against *E. coli* O157:H7 (ATCC 35150)**

Test Solution	Details / Concentration	Contact Time				
		5 seconds	10 seconds	15 seconds	20 seconds	30 seconds
Control	77° F	N/A	N/A	+	+	+
Hot Water	180° F	+	+	-	N/A	N/A
NaOCl	100 ppm as Cl <sub>2</sub>	+	N/A	N/A	N/A	-
MP-2	200 ppm as PAA	-	-	N/A	-	N/A
HB2	200 ppm as Bromine	-	-	N/A	-	N/A
HB2	300 ppm as Bromine	-	-	N/A	-	N/A

Note:    "-" indicates no bacterial growth                    "+" indicates bacterial growth  
               "N/A" indicates not tested at this time

The actual number of *E. coli* O157:H7 remaining on the knives can be seen in Table 2. The control averaged a  $\log_{10}$  of 4.86 CFU/half knife *E. coli* O157:H7 remaining after being submerged in Modesto City water. There was a >99.99% reduction in *E. coli* O157:H7 bacteria when the knives were submerged in the 200 ppm PAA, 200 ppm Br<sub>2</sub> and 300 ppm Br<sub>2</sub> for just five seconds, meaning there were no *E. coli* O157:H7 colonies present on the 3M *E. coli* plates. There was a  $\log_{10}$  average 2.69 CFU/ half knife and 1.43 CFU/ half knife *E. coli* O157:H7 remaining when hot water was used as a disinfectant at five and ten seconds, respectively. An average 2.58  $\log_{10}$  CFU/ knife remained when 100 ppm Cl<sub>2</sub> was used as a disinfectant at five seconds.

Table 2:

<b>Description</b>	<b>Log10 (CFU/mL) remaining</b>	<b>Log10 Reduction</b>	<b>% Reduction</b>
<b>Control 77° F</b>	<b>4.86</b>	N/A	N/A
Hot Water 180° F (5 sec.)	2.69	2.17	99.32
Hot Water 180° F (10 sec.)	1.43	3.43	99.96
Hot Water 180° F (15 sec.)	<4.86	>4.86	>99.99
NaOCl: 100 ppm as Cl <sub>2</sub> (5 sec.)	2.58	2.28	99.48
NaOCl: 100 ppm as Cl <sub>2</sub> (30 sec.)	<4.86	>4.86	>99.99
NaOCl: 100 ppm as Cl <sub>2</sub> (45 sec.)	<4.86	>4.86	>99.99
MP-2: 200 ppm as PAA (5 sec.)	<4.86	>4.86	>99.99
MP-2: 200 ppm as PAA (10 sec.)	<4.86	>4.86	>99.99
MP-2: 200 ppm as PAA (20 sec.)	<4.86	>4.86	>99.99
HB2 200 ppm as Br <sub>2</sub> (5 sec.)	<4.86	>4.86	>99.99
HB2 200 ppm as Br <sub>2</sub> (10 sec.)	<4.86	>4.86	>99.99
HB2 200 ppm as Br <sub>2</sub> (20 sec.)	<4.86	>4.86	>99.99
HB2: 300 ppm as Br <sub>2</sub> (5 sec.)	<4.86	>4.86	>99.99
HB2: 300 ppm as Br <sub>2</sub> (10 sec.)	<4.86	>4.86	>99.99
HB2: 300 ppm as Br <sub>2</sub> (20 sec.)	<4.86	>4.86	>99.99

Conclusions:

- One intervention step that meat and poultry processing facilities employ in order to assure that a safe and reliable food supply is delivered to the consumer occurs during the processing of the meat or poultry carcasses and involves disinfecting the knives, blades or other cutting tools. This step is significant because use of a microbiologically contaminated cutting tool can actually inoculate potentially harmful microbes into the deep tissue of the meat or poultry.
- Cutting tools used by these meat and poultry processing facilities are disinfected by either submerging them in hot water (180° F) between 11 and 15 seconds or by using sodium hypochlorite bleach (NaOCl) at 100 ppm as Cl<sub>2</sub> for at least 30 sec. It can get quite costly for these facilities to maintain a water temperature of 180° F for hours at a time in order to disinfect the cutting tools. NaOCl is extremely corrosive to the metal containing cutting tools and if they are not high grade stainless steel, the corrosion occurs quickly, damaging the instruments.

- The purpose of this study was to determine the available germicidal equivalence of 200 ppm PAA, 200 ppm Br<sub>2</sub> and 300 ppm Br<sub>2</sub> compared to hot water at 180° and 100 ppm Cl<sub>2</sub>.
- The knives were inoculated with *E. coli* O157:H7 buffer solution that contained a population of  $9.8 \times 10^7$  CFU/ml or log<sub>10</sub> 7.99 *E. coli* O157:H7.
- Table 1 demonstrates the efficacy of using various types of sterilization methods at different contact times against *E. coli* O157:H7. Modesto City water at 180° F, 100 ppm Cl<sub>2</sub> solution, 200 ppm PAA solution from Perasan MP-2, 200 ppm Br<sub>2</sub> and 300 ppm Br<sub>2</sub> were compared to a 77° F water control. It can be seen that all treatments except for the 100 ppm Cl<sub>2</sub> and hot water eradicated the *E. coli* bacteria after just 5 seconds. It took between 10 and 15 seconds in hot water and between 10 and 30 seconds in the 100 ppm Cl<sub>2</sub> solution to completely disinfect the knives.
- Table 2 also demonstrates the efficacy of the test solutions compared to the control. This table shows the log<sub>10</sub> CFU/half knife *E. coli* O157:H7 remaining after treatment.
- Tables 1 and 2 show that 200 ppm PAA from Perasan MP-2, 200 ppm Br<sub>2</sub> and 300 ppm Br<sub>2</sub> has far greater efficacy as an antimicrobial against *E. coli* O157:H7 on knives than the 180° F water treatment and the 100 ppm Cl<sub>2</sub> treatment.
- This data can be beneficial to meat and poultry processing facilities because the use of PAA or Br<sub>2</sub> to disinfect knives can be more cost effective. This is because these chemical treatments are cheaper than keeping water at such high temperatures for such a long period of time. Also, the corrosivity of using NaOCl on cutting tools results in replacement of these instruments frequently. Disinfecting the knives at five seconds rather than 15 seconds or more also saves time.

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