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Abstracts of articles about ELECTROLYZED WATER

Inactivation of Avian Flu Virus (AIV), Newcastle Disease Virus (NDV), Infectious Bronchitis (IBV), Fowl Adenovirus (FAV), Avian Reovirus and Avian Herpesvirus.

[Dr. Lu.](#)

This study concluded that all 40 subject viruses were 100% inactivated with HOCL within 5 min, 10 min, 15 min, 30 min, 60 min and 120 min reaction time tested. HOCL had a neutral pH and consisted approximately 300ppm FAC. The active ingredient of Neutral electrolyzed water is Hypochlorous Acid (the human body produces Hypochlorous Acid naturally as part of its immune system). Dilution of HOCL to the 1:2 and 1:5 ratio was accomplished using sterile distilled water or deionized water.

Inactivation of *Escherichia coli* O157:H7, *Salmonella enteritidis* and *Listeria monocytogenes* on the surface of tomatoes by neutral electrolyzed water

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Aims: To determine the efficacy of neutral electrolyzed water (**NEW**[®]) in killing *Escherichia coli* O157:H7, *Salmonella enteritidis* and *Listeria monocytogenes*, as well as non-pathogenic *E. coli*, on the surface of tomatoes, and to evaluate the effect of rinsing with **NEW**[®] on the organoleptic characteristics of the tomatoes.

Methods and Results: The bactericidal activity of **NEW**[®], containing 444 or 89 mg^l⁻¹ of active chlorine, was evaluated over pure cultures (8.5log CFU ml⁻¹) of the above-mentioned strains. All of them were reduced by more than 6 log CFU ml⁻¹ within 5 min of exposure to **NEW**[®]. Fresh tomatoes were surface-inoculated with the same strains, and rinsed in **NEW**[®] (89 mg^l⁻¹ of active chlorine) or in deionized sterile water (control), for 30 or 60 s. In the **NEW**[®] treatments, independent of the strain and of the treatment time, an initial surface population of about 5 log CFU sq.cm⁻¹ was reduced to 5log CFU sq.cm⁻¹, and no cells were detected in the washing solution by plating procedure. A sensory evaluation was conducted to ascertain possible alterations in organoleptic qualities, yielding no significant differences with regard to untreated tomatoes.

Significance and Impact of the Study: Rinsing in **NEW**[®] reveals as an effective method to control the presence of *E. coli* O157:H7, *S. enteritidis* and *L. monocytogenes* on the surface of fresh tomatoes, without affecting their organoleptic characteristics. This indicates its potential application for the decontamination of fresh produce surfaces.

Keywords: ANK-Anolyte, disinfectant, *E. coli* O 157 :H7 , *L. monocytogenes*, neutral electroly'zed water, organoleptic quality, rinsing fresh tomatoes, *S. enteritidis*.

Efficacy of electrolyzed water in the prevention and removal of fecal material attachment and its microbicidal effectiveness during simulated industrial poultry processing.

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This study was undertaken to investigate the efficacy of alkaline and acidic electrolyzed (EO) water in preventing and removing fecal contaminants and killing *Campylobacter jejuni* on poultry carcasses under simulated industrial processing conditions. New York dressed and defeathered chicken carcasses spot-inoculated with cecal material or *C. jejuni* were subjected to spraying treatment with alkaline EO or 10% trisodium phosphate (TSP) water or combinations of spraying and immersion treatments with alkaline EO and chlorinated water, respectively. Prespraying chicken carcasses with alkaline EO water significantly lowered cecal material attachment scores (3.77) than tap water (4.07) and 10% TSP (4.08) upon treatment of the dorsal area. Combinations of pre- and postspraying were significantly more effective than postspraying only, especially when using alkaline EO water in removing fecal materials on the surface of chicken carcasses. Although treatment by immersion only in EO and chlorinated water significantly reduced the initial population (4.92 log₁₀ cfu/g) of *C. jejuni* by 2.33 and

2.05 log₁₀ cfu/g, respectively, combinations of spraying and immersion treatment did not improve the bactericidal effect of sanitizers. The results indicated that alkaline EO water might provide an alternative to TSP in preventing attachment and removal of feces on the surface of chicken carcasses. The results also suggested that chicken carcasses containing pathogenic microorganisms may contribute to the cross-contamination of whole batches of chickens during processing in the chiller tank and afterward.

Enhancing the bactericidal effect of electrolyzed water on *Listeria monocytogenes* biofilms formed on stainless steel.

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Biofilms are potential sources of contamination to food in processing plants, because they frequently survive sanitizer treatments during cleaning. The objective of this research was to investigate the combined use of alkaline and acidic electrolyzed (EO) water in the inactivation of *Listeria monocytogenes* biofilms on stainless steel surfaces. Biofilms were grown on rectangular stainless steel (type 304, no. 4 finish) coupons (2 by 5 cm) in a 1:10 dilution of tryptic soy broth that contained a five-strain mixture of *L. monocytogenes* for 48 h at 25 degrees C. The coupons with biofilms were then treated with acidic EO water or alkaline EO water or with alkaline EO water followed by acidic EO water produced at 14 and 20 A for 30, 60, and 120 s. Alkaline EO water alone did not produce significant reductions in *L. monocytogenes* biofilms when compared with the control. Treatment with acidic EO water only for 30 to 120 s, on the other hand, reduced the viable bacterial populations in the biofilms by 4.3 to 5.2 log CFU per coupon, whereas the combined treatment of alkaline EO water followed by acidic EO water produced an additional 0.3- to 1.2-log CFU per coupon reduction. The population of *L. monocytogenes* reduced by treatments with acidic EO water increased significantly with increasing time of exposure. However, no significant differences occurred between treatments with EO water produced at 14 and 20 A. Results suggest that alkaline and acidic EO water can be used together to achieve a better inactivation of biofilms than when applied individually.

Investigation of the presence of OH radicals in electrolyzed NaCl solution by electron spin resonance spectroscopy.

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In the anode side of a two-chamber electrolyzer, electrolysis of a NaCl solution generates acidic electrolyzed oxidizing (EO) water, which exhibits bactericidal effects against a large number of pathogens. This study was undertaken to investigate whether OH radical species are present in EO water or are formed when EO water

reacts with iron ions. Electron spin resonance spectroscopy (ESR) coupled with the spin trapping technique was used for the detection of free radicals. Samples of EO water were collected at 0.5, 1, 2, 3, and 5 min of electrolysis and immediately mixed with the spin trapping agent 5,5-dimethyl-1-pyrroline-N-oxide (DMPO). The 5,5-dimethyl-2-hydroxypyrrolidine-N-oxyl (DMPO-OH) spin adduct, characteristic of OH radicals, was not observed. Starting with 2-min electrolysis, a seven-line spectrum characteristic of 5,5-dimethyl-2-pyrrolidone-N-oxyl (DMPOX) was formed. The reactions of EO water with Fe³⁺ and Fe²⁺ in the presence of DMPO yielded the spin adduct DMPO-OH. However, the addition of OH radical scavengers (ethanol and methanol) did not generate the characteristic DMPO-alkyl spin adducts. This indicated that the DMPO-OH spectrum was due to a nucleophilic addition of water to DMPO and not to trapping of OH radicals.

Efficacy of electrolyzed water in inactivating *Salmonella enteritidis* and *Listeria monocytogenes* on shell eggs.

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The efficacy of acidic electrolyzed (EO) water produced at three levels of total available chlorine (16, 41, and 77 mg/ liter) and chlorinated water with 45 and 200 mg/liter of residual chlorine was investigated for inactivating *Salmonella Enteritidis* and *Listeria monocytogenes* on shell eggs. An increasing reduction in *Listeria* population was observed with increasing chlorine concentration from 16 to 77 mg/liter and treatment time from 1 to 5 min, resulting in a maximal reduction of 3.70 log CFU per shell egg compared with a deionized water wash for 5 min. There was no significant difference in antibacterial activities against *Salmonella* and *Listeria* at the same treatment time between 45 mg/liter of chlorinated water and 14-A acidic EO water treatment ($P > \text{or} = 0.05$). Chlorinated water (200 mg/liter) wash for 3 and 5 min was the most effective treatment; it reduced mean populations of *Listeria* and *Salmonella* on inoculated eggs by 4.89 and 3.83 log CFU/shell egg, respectively. However, reductions (log CFU/shell egg) of *Listeria* (4.39) and *Salmonella* (3.66) by 1-min alkaline EO water treatment followed by another 1 min of 14-A acidic EO water (41 mg/liter chlorine) treatment had a similar reduction to the 1-min 200 mg/liter chlorinated water treatment for *Listeria* (4.01) and *Salmonella* (3.81). This study demonstrated that a combination of alkaline and acidic EO water wash is equivalent to 200 mg/liter of chlorinated water wash for reducing populations of *Salmonella Enteritidis* and *L. monocytogenes* on shell eggs.

Efficacy of electrolyzed oxidizing water for the microbial safety and quality of eggs.

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During commercial processing, eggs are washed in an alkaline detergent and then rinsed with chlorine to reduce dirt, debris, and microorganism levels. The alkaline and acidic fractions of electrolyzed oxidizing (EO) water have the ability to fit into the 2-step commercial egg washing process easily if proven to be effective. Therefore, the efficacy of EO water to decontaminate *Salmonella* Enteritidis and *Escherichia coli* K12 on artificially inoculated shell eggs was investigated. For the in vitro study, eggs were soaked in alkaline EO water followed by soaking in acidic EO water at various temperatures and times. Treated eggs showed a reduction in population between $> \text{ or } = 0.6$ to $> \text{ or } = 2.6$ log₁₀ cfu/g of shell for *S. Enteritidis* and $> \text{ or } = 0.9$ and $> \text{ or } = 2.6$ log₁₀ for *E. coli* K12. Log₁₀ reductions of 1.7 and 2.0 for *S. Enteritidis* and *E. coli* K12, respectively, were observed for typical commercial detergent-sanitizer treatments, whereas log₁₀ reductions of $> \text{ or } = 2.1$ and $> \text{ or } = 2.3$ for *S. Enteritidis* and *E. coli* K12, respectively, were achieved using the EO water treatment. For the pilot-scale study, both fractions of EO water were compared with the detergent-sanitizer treatment using *E. coli* K12. Log₁₀ reductions of $> \text{ or } = 2.98$ and $> \text{ or } = 2.91$ were found using the EO water treatment and the detergent-sanitizer treatment, respectively. The effects of 2 treatments on egg quality were investigated. EO water and the detergent-sanitizer treatments did not significantly affect albumen height or eggshell strength; however, there were significant affects on cuticle presence. These results indicate that EO water has the potential to be used as a sanitizing agent for the egg washing process.

Efficacy of acidic electrolyzed water ice for pathogen control on lettuce.

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Acidic electrolyzed water (AcEW) was used as frozen AcEW (AcEW-ice) for inactivation of *Listeria monocytogenes* and *Escherichia coli* O157:H7 on lettuce. AcEW-ice was prepared from AcEW with 20, 50, 100, and 200 ppm of available chlorine by freezing at -40 degrees C and generated 30, 70, 150, and 240 ppm of chlorine gas (Cl₂), respectively. Th AcEW-ice was placed into styrene-foam containers with lettuce samples at 20 degrees C for 24 h. Although AcEW-ice generating 30 ppm Cl₂ had no effect on *L. monocytogenes* cell counts, AcEW-ice generating 70 to 240 ppm of Cl₂ significantly ($P < 0.05$) reduced *L. monocytogenes* by ca. 1.5 log CFU/g. *E. coli* O157:H7 cell counts were reduced by 1.0 log CFU/g with AcEW-ice generating 30 ppm of Cl₂. AcEW-ice generating 70 and 150 ppm of Cl₂ reduced *E. coli* O157:H7 by 2.0 log CFU/g. Further significant reduction of *E. coli* O157:H7 (2.5 log CFU/g) was demonstrated by treatment with AcEW-ice generating 240 ppm of Cl₂. However, treatment with AcEW-ice generating 240 ppm of Cl₂ resulted in a physiological disorder resembling leaf burn. AcEW-ice that generated less than 150 ppm of Cl₂ had no effect on the surface color of the lettuce. AcEW-ice, regardless of the concentration of the emission of Cl₂, had no effect on the ascorbic acid content in the lettuce. The weight ratio of lettuce to AcEW-ice required was determined to be over 1:10. The bactericidal effect of AcEW-ice appeared within the first 2 h. The use of AcEW-ice provides simultaneously for low temperature storage and inactivation of bacteria.

The efficacy of function water (electrolyzed strong acid solution) on open heart surgery; postoperative mediastinitis due to methicillin-resistant *Staphylococcus aureus*]

[Article in Japanese]

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Methicillin-resistant *Staphylococcus aureus* (MRSA) infection after cardiac surgery has recently increased. We compared the anti-inflammatory effect of an electrolyzed strong acid solution and a warm saline solution in patients with open heart surgery. These solutions were used for mediastinal irrigation before closing the sternum. Group A patients were irrigated by a warm saline solution, and group B patients were irrigated by an electrolyzed strong acid solution, administration of this water is safe, feasible, and easy for the prevention of MRSA infection. Postoperative infection was significantly decreased in the group B as compared in the group A. An electrolyzed strong acid solution may be effective on postoperative infection, particularly MRSA infection following open heart surgery.

Effects of water source, dilution, storage, and bacterial and fecal loads on the efficacy of electrolyzed oxidizing water for the control of *Escherichia coli* O157:H7.

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To evaluate the potential of using electrolyzed oxidizing (EO) water for controlling *Escherichia coli* O157:H7 in water for livestock, the effects of water source, electrolyte concentration, dilution, storage conditions, and bacterial or fecal load on the oxidative reduction potential (ORP) and bactericidal activity of EO water were investigated. Anode and combined (7:3 anode:cathode, vol/vol) EO waters reduced the pH and increased the ORP of deionized water, whereas cathode EO water increased pH and lowered ORP. Minimum concentrations (vol/vol) of anode and combined EO waters required to kill 10(4) CFU/ml planktonic suspensions of *E. coli* O157:H7 strain H4420 were 0.5 and 2.0%, respectively. Cathode EO water did not inhibit H4420 at concentrations up to 16% (vol/vol). Higher concentrations of anode or combined EO water were required to elevate the ORP of irrigation or chlorinated tap water compared with that of deionized water. Addition of feces to EO water products (0.5% anode or 2.0% combined, vol/vol) significantly reduced ($P < 0.001$) their ORP values to < 700 mV in all water types. A relationship between ORP and bactericidal activity of EO water was observed. The dilute EO waters retained the capacity to eliminate a 10(4) CFU/ml inoculation of *E. coli* O157:H7 H4420 for at least 70 h regardless of exposure to UV light or storage temperature (4 versus 24 degrees C). At 95 h and beyond, UV exposure reduced ORP, significantly more so ($P < 0.05$) in open than in closed containers. Bactericidal activity of EO products (anode or combined) was lost in samples in which ORP value had fallen to $< \text{or} = 848$ mV. When stored in the dark, the

diluted EO waters retained an ORP of > 848 mV and bactericidal efficacy for at least 125 h; with refrigeration (4 degrees C), these conditions were retained for at least 180 h. Results suggest that EO water may be an effective means by which to control E. coli O157:H7 in livestock water with low organic matter content.

Efficacy of electrolyzed acid water in reprocessing patient-used flexible upper endoscopes: Comparison with 2% alkaline glutaraldehyde.

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BACKGROUND AND AIM: Two percent glutaraldehyde, the most widely used liquid chemical germicide (LCG), may be hazardous to patients and medical personnel. Alternatives to glutaraldehyde, such as electrolyzed acid water (EAW), are being developed, but data from well-controlled studies with patient-used endoscopes are rare. The purpose of the present paper was to evaluate the high-level disinfection capability of EAW and compare it with glutaraldehyde. METHODS: A random sample of 125 endoscopes was collected immediately after upper endoscopic examination. After careful manual cleaning, endoscopes were divided into a glutaraldehyde and EAW group. After the disinfection procedure, samples from working channel (S-1), insertion tube (S-2), umbilical cord (S-3), and angulation knob (S-4) were taken and cultured. Another twenty endoscopes were experimentally contaminated with hepatitis B virus (HBV) and samples were collected after contamination (T-1), after manual cleaning (T-2), and after final disinfection (T-3). Polymerase chain reaction (PCR) for HBV-DNA was performed. RESULTS: In the EAW group, culture-positive rates were 3.2% in S-1, 9.5% in S-2, 3.2% in S-3, and 27.0% in the S-4 samples. There was no significant difference between the EAW and glutaraldehyde groups for all sampling sites. However, in both groups, disinfection of the angulation knobs (S-4) was less efficient than the others. For the T-1 site, HBV-DNA was detected from all of them, and in 95% (19/20) of T-2. However, HBV-DNA was not detected from T-3 samples. CONCLUSIONS: Electrolyzed acid water is as efficient as glutaraldehyde in eliminating bacteria from patient-used endoscopes. After disinfection procedures using both methods, HBV-DNA was not detected from any endoscopes experimentally contaminated with HBV-positive mixed sera. However, some bacteria may remain on the surface of the endoscopes. Therefore, more careful precleaning of the endoscopes may help achieve high-level disinfection in the clinical setting.

Efficacy of acidic electrolyzed water for microbial decontamination of cucumbers and strawberries.

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An examination was made of the efficacy of acidic electrolyzed water (AcEW, 30 ppm free available chlorine), ozonated water (5 ppm ozone), and a sodium hypochlorite

solution (NaOCl, 150 ppm free available chlorine) for use as potential sanitizers of cucumbers and strawberries. AcEW and NaOCl reduced the aerobic mesophiles naturally present on cucumbers within 10 min by 1.4 and 1.2 log CFU per cucumber, respectively. The reduction by ozonated water (0.7 log CFU per cucumber) was significantly less than that of AcEW or NaOCl ($P < \text{or} = 0.05$). Cucumbers washed in alkaline electrolyzed water for 5 min and then treated with AcEW for 5 min showed a reduction in aerobic mesophiles that was at least 2 log CFU per cucumber greater than that of other treatments ($P < \text{or} = 0.05$). This treatment was also effective in reducing levels of coliform bacteria and fungi associated with cucumbers. All treatments offered greater microbial reduction on the cucumber surface than in the cucumber homogenate. Aerobic mesophiles associated with strawberries were reduced by less than 1 log CFU per strawberry after each treatment. Coliform bacteria and fungi associated with strawberries were reduced by 1.0 to 1.5 log CFU per strawberry after each treatment. Microbial reduction was approximately 0.5 log CFU per strawberry greater on the strawberry surface than in the strawberry homogenate. However, neither treatment was able to completely inactivate or remove the microorganisms from the surface of the cucumber or strawberry.

The bactericidal effects of electrolyzed oxidizing water on bacterial strains involved in hospital infections.

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The study is designed to investigate bactericidal actions of electrolyzed oxidizing water on hospital infections. Ten of the most common opportunistic pathogens are used for this study. Cultures are inoculated in 4.5 mL of electrolyzed oxidizing (EO) water or 4.5 mL of sterile deionized water (control), and incubated for 0, 0.5, and 5 min at room temperature. At the exposure time of 30 s the EO water completely inactivates all of the bacterial strains, with the exception of vegetative cells and spores of bacilli which need 5 min to be killed. The results indicate that electrolyzed oxidizing water may be a useful disinfectant for hospital infections, but its clinical application has still to be evaluated.

Bactericidal effects of acidic electrolyzed water on the dental unit waterline.

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Many studies have been conducted in the United States regarding the microbial contamination of dental unit waterline, but not in Japan. Recently, acidic electrolyzed water has been used in the medical and dental fields. In this study, we investigated the bactericidal effects of the temporary inflow of acidic electrolyzed water on microbial

contamination of the dental unit waterline. First, in order to observe the daily bacterial contamination of the dental unit waterline, water samples were collected at the end of handpieces and three-way syringes before the inflow of acidic electrolyzed water. They were cultured to detect viable bacteria. Later, the inflow of acidic electrolyzed water was conducted through the piping box of the dental unit. Before starting operation on next day, water samples were collected and cultured, as described above. The mean viable bacteria count was 910 \pm 190 CFU/ml at the end of handpieces, and 521 \pm 116 CFU/ml at the end of three-way syringes before the inflow of acidic electrolyzed water. However, bacteria were detected in only small numbers at the end of handpieces and three-way syringes on the next day. These results indicated that acidic electrolyzed water could be applied as an appropriate measure against bacterial contamination of the dental unit waterline.

Corrosion behavior of dental alloys in various types of electrolyzed water.

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The corrosion behavior of dental alloys was examined in electrolyzed strong acid water, weak acid water and neutral water using a 7-day immersion test. The precious metal alloys, gold alloy, Au-Ag-Pd alloy and silver alloy showed the greatest surface color change and dissolution of constituents in the strong acid water and the smallest in the neutral water. The release of Au from gold alloy was especially marked in the strong acid water. Co-Cr alloy showed greater corrosion and tarnish resistance in the strong acid water rather than in the weak acid water and the neutral water. X-ray microanalysis revealed that the corrosion products on the precious metal alloys were silver chloride crystals and the thin brown products on Co-Cr alloy were cobalt and chromium oxides. Ti was found in all three types of electrolyzed water. The neutral water appeared the least corrosive to metals among the three types showing equivalent bactericidal activity.

Effects of chlorine and pH on efficacy of electrolyzed water for inactivating *Escherichia coli* O157:H7 and *Listeria monocytogenes*.

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The effects of chlorine and pH on the bactericidal activity of electrolyzed (EO) water were examined against *Escherichia coli* O157:H7 and *Listeria monocytogenes*. The residual chlorine concentration of EO water ranged from 0.1 to 5.0 mg/l, and the pH effect was examined at pH 3.0, 5.0, and 7.0. The bactericidal activity of EO water increased with residual chlorine concentration for both pathogens, and complete inactivation was achieved at residual chlorine levels equal to or higher than 1.0 mg/l. The results showed that both pathogens are very sensitive to chlorine, and residual chlorine level of EO water should be maintained at 1.0 mg/l or higher for practical

applications. For each residual chlorine level, bactericidal activity of EO water increased with decreasing pH for both pathogens. However, with sufficient residual chlorine (greater than 2 mg/l), EO water can be applied in a pH range between 2.6 (original pH of EO water) and 7.0 while still achieving complete inactivation of *E. coli* O157:H7 and *L. monocytogenes*.

Inactivation of *Escherichia coli* O157:H7, *Salmonella enteritidis* and *Listeria monocytogenes* on the surface of tomatoes by neutral electrolyzed water.

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AIMS: To determine the efficacy of neutral electrolyzed water (**NEW**[®]) in killing *Escherichia coli* O157:H7, *Salmonella enteritidis* and *Listeria monocytogenes*, as well as nonpathogenic *E. coli*, on the surface of tomatoes, and to evaluate the effect of rinsing with **NEW**[®] on the organoleptic characteristics of the tomatoes. METHODS AND RESULTS: The bactericidal activity of **NEW**[®], containing 444 or 89 mg l(-1) of active chlorine, was evaluated over pure cultures (8.5 log CFU ml(-1)) of the above-mentioned strains. All of them were reduced by more than 6 log CFU ml(-1) within 5 min of exposure to **NEW**[®]. Fresh tomatoes were surface-inoculated with the same strains, and rinsed in **NEW**[®] (89 mg l(-1) of active chlorine) or in deionized sterile water (control), for 30 or 60 s. In the **NEW**[®] treatments, independent of the strain and of the treatment time, an initial surface population of about 5 log CFU sq.cm(-1) was reduced to <1 log CFU sq.cm(-1), and no cells were detected in the washing solution by plating procedure. A sensory evaluation was conducted to ascertain possible alterations in organoleptic qualities, yielding no significant differences with regard to untreated tomatoes. SIGNIFICANCE AND IMPACT OF THE STUDY: Rinsing in **NEW**[®] reveals as an effective method to control the presence of *E. coli* O157:H7, *S. enteritidis* and *L. monocytogenes* on the surface of fresh tomatoes, without affecting their organoleptic characteristics. This indicates its potential application for the decontamination of fresh produce surfaces.

Reduction of *Salmonella enterica* on alfalfa seeds with acidic electrolyzed oxidizing water and enhanced uptake of acidic electrolyzed oxidizing water into seeds by gas exchange.

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Alfalfa sprouts have been implicated in several salmonellosis outbreaks in recent years. The disinfectant effects of acidic electrolyzed oxidizing (EO) water against *Salmonella enterica* both in an aqueous system and on artificially contaminated alfalfa seeds were

determined. The optimum ratio of seeds to EO water was determined in order to maximize the antimicrobial effect of EO water. Seeds were combined with EO water at ratios (wt/vol) of 1:4, 1:10, 1:20, 1:40, and 1:100, and the characteristics of EO water (pH, oxidation reduction potential [ORP], and free chlorine concentration) were determined. When the ratio of seeds to EO water was increased from 1:4 to 1:100, the pH decreased from 3.82 to 2.63, while the ORP increased from +455 to +1,073 mV. EO water (with a pH of 2.54 to 2.38 and an ORP of +1,083 to +1,092 mV) exhibited strong potential for the inactivation of *S. enterica* in an aqueous system (producing a reduction of at least 6.6 log CFU/ml). Treatment of artificially contaminated alfalfa seeds with EO water at a seed-to-EO water ratio of 1:100 for 15 and 60 min significantly reduced *Salmonella* populations by 2.04 and 1.96 log CFU/g, respectively ($P < 0.05$), while a Butterfield's buffer wash decreased *Salmonella* populations by 0.18 and 0.23 log CFU/g, respectively. After treatment, EO water was *Salmonella* negative by enrichment with or without neutralization. Germination of seeds was not significantly affected ($P > 0.05$) by treatment for up to 60 min in electrolyzed water. The uptake of liquid into the seeds was influenced by the internal gas composition (air, N₂, or O₂) of seeds before the liquid was added.

Effectiveness of electrolyzed acidic water in killing *Escherichia coli* O157:H7, *Salmonella enteritidis*, and *Listeria monocytogenes* on the surfaces of tomatoes.

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A study was conducted to evaluate the efficacy of electrolyzed acidic water, 200-ppm chlorine water, and sterile distilled water in killing *Escherichia coli* O157:H7, *Salmonella*, and *Listeria monocytogenes* on the surfaces of spot-inoculated tomatoes. Inoculated tomatoes were sprayed with electrolyzed acidic water, 200-ppm chlorine water, and sterile distilled water (control) and rubbed by hand for 40 s. Populations of *E. coli* O157:H7, *Salmonella*, and *L. monocytogenes* in the rinse water and in the peptone wash solution were determined. Treatment with 200-ppm chlorine water and electrolyzed acidic water resulted in 4.87- and 7.85-log₁₀ reductions, respectively, in *Escherichia coli* O157:H7 counts and 4.69- and 7.46-log₁₀ reductions, respectively, in *Salmonella* counts. Treatment with 200-ppm chlorine water and electrolyzed acidic water reduced the number of *L. monocytogenes* by 4.76 and 7.54 log₁₀ CFU per tomato, respectively. This study's findings suggest that electrolyzed acidic water could be useful in controlling pathogenic microorganisms on fresh produce.

Stability of electrolyzed oxidizing water and its efficacy against cell suspensions of *Salmonella typhimurium* and *Listeria monocytogenes*.

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Electrolyzed oxidizing (EO) water has proved to be effective against foodborne pathogens attached to cutting boards and poultry surfaces and against spoilage organisms on vegetables; however, its levels of effectiveness against *Listeria monocytogenes* and *Salmonella Typhimurium* in cell suspensions have not been compared with those of other treatments. In this study, the oxidation reduction potentials (ORPs), chlorine concentrations, and pHs of acidic and basic EO water were monitored for 3 days at 4 and 25 degrees C after generation. There were no differences between the pHs or ORPs of acidic and basic EO waters stored at 4 or 25 degrees C. However, the free chlorine concentration in acidic EO water stored at 4 degrees C increased after 24 h. In contrast, the free chlorine concentration in acidic EO water stored at 25 degrees C decreased after one day. Cell suspensions of *Salmonella Typhimurium* and *L. monocytogenes* were treated with distilled water, chlorinated water (20 ppm), acidified chlorinated water (20 ppm, 4.5 pH), acidic EO water (EOA), basic EO water (EOB), or acidic EO water that was "aged" at 4 degrees C for 24 h (AEOA) for up to 15 min at either 4 or 25 degrees C. The largest reductions observed were those following treatments carried out at 25 degrees C. EOA and AEOA treatments at both temperatures significantly reduced *Salmonella Typhimurium* populations by $> 8 \log_{10}$ CFU/ml. EOA and AEOA treatments effectively reduced *L. monocytogenes* populations by $> 8 \log_{10}$ CFU/ml at 25 degrees C. These results demonstrate the stability of EO water under different conditions and that EO water effectively reduced *Salmonella Typhimurium* and *L. monocytogenes* populations in cell suspensions.

Treatment of *Escherichia coli* O157:H7 inoculated alfalfa seeds and sprouts with electrolyzed oxidizing water.

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Electrolyzed oxidizing water is a relatively new concept that has been utilized in agriculture, livestock management, medical sterilization, and food sanitation. Electrolyzed oxidizing (EO) water generated by passing sodium chloride solution through an EO water generator was used to treat alfalfa seeds and sprouts inoculated with a five-strain cocktail of nalidixic acid resistant *Escherichia coli* O157:H7. EO water had a pH of 2.6, an oxidation-reduction potential of 1150 mV and about 50 ppm free chlorine. The percentage reduction in bacterial load was determined for reaction times of 2, 4, 8, 16, 32, and 64 min. Mechanical agitation was done while treating the seeds at different time intervals to increase the effectiveness of the treatment. Since *E. coli* O157:H7 was released due to soaking during treatment, the initial counts on seeds and sprouts were determined by soaking the contaminated seeds/sprouts in 0.1% peptone water for a period equivalent to treatment time. The samples were then pummeled in 0.1% peptone water and spread plated on tryptic soy agar with 5 microg/ml of nalidixic acid (TSAN). Results showed that there were reductions between 38.2% and 97.1% (0.22-1.56 \log_{10} CFU/g) in the bacterial load of treated seeds. The reductions for sprouts were between 91.1% and 99.8% (1.05-2.72 \log_{10} CFU/g). An increase in treatment time increased the percentage reduction of *E. coli* O157:H7. However, germination of the treated seeds reduced from 92% to 49% as amperage to make EO

water and soaking time increased. EO water did not cause any visible damage to the sprouts.

Effect of electrolyzed oxidizing water and hydrocolloid occlusive dressings on excised burn-wounds in rats.

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OBJECTIVE: To study the efficacy of electrolyzed oxidizing water (EOW) and hydrocolloid occlusive dressings in the acceleration of epithelialization in excised burn-wounds in rats. **METHODS:** Each of the anesthetized Sprague-Dawley rats (n=28) was subjected to a third-degree burn that covered approximately 10% of the total body surface area. Rats were assigned into four groups: Group I (no irrigation), Group II (irrigation with physiologic saline), Group III (irrigation with EOW) and Group IV (hydrocolloid occlusive dressing after EOW irrigation). Wounds were observed macroscopically until complete epithelialization was present, then the epithelialized wounds were examined microscopically. **RESULTS:** Healing of the burn wounds was the fastest in Group IV treated with hydrocolloid occlusive dressing together with EOW. Although extensive regenerative epidermis was seen in each Group, the proliferations of lymphocytes and macrophages associated with dense collagen deposition were more extensive in Group II, III and IV than in Group I. These findings were particularly evident in Group III and IV. **CONCLUSIONS:** Wound Healing may be accelerated by applying a hydrocolloid occlusive dressing on burn surfaces after they are cleaned with EOW.

Reduced hemodialysis-induced oxidative stress in end-stage renal disease patients by electrolyzed reduced water.

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BACKGROUND: Increased oxidative stress in end-stage renal disease (ESRD) patients may oxidize macromolecules and consequently lead to cardiovascular events during chronic hemodialysis. Electrolyzed reduced water (ERW) with reactive oxygen species (ROS) scavenging ability may have a potential effect on reduction of hemodialysis-induced oxidative stress in ESRD patients. **METHODS:** We developed a chemiluminescence emission spectrum and high-performance liquid chromatography analysis to assess the effect of ERW replacement on plasma ROS (H₂O₂ and HOCl) scavenging activity and oxidized lipid or protein production in ESRD patients undergoing hemodialysis. Oxidized markers, dityrosine, methylguanidine, and phosphatidylcholine hydroperoxide, and inflammatory markers, interleukin 6 (IL-6), and C-reactive protein (CRP) were determined. **RESULTS:** Although hemodialysis efficiently removes dityrosine and creatinine, hemodialysis increased oxidative stress,

including phosphatidylcholine hydroperoxide, and methylguanidine. Hemodialysis reduced the plasma ROS scavenging activity, as shown by the augmented reference H₂O₂ and HOCl counts (Rh₂O₂ and Rhocl, respectively) and decreased antioxidative activity (expressed as total antioxidant status in this study). ERW administration diminished hemodialysis-enhanced Rh₂O₂ and Rhocl, minimized oxidized and inflammatory markers (CRP and IL-6), and partly restored total antioxidant status during 1-month treatment. CONCLUSION: This study demonstrates that hemodialysis with ERW administration may efficiently increase the H₂O₂- and HOCl-dependent antioxidant defense and reduce H₂O₂- and HOCl-induced oxidative stress.

[Cytotoxic effect of antiseptics: comparison In vitro. In vivo examination of strong acidic electrolyzed water, povidone-iodine, chlorhexidine and benzalkonium chloride]

[Article in Japanese]

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Cytotoxic effect and guinea pig wound cure stage, pus fabrication presence in infected wound were compared with strong acidic electrolyzed water (AcEW) and povidone-iodine solution (PVP-I), chlorhexidine (CHG) and benzalkonium chloride (BAC). It gave the following results: In a cytotoxic test, the toxicity was recognized in 0.1%-0.01% PVP-I, in 0.0002-0.0004% CHG, in 10-0.1 micrograms/ml BAC, but there was no toxicity in AcEW. By a guinea pig wound cure process, no significance was recognized between each pharmaceutical agent in epidermal cell migration, but by an inflammation locus area, the significance was considerable in comparison with no-treatment. The pyopoesis of *P. aeruginosa* infected wound was recognized in a ratio of 38.2% physiological saline, 27.3% CHG, 20.6% PVP-I and 12.1% AcEW. When pollution locus includes an infection image of bacteria, while draining AcEW instead of physiological saline, disinfection, indication was expected, and, as for the disorder in cure stage. I do not agree with that mentioned above. As for AcEW, availability by organism use was recognized for the cytotoxic effect of antiseptic instead of action of acceleration for wound cure.

Effectiveness of electrolyzed acidic water in killing *Escherichia coli* O157:H7, *Salmonella enteritidis*, and *Listeria monocytogenes* on the surfaces of tomatoes.

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A study was conducted to evaluate the efficacy of electrolyzed acidic water, 200-ppm chlorine water, and sterile distilled water in killing *Escherichia coli* O157:H7, *Salmonella*, and *Listeria monocytogenes* on the surfaces of spot-inoculated tomatoes.

Inoculated tomatoes were sprayed with electrolyzed acidic water, 200-ppm chlorine water, and sterile distilled water (control) and rubbed by hand for 40 s. Populations of *E. coli* O157:H7, *Salmonella*, and *L. monocytogenes* in the rinse water and in the peptone wash solution were determined. Treatment with 200-ppm chlorine water and electrolyzed acidic water resulted in 4.87- and 7.85-log₁₀ reductions, respectively, in *Escherichia coli* O157:H7 counts and 4.69- and 7.46-log₁₀ reductions, respectively, in *Salmonella* counts. Treatment with 200-ppm chlorine water and electrolyzed acidic water reduced the number of *L. monocytogenes* by 4.76 and 7.54 log₁₀ CFU per tomato, respectively. This study's findings suggest that electrolyzed acidic water could be useful in controlling pathogenic microorganisms on fresh produce.

Efficacy of electrolyzed oxidizing water in inactivating *Salmonella* on alfalfa seeds and sprouts.

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Studies have demonstrated that electrolyzed oxidizing (EO) water is effective in reducing foodborne pathogens on fresh produce. This study was undertaken to determine the efficacy of EO water and two different forms of chlorinated water (chlorine water from Cl₂ and Ca(OCl)₂ as sources of chlorine) in inactivating *Salmonella* on alfalfa seeds and sprouts. Tengram sets of alfalfa seeds inoculated with a five-strain cocktail of *Salmonella* (6.3 x 10⁴ CFU/g) were subjected to 90 ml of deionized water (control), EO water (84 mg/liter of active chlorine), chlorine water (84 mg/liter of active chlorine), and Ca(OCl)₂ solutions at 90 and 20,000 mg/liter of active chlorine for 10 min at 24 +/- 2 degrees C. The application of EO water, chlorinated water, and 90 mg/liter of Ca(OCl)₂ to alfalfa seeds for 10 min reduced initial populations of *Salmonella* by at least 1.5 log₁₀ CFU/g. For seed sprouting, alfalfa seeds were soaked in the different treatment solutions described above for 3 h. Ca(OCl)₂ (20,000 mg/liter of active chlorine) was the most effective treatment in reducing the populations of *Salmonella* and non-*Salmonella* microflora (4.6 and 7.0 log₁₀ CFU/g, respectively). However, the use of high concentrations of chlorine generates worker safety concerns. Also, the Ca(OCl)₂ treatment significantly reduced seed germination rates (70% versus 90 to 96%). For alfalfa sprouts, higher bacterial populations were recovered from treated sprouts containing seed coats than from sprouts with seed coats removed. The effectiveness of EO water improved when soaking treatments were applied to sprouts in conjunction with sonication and seed coat removal. The combined treatment achieved 2.3- and 1.5-log₁₀ CFU/g greater reductions than EO water alone in populations of *Salmonella* and non-*Salmonella* microflora, respectively. This combination treatment resulted in a 3.3-log₁₀ CFU/g greater reduction in *Salmonella* populations than the control (deionized water) treatment.

The effect of electrolyzed oxidative water applied using electrostatic spraying on pathogenic and indicator bacteria on the surface of eggs.

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Research was conducted to compare the effectiveness of electrolyzed oxidative (EO) water applied using an electrostatic spraying system (ESS) for killing populations of bacteria that are of concern to the poultry industry. Populations of pathogenic bacteria (*Salmonella typhimurium*, *Staphylococcus aureus*, and *Listeria monocytogenes*), and the indicator bacterium *Escherichia coli* were applied to eggs and allowed to attach for 1 h. EO water completely eliminated all *Salmonella typhimurium* on 3, 7, 1, and 8 out of 15 eggs in Repetitions (Rep) 1, 2, 3, and 4, respectively, even when very high inoculations were used. EO water completely eliminated all *Staphylococcus aureus* on 12, 11, 12, and 11 out of 15 eggs in Rep 1, 2, 3, and 4, respectively. EO water completely eliminated all *Listeria monocytogenes* on 8, 13, 12, and 14 out of 15 eggs in Reps 1, 2, 3, and 4, respectively. EO water completely eliminated all *Escherichia coli* on 9, 11, 15, and 11 out of 15 eggs in Reps 1, 2, 3, and 4, respectively. Even when very high concentrations of bacteria were inoculated onto eggs (many times higher than would be encountered in industrial situations), EO water was found to be effective when used in conjunction with electrostatic spraying for eliminating pathogenic and indicator populations of bacteria from hatching eggs.

Electrochemical removal of bromide and reduction of THM formation potential in drinking water.

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Trihalomethanes (THMs), a by-product of the chlorination of natural waters containing dissolved organic carbon and bromide, are the focus of considerable public health concern and regulation due to their potential as a carcinogen by ingestion. This paper presents a promising new water treatment process that lowers the concentration of bromide in drinking water and thus, lowers the THM formation potential. Bromide is oxidized by electrolysis to bromine and then the bromine apparently volatilized. The electrolyzed water, when chlorinated, produces measurably lower amounts of THMs and proportionately fewer brominated THMs, which are of greater public health concern than the chlorinated THMs. Removing bromide should also reduce the formation of other disinfection by-products such as bromate and haloacetic acids

Comparison of electrolyzed oxidizing water with various antimicrobial interventions to reduce *Salmonella* species on poultry.

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Foodborne pathogens in cell suspensions or attached to surfaces can be reduced by electrolyzed oxidizing (EO) water; however, the use of EO water against pathogens

associated with poultry has not been explored. In this study, acidic EO water [EO-A; pH 2.6, chlorine (CL) 20 to 50 ppm, and oxidation-reduction potential (ORP) of 1,150 mV], basic EO water (EO-B; pH 11.6, ORP of -795 mV), CL, ozonated water (OZ), acetic acid (AA), or trisodium phosphate (TSP) was applied to broiler carcasses inoculated with *Salmonella Typhimurium* (ST) and submerged (4 C, 45 min), spray-washed (85 psi, 25 C, 15 s), or subjected to multiple interventions (EO-B spray, immersed in EO-A; AA or TSP spray, immersed in CL). Remaining bacterial populations were determined and compared at Day 0 and 7 of aerobic, refrigerated storage. At Day 0, submersion in TSP and AA reduced ST 1.41 log₁₀, whereas EO-A water reduced ST approximately 0.86 log₁₀. After 7 d of storage, EO-A water, OZ, TSP, and AA reduced ST, with detection only after selective enrichment. Spray-washing treatments with any of the compounds did not reduce ST at Day 0. After 7 d of storage, TSP, AA, and EO-A water reduced ST 2.17, 2.31, and 1.06 log₁₀, respectively. ST was reduced 2.11 log₁₀ immediately following the multiple interventions, 3.81 log₁₀ after 7 d of storage. Although effective against ST, TSP and AA are costly and adversely affect the environment. This study demonstrates that EO water can reduce ST on poultry surfaces following extended refrigerated storage.

Durability of bactericidal activity in electrolyzed neutral water by storage.

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Electrolyzed strong and weak acid waters have been widely used for sterilization in clinical dentistry because of their excellent bactericidal activities. Electrolyzed neutral water was recently developed with a new concept of long-term good durability in addition to the excellent bactericidal activity similar to acid waters. The present study, evaluated the storage life of this water compared with the acid waters in terms of the changes in pH, oxidation-reduction potential (ORP), residual chlorine and bactericidal activity under several conditions using *Staphylococcus aureus* 209P. The strong acid water showed a rapid deterioration of its bactericidal activity. The weak acid and neutral waters exhibited excellent durability. Although all the bacteria were annihilated by the contact with the waters even stored for 40 days in the uncapped bottle, the neutral water was superior in further long-term duration.

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The effectiveness of electrolyzed (EO) water at killing *Enterobacter aerogenes* and *Staphylococcus aureus* in pure culture was evaluated. One milliliter (approximately 10⁹ CFU/ml) of each bacterium was subjected to 9 ml of EO water or control water (EO water containing 10% neutralizing buffer) at room temperature for 30 s. Inactivation (reduction of > 9 log₁₀ CFU/ml) of both pathogens occurred within 30 s after exposure to EO water containing approximately 25 or 50 mg of residual chlorine

per liter. The effectiveness of EO water in reducing *E. aerogenes* and *S. aureus* on different surfaces (glass, stainless steel, glazed ceramic tile, unglazed ceramic tile, and vitreous china) was also evaluated. After immersion of the tested surfaces in EO water for 5 min without agitation, populations of *E. aerogenes* and *S. aureus* were reduced by 2.2 to 2.4 log₁₀ CFU/cm² and by 1.7 to 1.9 log₁₀ CFU/cm², respectively, whereas washing with control water resulted in a reduction of only 0.1 to 0.3 log₁₀ CFU/cm². The washing of tested surfaces in EO water with agitation (50 rpm) reduced populations of viable cells on the tested surfaces to < 1 CFU/cm². For the control water treatment with agitation, the surviving numbers of both strains on the tested surfaces were approximately 3 log₁₀ CFU/cm². No viable cells of either strain were observed in the EO water after treatment, regardless of agitation. However, large populations of both pathogens were recovered from control wash solution after treatment.

[Antimicrobial effects and efficacy on habitually hand-washing of strong acidic electrolyzed water--a comparative study of alcoholic antiseptics and soap and tap water]

[Article in Japanese]

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The rate of bacterial elimination for the stamp method was compared with regular hand-washing (using soap and tap water), hygienic hand-washing (using alcoholic antiseptics), and hand-washing using strong acidic electrolyzed water (the SAEW method) in routine work. After routine work, the average number of bacteria remaining on the nurse's hands with using the SAEW-method, rubbing method and tap water method, were: 54 +/- 63, 89 +/- 190, 128 +/- 194 CFU/agar plate, respectively (n = 81). In this study. It was clarified that a much larger number of *Bacillus* sp. were detected for the rubbing method than for the other methods. After further nurse work, the most number of absorbed bacteria on a nurse's hands were counted after cleaning a patient's body. The rate of bacteria elimination for hand-washing with soap and tap water after taking care of a patient was insufficient, especially when before care was provided the number of bacteria on the nurse's hands were less than 100 CFU/agar plate. From these results, the following manual for sanitary hand washing is recommended: 1. At first, dirty hands should be cleaned and the number of bacteria should be reduced using soap and tap water or by scrubbing with disinfectants. 2. After the number of bacteria has been reduced, use the SAEW method routinely. 3. For care requiring a high level of cleanliness or if no tap water facilities are available, use the rubbing method. Finally, routine use of the SAEW method in ICU could be recommended with conventional disinfectants and soap and tap water on a case by case basis for less than adverse reactions, such as in the case of rough-hands or keeping a low level of bacteria on hands.

Bactericidal activity of electrolyzed acid water from solution containing sodium chloride at low concentration, in comparison with that at high concentration.

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Electrolyzed strong acid water (ESW) containing free chlorine at various concentrations is becoming to be available in clinical settings as a disinfectant. ESW is prepared by electrolysis of a NaCl solution, and has a corrosive activity against medical instruments. Although lower concentrations of NaCl and free chlorine are desired to eliminate corrosion, the germicidal effect of ESW with low NaCl and free-chlorine concentrations (ESW-L) has not been fully clarified. In this study, we demonstrated that ESW-L possesses bactericidal activity against Mycobacteria and spores of *Bacillus subtilis*. The effect was slightly weaker than that of ESW containing higher NaCl and free-chlorine concentrations (ESW-H), but acceptable as a disinfectant. To clarify the mechanism of the bactericidal activity, we investigated ESW-L-treated *Pseudomonas aeruginosa* by transmission electron microscopy, a bacterial enzyme assay and restriction fragment length polymorphism pattern (RFLP) assay. Since the bacterium, whose growth was completely inhibited by ESW-L, revealed the inactivation of cytoplasmic enzyme, blebs and breaks in its outer membrane and remained complete RFLP of DNA, damage of the outer membrane and inactivation of cytoplasmic enzyme are the important determinants of the bactericidal activity.

[Observation on the effect of disinfection to HBsAg by electrolyzed oxidizing water]

[Article in Chinese]

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OBJECTIVE: Observation on the effect of disinfection on gastroscope, contaminated by hepatitis B surface antigen (HBsAg) in the electrolyzed oxidizing water (EOW). METHODS: Contaminated gastric juice and serum was added to EOW for 1 minute. Positive control samples were treated with PBS instead of EOW in the same way. Gastroscopes used for hepatitis patients were immersed in the EOW for 1 minute after cleaning. Samples were collected before and after treatment. ELISA was used to test HBsAg. RESULTS: With mixed samples (average S/N = 42.16) of EOW, HBsAg became negative when diluted in 100 times. However, the HBsAg of positive control samples remained positive. After cleaning the gastroscope (average S/N = 5.99) immersed in EOW, HBsAg became negative. CONCLUSION: EOW was effective in destroying HBsAg which could be used for gastroscope disinfection.

Decontaminative effect of frozen acidic electrolyzed water on lettuce.

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We investigated the effects of frozen acidic electrolyzed water (AcEW) on lettuce during storage in a styrene-foam container. The lettuce was kept at 2 to 3 degrees C for 24 h. Populations of aerobic bacteria associated with lettuce packed in frozen AcEW were reduced by 1.5 log CFU/g after storage for 24 h. With frozen tap water, no microorganism populations tested in this study were reduced. A frozen mixture of AcEW and alkaline electrolyzed water (AIEW) also failed to reduce populations of microorganisms associated with lettuce. Although chlorine gas was produced by frozen AcEW, it was not produced by the AcEW-AIEW mixture. This result indicates that the main factor in the decontaminative effect of frozen AcEW was the production of chlorine gas. Accordingly, low-temperature storage and decontamination could be achieved simultaneously with frozen AcEW during distribution.

Antimicrobial effect of electrolyzed water for inactivating *Campylobacter jejuni* during poultry washing.**Park H, Hung YC, Brackett RE.**

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The effectiveness of electrolyzed (EO) water for killing *Campylobacter jejuni* on poultry was evaluated. Complete inactivation of *C. jejuni* in pure culture occurred within 10 s after exposure to EO or chlorinated water, both of which contained 50 mg/l of residual chlorine. A strong bactericidal activity was also observed on the diluted EO water (containing 25 mg/l of residual chlorine) and the mean population of *C. jejuni* was reduced to less than 10 CFU/ml (detected only by enrichment for 48 h) after 10-s treatment. The diluted chlorine water (25 mg/l residual chlorine) was less effective than the diluted EO water for inactivation of *C. jejuni*. EO water was further evaluated for its effectiveness in reducing *C. jejuni* on chicken during washing. EO water treatment was equally effective as chlorinated water and both achieved reduction of *C. jejuni* by about 3 log₁₀ CFU/g on chicken, whereas deionized water (control) treatment resulted in only 1 log₁₀ CFU/g reduction. No viable cells of *C. jejuni* were recovered in EO and chlorinated water after washing treatment, whereas high populations of *C. jejuni* (4 log₁₀ CFU/ml) were recovered in the wash solution after the control treatment. Our study demonstrated that EO water was very effective not only in reducing the populations of *C. jejuni* on chicken, but also could prevent cross-contamination of processing environments.

Effects of storage conditions and pH on chlorine loss in electrolyzed oxidizing (EO) water.**Len SV, Hung YC, Chung D, Anderson JL, Morita K.**

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The chlorine loss of electrolyzed oxidizing (EO) water was examined during storage under different light, agitation, and packaging conditions. The chlorine loss of pH-adjusted EO water was also examined. Under open conditions, the chlorine loss through evaporation followed first-order kinetics. The rate of chlorine loss was increased about 5-fold with agitation, but it was not significantly affected by diffused light. Under closed conditions, the chlorine loss did not follow first-order kinetics, because the primary mechanism of chlorine loss may be self-decomposition of chlorine species rather than chlorine evaporation. The effect of diffused light was more significant compared to agitation after two months of storage under closed conditions. The chlorine loss of EO water and commercial chlorinated water decreased dramatically with the increase of pH from the acidic (pH 2.5) to the alkaline (pH 9.0) region.

Application of electrolyzed acid water to sterilization of denture base part 1. Examination of sterilization effects on resin plate.

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Bactericidal activities of electrolyzed strong and weak acid waters for acrylic denture base resin were evaluated in order to discuss the applicability of these waters for sterilization of denture base. Only 1-minute immersion in the electrolyzed strong or weak acid water could completely eliminate the attached bacteria, *Staphylococcus aureus* 209P, on the resin plate. When the resin was relined with tissue conditioner, 5-minute immersion or 1- to 2-minute ultrasonic cleaning reduced the number of the bacteria from 10(5)/cm² level to 10(1)/cm² and no surviving bacteria could be detected after 10-minute treatment. These findings suggest that both the electrolyzed strong and weak acid waters are well applicable to the disinfectant for acrylic denture base showing excellent bactericidal activities in a significantly shorter treatment as compared with the conventional denture cleaning.

Decontamination of lettuce using acidic electrolyzed water.

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The disinfectant effect of acidic electrolyzed water (AcEW), ozonated water, and sodium hypochlorite (NaOCl) solution on lettuce was examined. AcEW (pH 2.6; oxidation reduction potential, 1140 mV; 30 ppm of available chlorine) and NaOCl solution (150 ppm of available chlorine) reduced viable aerobes in lettuce by 2 log CFU/g within 10 min. For lettuce washed in alkaline electrolyzed water (AIEW) for 1 min and then disinfected in AcEW for 1 min, viable aerobes were reduced by 2 log CFU/g. On the other hand, ozonated water containing 5 ppm of ozone reduced viable aerobes in lettuce 1.5 log CFU/g within 10 min. It was discovered that AcEW showed a

higher disinfectant effect than did ozonated water significantly at $P < 0.05$. It was confirmed by swabbing test that AcEW, ozonated water, and NaOCl solution removed aerobic bacteria, coliform bacteria, molds, and yeasts on the surface of lettuce. Therefore, residual microorganisms after the decontamination of lettuce were either in the inside of the cellular tissue, such as the stomata, or making biofilm on the surface of lettuce. Biofilms were observed by a scanning electron microscope on the surface of the lettuce treated with AcEW. Moreover, it was shown that the spores of bacteria on the surface were not removed by any treatment in this study. However, it was also observed that the surface structure of lettuce was not damaged by any treatment in this study. Thus, the use of AcEW for decontamination of fresh lettuce was suggested to be an effective means of controlling microorganisms

Effect of electrolyzed water on wound healing.

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Electrolyzed water accelerated the healing of full-thickness cutaneous wounds in rats, but only anode chamber water (acid pH or neutralized) was effective. Hypochlorous acid (HOCl), also produced by electrolysis, was ineffective, suggesting that these types of electrolyzed water enhance wound healing by a mechanism unrelated to the well-known antibacterial action of HOCl. One possibility is that reactive oxygen species, shown to be electron spin resonance spectra present in anode chamber water, might trigger early wound healing through fibroblast migration and proliferation.

The use of electrolyzed solutions for the cleaning and disinfecting of dialyzers.

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Recently, the use of electrolyzed solutions has attracted considerable interest in Japan. This study investigates the efficiency of electrolyzed solutions as disinfecting agents (DA) in the reuse of dialyzers and compares their efficiency to that of other disinfectants currently in use. The following 3 methods were employed. First, the rinsing time and rebound release of reused dialyzers were measured and compared after electrolyzed solutions, electrolyzed strong acid aqueous solution (ESAAS) and electrolyzed strong basic aqueous solution (ESBAS), made from reverse osmosis (RO) water (ESAAS, ESBAS; Generating apparatuses: Super Oxseed alpha 1000, Amano Corporation, Yokohama, Japan), 2% Dialox-cj (Teijin Gambro Medical, Tokyo, Japan), and 3.8% formalin were used as DAs. This involved performing dialysis with 2 types of dialyzers: a cellulose acetate membrane (CAM) dialyzer and a polysulfone membrane (PSM) dialyzer. The dialyzers were cleaned and disinfected using the different DA and left for 48 h. Next, after performing dialysis the dialyzer membranes were cleaned with a saline solution (0.9% NaCl) and RO water and then cleaned with the various DA.

These membranes were observed using a scanning electron microscope (SEM) to check for the presence of physical and biological contaminants. Finally, in vitro tests were performed to determine the level of dialyzer clearance when PSM dialyzers were reused after having been cleaned and disinfected with the electrolyzed solutions. The rinsing time results for both the CAM and PSM dialyzers showed the electrolyzed solutions (ESBAS and ESAAS) as being undetectable within 10 min. With regard to the rebound release, for both the CAM and PSM dialyzers, the electrolyzed solutions were undetectable at all checking times between 30 and 240 min. Observation by SEM showed that cleaning with both ESAAS and ESBAS left the fewest contaminants, and cleaning with 2% Dialox-cj left the highest level of contaminants in the CAM dialyzers. With regard to experiments concerning use in vitro, no major changes in the dialyzer clearance were noticed after 6 uses. In every experiment, the previous investigations showed the electrolyzed solutions to be superior to 3.8% formalin and 2% Dialox-cj DA for the reuse of dialyzers.

Ultraviolet spectrophotometric characterization and bactericidal properties of electrolyzed oxidizing water as influenced by amperage and pH.

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To identify the primary component responsible in electrolyzed oxidizing (EO) water for inactivation, this study determined the concentrations of hypochlorous acid (HOCl) and hypochlorite ions (OCl⁻) and related those concentrations to the microbicidal activity of the water. The ultraviolet absorption spectra were used to determine the concentrations of HOCl and OCl⁻ in EO water and the chemical equilibrium of these species with change in pH and amperage. EO water generated at higher amperage contained a higher chlorine concentration. The maximum concentration of HOCl was observed around pH 4 where the maximum log reduction (2.3 log₁₀ CFU/ml) of *Bacillus cereus* F4431/73 vegetative cells also occurred. The high correlation ($r = 0.95$) between HOCl concentrations and bactericidal effectiveness of EO water supports HOCl's role as the primary inactivation agent. Caution should be taken with standard titrimetric methods for measurement of chlorine as they cannot differentiate the levels of HOCl present in EO water of varying pHs.

Efficacy of electrolyzed oxidizing (EO) and chemically modified water on different types of foodborne pathogens.

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This study was undertaken to evaluate the efficacy of electrolyzed oxidizing (EO) and chemically modified water with properties similar to the EO water for inactivation of different types of foodborne pathogens (*Escherichia coli* O157:H7, *Listeria*

monocytogenes and *Bacillus cereus*). A five-strain cocktail of each microorganism was exposed to deionized water (control), EO water and chemically modified water. To evaluate the effect of individual properties (pH, oxidation-reduction potential (ORP) and residual chlorine) of treatment solutions on microbial inactivation, iron was added to reduce ORP readings and neutralizing buffer was added to neutralize chlorine. Inactivation of *E. coli* O157:H7 occurred within 30 s after application of JAW EO water with 10 mg/l residual chlorine and chemically modified solutions containing 13 mg/l residual chlorine. Inactivation of Gram-positive and -negative microorganisms occurred within 10 s after application of ROX EO water with 56 mg/l residual chlorine and chemically modified solutions containing 60 mg/l residual chlorine. *B. cereus* was more resistant to the treatments than *E. coli* O157:H7 and *L. monocytogenes* and only 3 log₁₀ reductions were achieved after 10 s of ROX EO water treatment. *B. cereus* spores were the most resistant pathogen. However, more than 3 log₁₀ reductions were achieved with 120-s EO water treatment

Effectiveness of electrolyzed oxidized water irrigation in a burn-wound infection model.

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OBJECTIVE: The purpose of the study was to determine whether electrolyzed oxidized water (EOW) functions as a bactericide in burn injury with *Pseudomonas aeruginosa* infection in a rat burn-wound model. **METHODS:** Anesthetized Sprague-Dawley rats (n = 31) were subjected to third-degree burns to 30% of total body surface area. Two days after injury, all rats were infected with *P. aeruginosa* using 1 mL of a suspension containing 1 x 10⁸ colony-forming units. Rats were assigned to one of three groups: no irrigation (group I), irrigation with physiologic saline (group II), or irrigation with EOW (group III). Blood culture, endotoxin levels, and survival rates were determined. **RESULTS:** Survival rate was significantly higher in group III than in groups I or II (p < 0.0001). Serum endotoxin levels on day 3 after infection in group III were significantly lower than the levels in group I (p < 0.01) and group II (p < 0.01). There were significant differences between the three groups in the culture of *P. aeruginosa* (p < 0.05). **CONCLUSION:** Irrigation and disinfection with EOW may become useful in preventing burn-wound sepsis.

Disinfection potential of electrolyzed solutions containing sodium chloride at low concentrations.

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Electrolyzed products of sodium chloride solution were examined for their disinfection

potential against hepatitis B virus (HBV) and human immunodeficiency virus (HIV) in vitro. Electrolysis of 0.05% NaCl in tap water was carried out for 45 min at room temperature using a 3 A electric current in separate wells installed with positive and negative electrodes. The electrolyzed products were obtained from the positive well. The oxidation reduction potential (ORP), pH and free chlorine content of the product were 1053 mV, pH 2.34 and 4.20 ppm, respectively. The products modified the antigenicity of the surface protein of HBV as well as the infectivity of HIV in time- and concentration-dependent manner. Although the inactivating potential was decreased by the addition of contaminating protein, recycling of the product or continuous addition of fresh product may restore the complete disinfection against bloodborne pathogens.

Newer technologies for endoscope disinfection: electrolyzed acid water and disposable-component endoscope systems.

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Novel technologies have been designed to improve or replace more conventional methods of endoscope disinfection. Electrolyzed acid water has the potential to decrease the time, toxicity, and cost of endoscope disinfection. Disposable-component endoscope systems have the potential to improve the ease of cleaning and disinfection, or eliminate the need altogether

Roles of oxidation-reduction potential in electrolyzed oxidizing and chemically modified water for the inactivation of food-related pathogens.

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This study investigates the properties of electrolyzed oxidizing (EO) water for the inactivation of pathogen and to evaluate the chemically modified solutions possessing properties similar to EO water in killing *Escherichia coli* O157:H7. A five-strain cocktail (10(10) CFU/ml) of *E. coli* O157:H7 was subjected to deionized water (control), EO water with 10 mg/liter residual chlorine (J.A.W-EO water), EO water with 56 mg/liter residual chlorine (ROX-EO water), and chemically modified solutions. Inactivation (8.88 log₁₀ CFU/ml reduction) of *E. coli* O157:H7 occurred within 30 s after application of EO water and chemically modified solutions containing chlorine and 1% bromine. Iron was added to EO or chemically modified solutions to reduce oxidation-reduction potential (ORP) readings and neutralizing buffer was added to neutralize chlorine. J.A.W-EO water with 100 mg/liter iron, acetic acid solution, and chemically modified solutions containing neutralizing buffer or 100 mg/liter iron were ineffective in reducing the bacteria population. ROX-EO water with 100 mg/liter iron was the only solution still effective in inactivation of *E. coli* O157:H7 and having high ORP readings regardless of

residual chlorine. These results suggest that it is possible to simulate EO water by chemically modifying deionized water and ORP of the solution may be the primary factor affecting microbial inactivation.

Cytotoxicity and microbicidal activity of electrolyzed strong acid water and acidic hypochlorite solution under isotonic conditions.

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The cytotoxic effects of electrolyzed strong acid water and acidic hypochlorite solution, as well as these solutions after isotonization, against cultivated L cells were compared along with their microbicidal activities. Isotonization was accompanied by a reduction in the cytotoxic effects of these solutions against L cells. Microbicidal activity was also reduced somewhat but was still retained after isotonization. No difference was observed in these properties between these antiseptic solutions. The results obtained indicate that acidic hypochlorite solution may be useful as well as acidic electrolyzed water.

Efficacy of electrolyzed oxidizing water for inactivating *Escherichia coli* O157:H7, *Salmonella enteritidis*, and *Listeria monocytogenes*.

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The efficacy of electrolyzed oxidizing water for inactivating *Escherichia coli* O157:H7, *Salmonella enteritidis*, and *Listeria monocytogenes* was evaluated. A five-strain mixture of *E. coli* O157:H7, *S. enteritidis*, or *L. monocytogenes* of approximately 10(8) CFU/ml was inoculated in 9 ml of electrolyzed oxidizing water (treatment) or 9 ml of sterile, deionized water (control) and incubated at 4 or 23 degrees C for 0, 5, 10, and 15 min; at 35 degrees C for 0, 2, 4, and 6 min; or at 45 degrees C for 0, 1, 3, and 5 min. The surviving population of each pathogen at each sampling time was determined on tryptic soy agar. At 4 or 23 degrees C, an exposure time of 5 min reduced the populations of all three pathogens in the treatment samples by approximately 7 log CFU/ml, with complete inactivation by 10 min of exposure. A reduction of ≥ 7 log CFU/ml in the levels of the three pathogens occurred in the treatment samples incubated for 1 min at 45 degrees C or for 2 min at 35 degrees C. The bacterial counts of all three pathogens in control samples remained the same throughout the incubation at all four temperatures. Results indicate that electrolyzed oxidizing water may be a useful disinfectant, but appropriate applications need to be validated.

Inactivation of *Escherichia coli* O157:H7 and *Listeria monocytogenes* on plastic kitchen cutting boards by electrolyzed oxidizing water.

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One milliliter of culture containing a five-strain mixture of *Escherichia coli* O157:H7 (approximately 10(10) CFU) was inoculated on a 100-cm² area marked on unscarred cutting boards. Following inoculation, the boards were air-dried under a laminar flow hood for 1 h, immersed in 2 liters of electrolyzed oxidizing water or sterile deionized water at 23 degrees C or 35 degrees C for 10 or 20 min; 45 degrees C for 5 or 10 min; or 55 degrees C for 5 min. After each temperature-time combination, the surviving population of the pathogen on cutting boards and in soaking water was determined. Soaking of inoculated cutting boards in electrolyzed oxidizing water reduced *E. coli* O157:H7 populations by $> \text{ or } = 5.0 \text{ log CFU/100 cm}^2$ on cutting boards. However, immersion of cutting boards in deionized water decreased the pathogen count only by 1.0 to 1.5 log CFU/100 cm². Treatment of cutting boards inoculated with *Listeria monocytogenes* in electrolyzed oxidizing water at selected temperature-time combinations (23 degrees C for 20 min, 35 degrees C for 10 min, and 45 degrees C for 10 min) substantially reduced the populations of *L. monocytogenes* in comparison to the counts recovered from the boards immersed in deionized water. *E. coli* O157:H7 and *L. monocytogenes* were not detected in electrolyzed oxidizing water after soaking treatment, whereas the pathogens survived in the deionized water used for soaking the cutting boards. This study revealed that immersion of kitchen cutting boards in electrolyzed oxidizing water could be used as an effective method for inactivating foodborne pathogens on smooth, plastic cutting board

Bactericidal effect of electrolyzed neutral water on bacteria isolated from infected root canals.**Horiba N, Hiratsuka K, Onoe T, Yoshida T, Suzuki K, Matsumoto T, Nakamura H.**

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OBJECTIVE: The purposes of this study were to examine the time-related changes in pH, oxidation-reduction potential, and concentration of chlorine of electrolyzed neutral water and to evaluate the bactericidal effect of electrolyzed neutral water against bacteria from infected root canals. **STUDY DESIGN:** Various properties of electrolyzed neutral water--pH value, oxidation-reduction potential, and concentration of chlorine--were measured at different times after storage of the water in the open state, the closed state, or the closed-and-dark state. The bactericidal effect of the various electrolyzed neutral water samples was then tested against 17 strains of bacteria, including 15 strains isolated from infected canals, as well as against 1 strain of fungus. Each bacterial or fungal suspension was mixed with electrolyzed neutral water, and the 2 substances were reacted together for 1 minute. After incubation for 1 to 7 days, the bactericidal effect of the electrolyzed neutral water was determined. **RESULTS:** The pH value and oxidation-reduction potential of electrolyzed neutral water remained almost unchanged when the water was stored in a dark, closed container. However, the concentration of chlorine decreased from 18.4 ppm to 10.6 ppm. Electrolyzed neutral

water showed a bactericidal or growth-inhibitory effect against the bacteria.

CONCLUSIONS: The results indicate that electrolyzed neutral water maintains a constant pH and oxidation-reduction potential when kept in a closed container without light and that it exhibits a bacteriostatic/bactericidal action against isolates obtained from infected root canals.

Electrolyzed-reduced water scavenges active oxygen species and protects DNA from oxidative damage.

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Active oxygen species or free radicals are considered to cause extensive oxidative damage to biological macromolecules, which brings about a variety of diseases as well as aging. The ideal scavenger for active oxygen should be 'active hydrogen'. 'Active hydrogen' can be produced in reduced water near the cathode during electrolysis of water. Reduced water exhibits high pH, low dissolved oxygen (DO), extremely high dissolved molecular hydrogen (DH), and extremely negative redox potential (RP) values. Strongly electrolyzed-reduced water, as well as ascorbic acid, (+)-catechin and tannic acid, completely scavenged O_2^- produced by the hypoxanthine-xanthine oxidase (HX-XOD) system in sodium phosphate buffer (pH 7.0). The superoxide dismutase (SOD)-like activity of reduced water is stable at 4 degrees C for over a month and was not lost even after neutralization, repeated freezing and melting, deflation with sonication, vigorous mixing, boiling, repeated filtration, or closed autoclaving, but was lost by opened autoclaving or by closed autoclaving in the presence of tungsten trioxide which efficiently adsorbs active atomic hydrogen. Water bubbled with hydrogen gas exhibited low DO, extremely high DH and extremely low RP values, as does reduced water, but it has no SOD-like activity. These results suggest that the SOD-like activity of reduced water is not due to the dissolved molecular hydrogen but due to the dissolved atomic hydrogen (active hydrogen). Although SOD accumulated H_2O_2 when added to the HX-XOD system, reduced water decreased the amount of H_2O_2 produced by XOD. Reduced water, as well as catalase and ascorbic acid, could directly scavenge H_2O_2 . Reduced water suppresses single-strand breakage of DNA by active oxygen species produced by the Cu(II)-catalyzed oxidation of ascorbic acid in a dose-dependent manner, suggesting that reduced water can scavenge not only O_2^- and H_2O_2 , but also $1O_2$ and $\cdot OH$.

Trial of electrolyzed strong acid aqueous solution lavage in the treatment of peritonitis and intraperitoneal abscess.

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Electrolyzed strong acid aqueous solution is acidic water that contains active oxygen

and active chlorine and possesses a redox potential. We performed peritoneal and abscess lavages with an electrolyzed strong acid aqueous solution to treat 7 patients with peritonitis and intraperitoneal abscesses, who were seen in our department between December 1994 and April 1995. The underlying disease was duodenal ulcer perforation in 4 of these 7 patients and gastric ulcer perforation, acute enteritis, and intraperitoneal perforation of pyometrium in 1 patient each. Irrigation was performed twice a day. Microbiological studies of the paracentesis fluid were negative in 3 cases, and the irrigation period was 2-4 days. Anaerobic bacteria were isolated in 3 of the 4 positive cases (Bacteroides in 2, Prevotella in 1), and a fungus (Candida) was isolated in the remaining patient. The period of irrigation in these patients ranged from 9 to 12 days, but conversion to a microorganism negative state was observed in 3-7 days.

[Bactericidal effect of acidic electrolyzed water--comparison of chemical acidic sodium hydrochloride (NaOCl) solution]

[Article in Japanese]

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Acidic electrolyzed water is made recently by various kinds of machines and is widely utilized. In this study, we intended to clarify the relationship between the concentration of chloride and pH in the bactericidal effects with acidic electrolyzed water. The effects of weak or strong acidic electrolyzed water were compared with a pseudo-acidic water of pH adjusted by diluted hydrochloric acid and sodium hydroxide, on *Staphylococcus aureus*, *Staphylococcus epidermidis* and *Pseudomonas aeruginosa*. At pH 5.0 approximately 6.0, 3 bacterial strains were killed soon after being exposed to the acidic water containing chloride 50 mg/liter, and the amount of chloride did not change after allowing to stand open for 6 hours. At pH 2.67 approximately 2.80, the bactericidal effects was observed at the concentration of chloride 5 mg/liter, and 80% of chloride remained after allowing to stand for 6 hours. These results indicated that newly made strong acidic water is more effective under a smaller amount of chloride at pH 2.7, and that weak acidic electrolyzed water should be used, if stable bactericidal effect is expected in cleaning the surroundings.

[Preliminary study of microbiocide effect and its mechanism of electrolyzed oxidizing water]

[Article in Chinese]

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Electrolyzed Oxidizing water (EO Water) is characterized by possessing higher oxidizing reduction potential (ORP), lower pH value and oxidizing potential. Under conditions of

free organic matter, it was tested for microbiocide efficacy in laboratory. The results showed that EO water could completely kill all of the staphylococcus aureus and E. coli within 15 seconds, while for completely killing of spores of Bacillus subtilis Var. niger it would take 10 min. When it was used to destroy the antigenicity of HBsAg, 30 seconds was needed. The ORP and pH values of EO water were not obviously changed when stored in room-temperature with, airtight and light-free conditions for three weeks. Distilled water and physiological saline had little influence on the ORP and pH value of EO water, but organic matters and phosphates had greater influence upon the two values.

Cleaning effectiveness of root canal irrigation with electrochemically activated anolyte and catholyte solutions: a pilot study.

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AIM: The aim of this study was to evaluate the potential of electrochemically activated (ECA) anolyte and catholyte solutions to clean root canals during conventional root canal preparation.

METHODOLOGY: Twenty extracted single-rooted human mature permanent teeth were allocated randomly into four groups of five teeth. The pulp chambers were accessed and the canals prepared by hand with conventional stainless steel endodontic instruments using a double-flared technique. One or other of the following irrigants was used during preparation: distilled water, 3% NaOCl, anolyte neutral cathodic (ANC) (300 mg L⁻¹ of active chlorine), and a combination of anolyte neutral cathodic (ANC) (300 mg L⁻¹ of active chlorine) and catholyte. The teeth were split longitudinally and the canal walls examined for debris and smear layer by scanning electron microscopy. SEM photomicrographs were taken separately in the coronal, middle and apical parts of canal at magnification of x800 to evaluate the debridement of extracellular matrix and at a magnification of x2500 to evaluate the presence of smear layer. RESULTS: Irrigation with distilled water did not remove debris in the apical part of canals and left a continuous and firm smear layer overlying compressed low-mineralized predentine. All chemically active irrigants demonstrated improved cleaning potential compared to distilled water. The quality of loose debris elimination was similar for NaOCl and the anolyte ANC solution. The combination of anolyte ANC and catholyte resulted in improved cleaning, particularly in the apical third of canals. The evaluation of smear layer demonstrated that none of the irrigants were effective in its total removal; however, chemically active irrigants affected its surface and thickness. Compared to NaOCl, the ECA solutions left a thinner smear layer with a smoother and more even surface. NaOCl enhanced the opening of tubules predominantly in the coronal and middle thirds of canals, whereas combination of ANC and catholyte resulted in more numerous open dentine tubules throughout the whole length of canals. CONCLUSIONS: Irrigation with electrochemically activated solutions cleaned root canal walls and may be an alternative to NaOCl in conventional root canal treatment. Further investigation of ECA solutions for root canal irrigation is warranted.

Inactivation of Cryptosporidium parvum oocysts and Clostridium perfringens spores by a mixed-oxidant disinfectant and by free chlorine.

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Cryptosporidium parvum oocysts and Clostridium perfringens spores are very resistant to chlorine and other drinking-water disinfectants. Clostridium perfringens spores have been suggested as a surrogate indicator of disinfectant activity against Cryptosporidium parvum and other hardy pathogens in water. In this study, an alternative disinfectant system consisting of an electrochemically produced mixed-oxidant solution was evaluated for inactivation of both Cryptosporidium parvum oocysts and Clostridium perfringens spores. The disinfection efficacy of the mixed-oxidant solution was compared to that of free chlorine on the basis of equal weight per volume concentrations of total oxidants. Batch inactivation experiments were done on purified oocysts and spores in buffered, oxidant demand-free water at pH 7 and 25 degrees C by using a disinfectant dose of 5 mg/liter and contact times of up to 24 h. The mixed-oxidant solution was considerably more effective than free chlorine in inactivating both microorganisms. A 5-mg/liter dose of mixed oxidants produced a > 3-log₁₀-unit (> 99.9%) inactivation of Cryptosporidium parvum oocysts and Clostridium perfringens spores in 4 h. Free chlorine produced no measurable inactivation of Cryptosporidium parvum oocysts by 4 or 24 h, although Clostridium perfringens spores were inactivated by 1.4 log₁₀ units after 4 h. The on-site generation of mixed oxidants may be a practical and cost-effective system of drinking water disinfection protecting against even the most resistant pathogens, including Cryptosporidium oocysts.

Activity of Electrolyzed Oxidizing Water Against Penicillium expansum in Suspension and on Wounded Apples

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Spores of Penicillium expansum, the primary organism responsible for the occurrence of patulin in

apple juice, were exposed to electrolyzed oxidizing (EO) water in an aqueous suspension and on wounded apples. Full-strength and 50% EO water decreased viable spore populations by greater than 4 and 2 log units, respectively. Although EO water did not prevent lesion formation on fruit previously inoculated with P. expansum, cross-contamination of wounded apples from decayed fruit or by direct addition of spores to a simulated dump tank was substantially reduced. EO water, therefore, has potential as an alternative to chlorine disinfectants for controlling infection of apples by P. expansum during handling and processing operations.

Keywords: Penicillium expansum, electrolyzed oxidizing water, apples, patulin

Effectiveness of Electrolyzed Water Irrigation in a burn-wound infection model.

[Hajime Nakae, MD, PhD](#) and [Hideo Inaba, MD, PhD](#)

Journal of Trauma injury, infection and critical care

Antioxidant effect of Reduced Water (Alkaline Water) produced by the Electrolyses of Sodium chloride solutions.

K. Hanaoko

Journal of Applied Electrochemistry

Bactericidal Activity of Electrolyzed Acid Water from solution containing sodium chloride at low concentration in comparison with that at high concentration.

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Journal of Microbiological Methods

Enhanced disinfection efficiency of mechanically mixed oxidants with free chlorine.

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To the best of our knowledge, this study is the first investigation to be performed into the potential benefits of mechanically mixed disinfectants in controlling bacterial inactivation. The purpose of this study was to evaluate the disinfection efficiency of mechanically mixed oxidants with identical oxidant concentrations, which were made by adding small amounts of subsidiary oxidants, namely ozone (O₃), chlorine dioxide (ClO₂), hydrogen peroxide (H₂O₂) and chlorite (ClO₂(-)), to free available chlorine (Cl₂), using *Bacillus subtilis* spores as the indicator microorganisms. The mechanically mixed oxidants containing Cl₂/O₃, Cl₂/ClO₂ and Cl₂/ClO₂(-) showed enhanced efficiencies (of up to 52%) in comparison with Cl₂ alone, whereas no significant difference was observed between the mixed oxidant, Cl₂/H₂O₂, and Cl₂ alone. This enhanced disinfection efficiency can be explained by the synergistic effect of the mixed oxidant itself and the effect of intermediates such as ClO₂(-)/ClO₂, which are generated from the reaction between an excess of Cl₂ and a small amount of O₃/ClO₂(-). Overall, this study suggests that mechanically mixed oxidants incorporating excess chlorine can constitute a new and moderately efficient method of disinfection.

Effects of electrolyzed oxidizing water on reducing *Listeria monocytogenes* contamination on seafood processing surfaces.

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The effects of electrolyzed oxidizing (EO) water on reducing *Listeria monocytogenes* contamination on seafood processing surfaces were studied. Chips (5 x 5 cm²) of stainless steel sheet (SS), ceramic tile (CT), and floor tile (FT) with and without crabmeat residue on the surface were inoculated with *L. monocytogenes* and soaked in tap or EO water for 5 min. Viable cells of *L. monocytogenes* were detected on all chip surfaces with or without crabmeat residue after being held at room temperature for 1 h. Soaking contaminated chips in tap water resulted in small-degree reductions of the organism (0.40-0.66 log cfu/chip on clean surfaces and 0.78-1.33 log cfu/chip on dirty surfaces). Treatments of EO water significantly ($p < 0.05$) reduced *L. monocytogenes* on clean surfaces (3.73 log on SS, 4.24 log on CT, and 5.12 log on FT). Presence of crabmeat residue on chip surfaces reduced the effectiveness of EO water on inactivating *Listeria* cells. However, treatments of EO water also resulted in significant reductions of *L. monocytogenes* on dirty surfaces (2.33 log on SS and CT and 1.52 log on FT) when compared with tap water treatments. The antimicrobial activity of EO water was positively correlated with its chlorine content. High oxidation-reduction potential (ORP) of EO water also contributed significantly to its antimicrobial activity against *L. monocytogenes*. EO water was more effective than chlorine water on inactivating *L. monocytogenes* on surfaces and could be used as a chlorine alternative for sanitation purpose. Application of EO water following a thorough cleaning process could greatly reduce *L. monocytogenes* contamination in seafood processing environments.

Efficacy of ozonated and electrolyzed oxidative waters to decontaminate hides of cattle before slaughter.

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The hides of cattle are the primary source of pathogens such as *Escherichia coli* O157:H7 that contaminate previsceration carcasses during commercial beef processing. A number of interventions that reduce hide contamination and subsequent carcass contamination are currently being developed. The objective of this study was to determine the efficacy of ozonated and electrolyzed oxidizing (EO) waters to decontaminate beef hides and to compare these treatments with similar washing in water without the active antimicrobial compounds. Cattle hides draped over barrels were used as the model system. Ozonated water (2 ppm) was applied at 4,800 kPa (700 lb in²) and 15 degrees C for 10 s. Alkaline EO water and acidic EO water were sequentially applied at 60 degrees C for 10 s at 4,800 and 1,700 kPa (250 lb in²), respectively. Treatment using ozonated water reduced hide aerobic plate counts by 2.1 log CFU/100 cm² and reduced Enterobacteriaceae counts by 3.4 log CFU/100 cm². EO water treatment reduced aerobic plate counts by 3.5 log CFU/100 cm² and reduced

Enterobacteriaceae counts by 4.3 log CFU/100 cm². Water controls that matched the wash conditions of the ozonated and EO treatments reduced aerobic plate counts by only 0.5 and 1.0 log CFU/100 cm², respectively, and each reduced Enterobacteriaceae counts by 0.9 log CFU/100 cm². The prevalence of *E. coli* O157 on hides was reduced from 89 to 31% following treatment with ozonated water and from 82 to 35% following EO water treatment. Control wash treatments had no significant effect on the prevalence of *E. coli* O157:H7. These results demonstrate that ozonated and EO waters can be used to decontaminate hides during processing and may be viable treatments for significantly reducing pathogen loads on beef hides, thereby reducing pathogens on beef carcasses.

The Anti-microbial Activity of Electrolysed Oxidizing Water against Microorganisms relevant in Veterinary Medicine

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Summary

Standards of the German Association of Veterinary Medicine (DVG) for the evaluation of chemical disinfectants were used to assess the anti-microbial efficacy of electrolysed oxidizing water (EOW). *Enterococcus faecium*, *Mycobacterium avium* subspecies *avium*, *Proteus mirabilis*, *Pseudomonas aeruginosa*, *Staphylococcus aureus* and *Candida albicans* were exposed to anode EOW (pH, 3.0 ± 0.1; oxidation-reduction potential (ORP), +1100 ± 50 mV; free chlorine, 400 ± 20 mg/l Cl₂) and combined EOW (7 : 3 anode : cathode, v/v; pH, 8.3 ± 0.1; ORP, 930–950 mV; free chlorine, 271 ± 20 mg/l Cl₂).

In water of standardized hardness (WSH), all bacterial strains were completely inactivated by a 30 min exposure to maximum 10.0% anode EOW (40.0 mg/l Cl₂) or 50.0% combined EOW (135.5 mg/l Cl₂). The sensitivity ranking order for anode EOW to the bacterial test strains was *P. mirabilis* > *S. aureus* > *M. avium* ssp. *avium* > *E. faecium* > *P. aeruginosa*. *P. mirabilis* and *S. aureus* decreased to undetectable levels after 5 min of exposure to 7.5% anode EOW (30.0 mg/l Cl₂). *Candida albicans* was completely inactivated by a 5-min exposure to 5.0% anode EOW. Both, anode and combined EOW exhibited no anti-microbial activities in standardized nutrient broth or after addition of 20.0% bovine serum to the WSH. Further research is necessary to evaluate the efficacy of EOW as a disinfectant under operating conditions in animal production facilities.

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