Study Title

Efficacy of Several Antimicrobial Processing Aids
Sprayed on Meat and Pork Products Against *E. coli O157:H7*

Data Requirements

Efficacy Data for FSIS, USDA, FDA

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Background

The contamination of food products by pathogenic organisms such as *E. coli* O157:H7 is an on-going problem that is addressed within the processing plant using antimicrobial products. The efficacy of these Food Contact Substances (FCS) is important to assure a safe and reliable food supply. Meat processing facilities are adopting new and improved chemical intervention steps of treating their meat carcasses with FDA approved sanitizers as part of their HACCP programs. *E. coli* is one of the primary pathogens of interest in most meat and pork processing plants. It can be seen in Table 1 that as little as 10 to 100 CFU/ml (Colony Forming Units/milliliter) *E. coli 0157:H7* bacteria cells can cause hemorrhagic colitis.

The primary method of applying the FCS in meat and pork processing plants on carcasses, parts, trim and organs, is using coarse spray technologies. Therefore, the purpose of this study was to determine the relative efficacy of a 30 second spray bar application of peroxyacetic acid (PAA) from Perasan MP-2®, DBDMH (from FCN No. 792), and HB2, which is a proposed Food Contact Substance based on liquid hypobromous acid as the active ingredient, against the bacteria, *E. coli 0157:H7.*

Table 1: Estimated infectious dose of bacteria species

Bacteria Species	**Estimated infectious dose (bacteria cell number)**	**Disease**
E. coli O157:H7	10 to 100	Hemorrhagic colitis
E. coli	1,000,000 to 100,000,000	Traveler's diarrhea
Salmonella	100 to 1,000,000,000	Salmonellosis
Principal source: Foodborne Pathogens: Risks and Consequences, Report No. 122, CAST- Council for Agricultural Science and Technology, September 1994.		

Methods

Meat processing facilities commonly treat beef and pork with antimicrobial solutions for ~30 seconds by spraying the beef and pork carcasses and trim with the solution in a spray cabinet. To simulate this process, a small spray cabinet was created for this study. A one inch air pump was used to deliver the test solution into half inch PVC tubing, which in turn allowed the test solutions to be dispensed out of six nozzles placed four and a half inches apart in a 30 gallon drum. Each nozzle delivered 0.6 gpm of test solution. A regulator on the air pump was used to adjust the pressure of the spray as needed. The air

pump used in this experiment can be seen in Image 1. The spray cabinet was calibrated using city water by adjusting the nozzles and pressure to ensure even distribution of the test solutions. Images 2 and 3 show the spray cabinet in operation at low pressure (10 psi) and high pressure (70 psi), respectively.

Image 1: One Inch Air Pump

Image 2: Low Pressure Spray (10 psi)

Image 3: High Pressure Spray (70 psi)

Due to variables such as temperature, spray pressures, meat types and sizes, operator techniques, and equipment that can alter the results of this study, two separate study days were set aside, the first day called Experiment 1 and the second repeat test, termed Experiment 2.

Experiment 1 was performed using duplicate cuts of meat and pork at 10 psi and 70 psi. After enumeration the experimenters found that there was no statistical difference between the low and high pressure results for the antimicrobial products (FCS). Therefore, it was determined to perform Experiment 2 using an average of 40 psi pressure on triplicate cuts of meat and pork. In all cases, the controls and challenges were plated on duplicate sets of 3M Petrifilm E. coli Plates.

The Food Contact Substances:

The MP-2 used to prepare the test solutions containing PAA was made from MP-2. 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 carcasses, parts, trim and organs.

DBDMH is a dry material and comes in granular form at 99.5% activity. The product was obtained at a local store. Albemarle Corporation is the only US manufacturer of DBDMH and is the owner of Food Contact Notifications #334, #357, #453, #775, and #792 for use of DBDMH on meat, pork and poultry. The product was dissolved in water to obtain a concentrate which was subsequently filtered to remove any undissolved solids and added to potable water to obtain the appropriate concentration needed for these experiments.

The HB2 is the subject of an application for a Food Contact Notification by the FDA. It is pure hypochlorous acid generated on-site by blending hydrogen bromide with a hypochlorite source. For this study, hypobromous acid was created on-site by combining hydrogen bromide and sodium hypochlorite.

Experiment 1:

A stock solution of a field strain of *E. coli 0157:H7* was incubated at 35 degrees C for four days in Sigma Nutrient Broth for microbial culture. Three daily, consecutive transfers of the inoculums were made to ensure a sufficient concentration of *E. coli 0157:H7* was available for the study. The broth and bacteria mixture was then centrifuged leaving the *E. coli 0157: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 48 hours where it was determined that the E. *coli 0157:H7* population was 6.76×10^7 CFU/ml or $\log_{10}$ 7.83.

1. Twelve uncooked, boneless, London broil beef strips of equal size and twelve uncooked, boneless, pork chops of equal size were dried, weighed and marinated in the 500 ml *E. coli* Butterfield's Buffer solution overnight, turning occasionally.

See Image 4. The beef weights ranged from 180.4g to 260.0g, the average being 209.5g. The average pork weight was 240.8g, the low being 182.8g and the high being 289.0g.

Image 4: Preparation of Beef and Pork

The twelve beef pieces and twelve pork pieces were removed from the *E. coli*. Butterfield's Buffer bath, shaken dry using sterile gloves, introduced into sterile poultry rinse bags and taken to an isolated area outside where the simulated spray bar portion of the study was taken place. This study was performed in duplicate, i.e., two pieces of each meat type was subjected to each test substance either at low or high pressure. During the 30 second spray, a piece of meat was held by a hook and moved up and down while rotating to ensure even distribution of the test spray at low pressure (10 psi) or high pressure (70 psi). The FCS concentration was measured prior to spraying the meat pieces by using a HACH DR/700 Colorimeter and HACH 10 ml Total Chlorine pillow packets (see Attachment 'A'). The value was multiplied by the molecular weight difference vs. chlorine and bromine (PAA = 1.07; DBDMH and HB2 = 2.25), respectively, and the dilution factor of each FCS.

In summary:

Beef-

a) Control: Two beef pieces- low pressure- city water
b) PAA: Two beef pieces- low pressure- 212 ppm PAA
c) DBDMH: Two beef pieces- low pressure- 308 ppm total bromine
d) HB2: Two beef pieces- high pressure- 276 ppm total bromine

Pork-

a) Control: Two pork pieces- low pressure- city water
b) PAA: Two pork pieces- low pressure- 212 ppm PAA
c) DBDMH: Two beef pieces- low pressure- 308 ppm total bromine
d) HB2: Two beef pieces- high pressure- 276 ppm total bromine

2. Immediately after each piece was sprayed a sample of the wash solution was taken from the bottom of the spray cabinet drum for microbial analysis. This test solution was plated on 3M Petrifilm E. coli Plates and incubated at 35 degrees C for 48 hours.
3. The drum was then rinsed clean with the test spray and made ready for use on the next piece of meat.
4. After challenge testing, the meat piece was gently shaken three times to remove excess liquid and returned to a new, sterile bag and taken to the lab where 200g of city water was introduced to the bag and subsequently tumbled gently for one minute to dislodge remaining *E. coli* bacteria. The water left at the bottom of the bag was plated using 3M Petrifilm E. coli Plates and incubated at 35 degrees C for 48 hours, upon which the plates were enumerated. All plating for *E. coli* was performed within 10 minutes after the challenge testing.

Experiment 2:

This test was performed subsequent to Experiment 1 in order to confirm that the results obtained the first time were not subjected to variables such as temperature, operator, equipment etc. Because there was no significant difference in the microbiological results between low pressure (10 psi) and high pressure (70 psi), the air pressure was set at 40 psi throughout Experiment 2 using triplicate meat and pork samples, rather than duplicates.

Another step omitted in Experiment 2 was the microbiological analysis of the solutions collected at the bottom of the drum (rinse water) immediately after the meat was sprayed. This step was omitted because there was such a dramatic decrease in *E. coli* present in Experiment 1 it seemed unnecessary to repeat this step. Also, it is known that what is left on the meat after treatment is more critical than what comes off of it during the wash.

The inoculums used in Experiment 2 were prepared in the same manner as Experiment 1 and yielded an *E. coli 0157:H7* population of 8.51×10^7 CFU/ml, or $\log_{10}$ 7.93. This was then sprayed onto the beef and pork pieces and left to marinate for two hours, see Image 5. The type of beef used this time was chuck roast, which was cut into twelve equal pieces. The average weight of beef piece used in this portion of the study was 257.1g and the average weight of pork was 142.9g.

All other steps performed in Experiment 2 were conducted in the same manner as Experiment 1, this time the study was performed in triplicate. In summary:

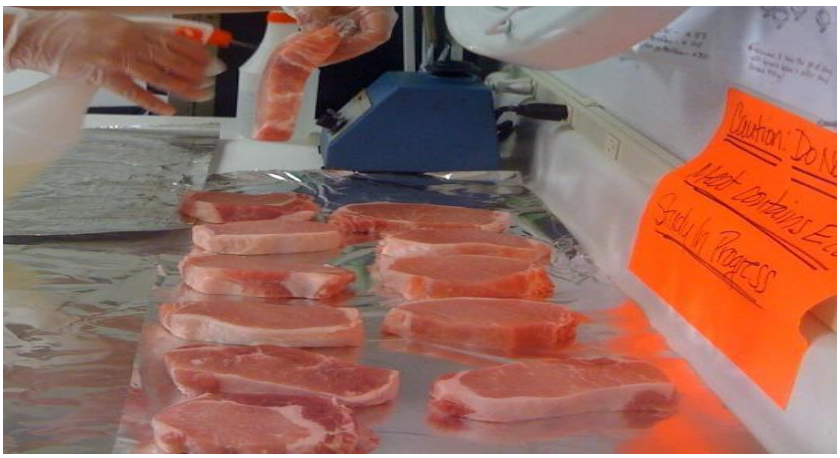
Beef-

a) Control: Three beef pieces- city water
b) PAA: Three beef pieces- 215 ppm PAA
c) DBDMH: Three beef pieces- 288 ppm available bromine
d) HB2: Three beef pieces- 279 ppm available bromine

Pork-

a) Control: Three beef pieces- city water
b) PAA: Three beef pieces- 215 ppm PAA
c) DBDMH: Three beef pieces- 288 ppm available bromine
d) HB2: Three beef pieces- 279 ppm available bromine

Image 5: *E. coli 0157 H:7* Inoculum Sprayed on Pork



Results and Discussion

Note: This study was conducted on two separate days to rule out variables that can interfere with the outcome of the study. Because the two experiment days resulted in very little difference in inoculum concentrations, spray pressures, and microbiological results, it was decided that the data from the two experiment days, as well as the duplicate and triplicate testing of the meats be consolidated and the average results would be reported in this study. The average of all beef control results, the average of all pork control results, the average of all PAA, DBDMH and HB2-hypobromous acid-treated beef and pork results are reported below.

A nominal 212-215 ppm PAA from Perasan MP-2® was used as the test solution to be sprayed onto the meat by the spray bar apparatus created for this study. The actual concentrations were measured by DPD method (see Attachment 'A') . Experiment 1 used a PAA solution of 212 ppm and Experiment 2 used a 215 ppm PAA solution. The DBDMH concentrations were determined to be 308 ppm available bromine in Experiment 1 and 288 ppm available bromine in Experiment 2. The HB2 concentration was 276 ppm in Experiment 1, and 279 ppm in Experiment 2.

In Experiment 1, immediately after each piece of meat was treated in the spray cabinet for 30 seconds, a water sample from the bottom of the drum was subjected to microbiological analysis. The results can be seen in Table 2. The controls contained an average of 5.01 CFU/ml of *E. coli 0157:H7* in the beef wash water and a $\log_{10}$ average 5.18 CFU/ml in the pork wash water. On the other hand, the PAA wash water, DBDMH wash water and HB2 wash water had very little *E. coli 0157:H7* present. There was an average $\log_{10}$ reduction of 4.77 CFU/ml (99.998%) in *E. coli 0157:H7* in the PAA wash water that was used to spray the beef and an average $\log_{10}$ reduction of >5.18 CFU/ml (>99.999%) in the pork wash water, meaning there were no bacteria colonies present. The DBDMH treated beef had an average $\log_{10}$ reduction of 4.53 CFU/ml (99.997%) in *E. coli 0157:H7* in the wash water and an average $\log_{10}$ reduction of 4.19 CFU/ml (99.994%) in the pork wash water. The HB2-treated beef wash water resulted in reductions of $\log_{10}$ 4.86 CFU/ml (99.999%) and in the pork-treated wash water, an

average reduction of $\log_{10}$ 4.79 CFU/ml (99.998%) was seen. These are significant reductions in bacterial numbers but what is washed off the meat is not as significant as what is left on the meat.

Table 2: Wash water microbiological results

Description	log10 (remaining)	log10 reduction	% reduction
Control Beef	5.01	N/A	N/A
PAA Beef	0.24	4.77	99.998
DBDMH Beef	0.48	4.53	99.997
HB2 Beef	0.15	4.86	99.999
Description	**log10 (remaining)**	**log10 reduction**	**% reduction**
Control Pork	5.18	N/A	N/A
PAA Pork	0.00	>5.18	>99.999
DBDMH Pork	0.99	4.19	99.994
HB2	0.39	4.79	99.998

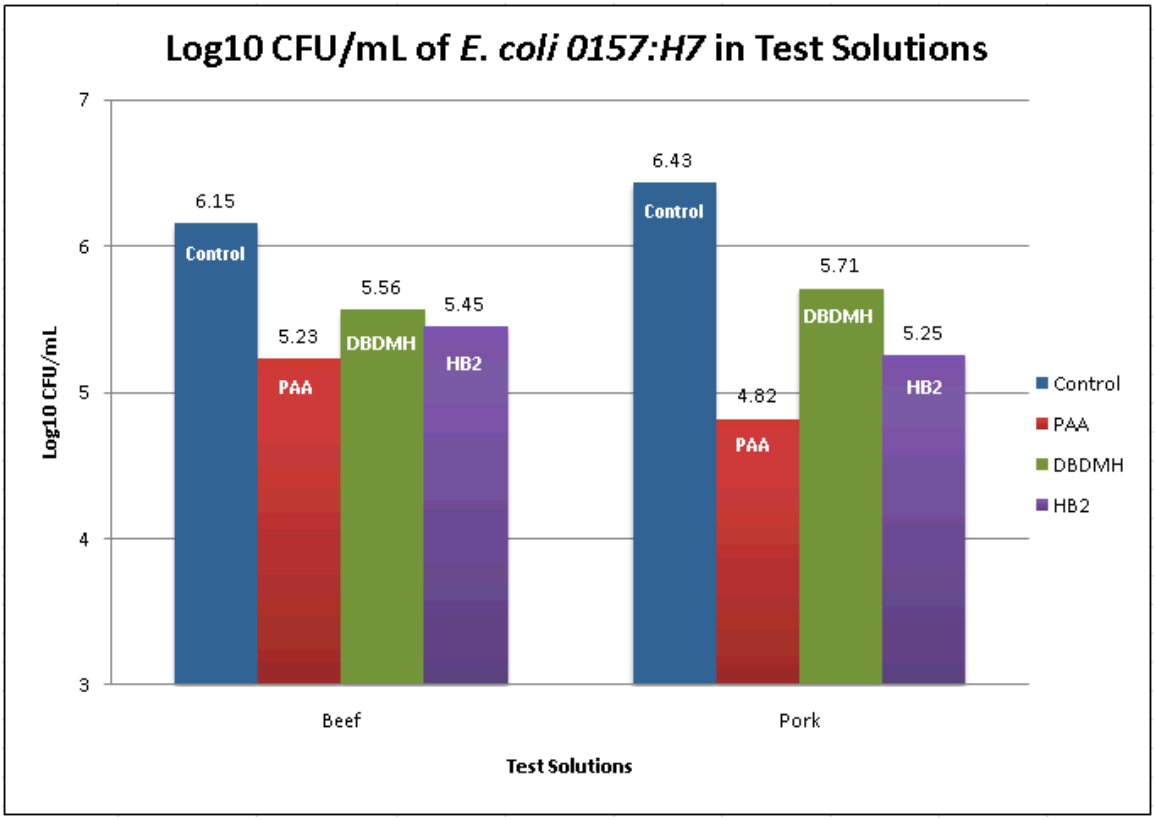
After each piece of meat was sprayed with the test solution and taken back to the lab in sterile bags, 200g of city water was added to the bag and subsequently tumbled for one minute to dislodge any viable *E. coli 0157:H7* bacteria. Table 3 contains the average number of bacteria left on the meat after being sprayed for 30 seconds with either city water (control), PAA, DBDMH and HB2 solutions. It can be seen that the controls averaged a $\log_{10}$ of 6.15 for beef and $\log_{10}$ 6.43 CFU/ml for pork. The $\log_{10}$ reduction in *E. coli 0157:H7* bacteria when PAA was sprayed onto beef, compared to the control, was 0.92 (87.98%). The $\log_{10}$ reduction when PAA was sprayed onto pork, compared to the control, was 1.61 (97.55%). The $\log_{10}$ reduction in *E. coli 0157:H7* bacteria when the DBDMH solution was sprayed onto beef, compared to the control, was 0.59 (74.30%). The $\log_{10}$ reduction when the DBDMH solution was sprayed onto pork, compared to the control, was 0.72 (80.95%). The HB2 challenge resulted in reductions of 0.70 $\log_{10}$ CFU/ml for beef and 1.18 $\log_{10}$ CFU/ml for pork.

The average concentrations of *E. coli 0157:H7* bacteria present on both the beef and the pork after being sprayed is charted in Figure 1 on the next page.

Table 3: Enumeration Results of Microbiological Analysis

Description	log10 (remaining)	log10 reduction	% reduction
Control Beef	6.15	N/A	N/A
PAA Beef	5.23	0.92	87.98
DBDMH	5.56	0.59	74.30
HB2 Beef	5.45	0.70	80.03
Description	**log10 (remaining)**	**log10 reduction**	**% reduction**
Control Pork	6.43	N/A	N/A
PAA Pork	4.82	1.61	97.55
DBDMH	5.71	0.72	80.95
HB2	5.25	1.18	93.39

Figure 1: Control vs. treatment, Log remaining



Conclusions:

- Due to variables such as temperature, spray pressures, meat types and sizes, operator techniques, and equipment that can alter the results of this study, two separate study days were set aside, the first day called Experiment 1 (which was conducted in duplicate) and the second a repeat test, called Experiment 2 (conducted in triplicate). The data from the two experiment days, as well as the duplicate and triplicate testing of meats were consolidated and the average results reported in this study because the two experiment days resulted in very little difference in inoculum concentrations, spray pressures, and microbiological results.
- Meat processing facilities commonly treat beef and pork with antimicrobial solutions for 30-60 seconds by spraying the beef and pork carcasses with the solution in a spray cabinet. To simulate this process, a small spray cabinet was created for this study.
- Immediately after each meat piece was sprayed a sample of the wash solution was taken from the bottom of the spray cabinet drum for microbial analysis. The results are very acceptable reductions in bacterial numbers for the PAA, DBDMH and HB2, but what is washed off the meat is not a significant factor in terms of food efficacy, but it is significant for the over-all health and safety of workers in and around the processing facility, as well as mitigating sanitation and wastewater

issues. The PAA wash water had very little *E. coli 0157:H7* present. There was an average $\log_{10}$ reduction of 4.77 CFU/ml (99.998%) in *E. coli 0157:H7* in the wash water that was used to spray the beef and an average $\log_{10}$ reduction of >5.18 (>99.999%) in the pork wash water. The DBDMH-treated beef had an average $\log_{10}$ reduction of 4.53 (99.997%) in *E. coli 0157:H7* in the wash water and an average $\log_{10}$ reduction of 4.19 (99.994%) in the pork wash water. HB2 yielded $\log_{10}$ reductions of 4.86 (beef) and 4.79 (pork) CFU/ml, respectively. All three test substances were effective in eradicating the planktonic (suspended) bacteria in the wash water retain challenge.

- 200 g of city water was added to the bag containing each piece of meat that was sprayed with the test solution and tumbled for one minute to dislodge any viable *E. coli 0157:H7* bacteria. Table 3 shows the average number of bacteria left on the meat after being sprayed for 30 seconds with either city water (control), the PAA solution or the DBDMH solution.
- The microbiological profile for the meat and pork challenges showed the controls averaged a $\log_{10}$ CFU/ml of 6.15 for beef and 6.43 for pork. The $\log_{10}$ reduction in *E. coli 0157:H7* bacteria when PAA was sprayed onto beef, compared to the control was 0.92 (87.98%). The $\log_{10}$ reduction when PAA was sprayed onto pork, compared to the control was 1.61 (97.55%). The $\log_{10}$ reduction in *E. coli 0157:H7* bacteria when the DBDMH solution was sprayed onto beef, compared to the control, was 0.59 (74.30%). The $\log_{10}$ reduction when the DBDMH solution was sprayed onto pork, compared to the control, was 0.72 (80.95%). The HB2 solution yielded reductions of 0.7 $\log_{10}$ CFU/ml and 1.18 $\log_{10}$ CFU/ml for beef and pork, respectively. The average concentration of *E. coli 0157:H7* bacteria present on both the beef and the pork after being sprayed with each test solution is charted in Figure 1.
- This study was designed to give the reader a reasonably accurate idea of the relative qualitative efficacy of peroxyacetic acid (PAA), DBDMH and hypobromous acid (HB2) against *E. coli 0157:H7* beef and pork when applied as a spray for 30 seconds.
- The PAA and liquid hypobromous acid (HB2) solution, when sprayed on both beef and pork, outperforms DBDMH in short 30 second contact times when the products are employed at the upper limits of their tolerance ceiling. These shorter contact time scenarios represent those typically encountered when meat carcasses are sprayed at meat processing facilities during commercial processing. Both treatments exhibit demonstrable efficacy on meat and pork surfaces compared to the control group.

References:

Bell, Kristen Y, et al*; *"Reductions of Foodborne Micro-Organisms on Beef Carcass Tissue Using Acetic Acid, Sodium Bicarbonate, and Hydrogen Peroxide Spray Washes";* Journal of Food Microbiology, 1997, 14, 439-448.

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ENVIRO TECH CHEMICAL SERVICES STANDARD OPERATING PROCEDURE

Original SOP Effective Date: No Date	**Superseded SOP Dated:** N/A	Effective Date: 7/08/09	Procedure No.: ETQC28
Facility: Modesto	Approval Name & Signature: Tina Rodrigues		Revision No.: 1
Review Frequency: 2 years	Approval Title: Lab Manager		Page 1 of 3
Without a yellow control stamp to the right of this statement, this procedure is a draft. A draft or an uncontrolled copy cannot be used to manage a process or task.			JMM-7/9/2009
Revised Section(s): Transferred SOP to the new SOP format.			

I. TITLE: DPD ANALYSIS OF PRODUCTS USING HACH DR/700 COLORIMETER

II. PURPOSE: This document is to be used by any lab personnel involved in the analysis of products using the HACH DR/700 Colorimeter.

III. EQUIPMENT / REAGENTS:
-HACH DR/700 Colorimeter- Model number 46700-00
-DPD ***TOTAL*** Chlorine Reagent Pillow Packets (for 10mL)- number 21056-69
-Hydrogen peroxide Activator 1 (15% KI solution)
-Hydrogen Peroxide Activator 2 (5% ammonium molybdate solution)
-De-ionized or reversed osmosis water

IV. PROCEDURE:

Before testing make sure the instrument is in the low (LO) range mode by checking that the display reads to the hundredths (0.00).

1. Make an appropriate dilution if needed.

2. Fill both 10mL sample cells with 10 mls of the water sample. Designate one of these to be the blank and the other to be the prepared sample. Make sure the cells are not wet and they are free of fingerprints or smudges.

3. Cap the blank cell and place it in the cell holder with the diamond mark facing you. Cover the cell compartment and press ZERO. The instrument will display 0.00. Remove the blank.

4. Add the contents of one DPD Total Chlorine pillow packet to the prepared sample. Cap and shake vigorously. A pink color will develop.

5. Quickly place the sample cell in the compartment with the diamond mark facing you, close the cover and press READ.

6. The instrument display will show-- followed by the results in ppm total chlorine.

7. Note: if the instrument reads a blinking 3.67, the sample concentration is too high and needs to be diluted.

Calculations:

Total Chlorine: no calculation needed, the instrument reading is the ppm total Cl_2.

Bromine: ppm Br_2= 2.25 X total Cl_2

PAA: ppm PAA= 1.07 X total Cl_2

DBNPA: ppm DBNPA = X total Cl_2
(For DBNPA- follow steps 1 – 7 but let react 3 minutes before taking a reading)

V. PROCEDURE FOR HYDROGEN PEROXIDE ONLY

1. Make an appropriate dilution if needed.

2. Fill both 10mL sample cells with 10 mls of the water sample. Designate one of these to be the blank and the other to be the prepared sample. Make sure the cells are not wet and they are free of fingerprints or smudges.

3. Cap the blank cell and place it in the cell holder with the diamond mark facing you. Cover the cell compartment and press ZERO. The instrument will display 0.00. Remove the blank.

4. Add 3 drops of Hydrogen Peroxide Activator 1 and 3 drops of Hydrogen Peroxide Activator 2 to the prepared sample cell.

5. Swirl the prepared sample cell and let react for 6 minutes.

6. Add the contents of one DPD Total Chlorine pillow packet to the prepared sample. Cap and shake vigorously. A pink color will develop.

7. Quickly place the sample cell in the compartment with the diamond mark facing you, close the cover and press READ.

8. The instrument display will show -- followed by the results in ppm total chlorine. This is the total Cl_2 peroxygen value.

9. Note: if the instrument reads a blinking 3.67, the sample concentration is too high and needs to be diluted.

Calculation:

ppm H_2O_2- 0.478 X (total Cl_2 peroxygen – total Cl_2 as PAA)