

Endoscope Disinfection Using Acidic Electrolytic Water

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Background and Study Aims: The aim of the present study was to evaluate a new endoscope disinfectant (WM-1) that uses acidic electrolytic water (AM).

Materials and Methods: AEW was produced by electrolysis of a 0.05% NaCl-water mixture, with a redox potential greater than 1000 mV and a pH lower than 2.7. In the first study, an endoscope artificially contaminated with 15 species of bacteria and four strains of viruses was treated using the WM-1. In the second study, endoscopic contamination after clinical use was examined by culture for *Helicobacter pylori* and other bacteria, and by polymerase chain reaction for the *H. pylori* urease gene and hepatitis C virus. The extent of contamination was then examined after exposing the WM-1 to AEW. The safety

of AEW was examined using both in vivo and in vitro studies.

Results: All of the bacteria and viruses were destroyed or inactivated after the instrument had been exposed to AEW. Clinical contamination was detected from the instrument in 19 of 30 endoscopic procedures, whereas no bacteria or viruses were detected after five minutes' exposure to AEW. AEW was found to be nonirritant, nontoxic to cells, and nonmutagenic.

Conclusion: The WM-1 successfully and safely disinfected the endoscopes. With running costs of ¥24 per day (\$0.21 per day), the WM-1 provides an effective and inexpensive alternative to conventional disinfection equipment.

Introduction

Contamination of endoscopes with bacteria and viruses is an important issue [1-16], particularly since transmission *Helicobacter pylori* via endoscopic instruments has been documented [17-21]. After ten minutes' exposure [7], disinfectants such as glutaraldehyde solution can eliminate 85 - 100 % of the bacterial contamination caused by endoscopy [6- 16]. However, the disinfectants currently available are expensive, hazardous, and often require long periods of exposure. Due to patient requests and screening measures, the frequency of endoscopic examinations may become more frequent in areas in which there is a high prevalence of *H. pylori* infection, in order to investigate *H. pylori* associated disorders and to detect the bacterium. Repeated use of endoscopes means that rapid, convenient, and safe disinfection of endoscopic instruments is extremely important; however, equipment meeting these requirements for cleaning and disinfection has so far not been available.

Acidic electrolytic water (AEW) is generated by electrolysis of water that contains 0.05% of NaCl (Figures 1,2). AEW, as well as solutions with similar properties (such as "oxidative potential water", "superoxidized water", etc.), consists of water, excess protons (H^+) and a small amount of chlorine and hypochlorous acid (Cl_2 , $HClO$). AEW has a strong oxidizing ability, with a redox potential greater than 1000 mV and a pH lower than 2.7 [22-25]. Bacteria are unlikely to survive in this solution, since $HClO$ and Cl_2 oxidize cell membranes, inactivate enzymes, and denature the nucleic acids of pathogens. Thus, AEW may be useful as a potent disinfectant. However, if AEW is not continuously supplied with H^+ , $HClO$, and Cl_2 by electrolysis, the solution rapidly loses its oxidative and acidic properties, and it is therefore safe in the environment and does not harm human tissue.

A new apparatus (WM-1, Asahi Denshi Co., Higashiosaka, Japan) for endoscope disinfection using AEW has been developed. The apparatus provides an alternative to the washer/disinfector using glutaraldehyde solution that is currently applied after mechanical or manual washing. With the WM-1, not only is the insertion tube immersed in AEW, but the biopsy channel, and the air insufflation and aspiration channels are also irrigated. The solution is then collected, filtered, electrolyzed again, and recirculated toward the endoscope in the WM-1. Because the WM-1 is continuously supplied with H^+ , Cl_2 and $HClO$ during operation, the AEW in the machine does not lose its oxidizing ability and acidic nature throughout repeated disinfection cycles. Consequently, it is possible to use the WM-1 to destroy pathogens without needing to empty and clean the device after each cycle. On the other hand, the cathode produces a small amount of hydrogen (H_2) and excess hydroxide anions (OH^-), making the solution alkaline. A cation exchange membrane allows movement of cations such as Na^+ but not anions, including OH^- , thus maintaining the acidic nature of the AEW on the anode side of the chamber. The WM-1 allows daily draining of the AEW and alkaline water from each chamber.

The present study investigated the ability of the WM-1 endoscope disinfector to destroy potential pathogens, including *H. pylori* and a range of viruses, using AEW. The safety of AEW was also examined in animal studies in vivo, and in cultured cells and mutagenic assays in vitro

Fig.1

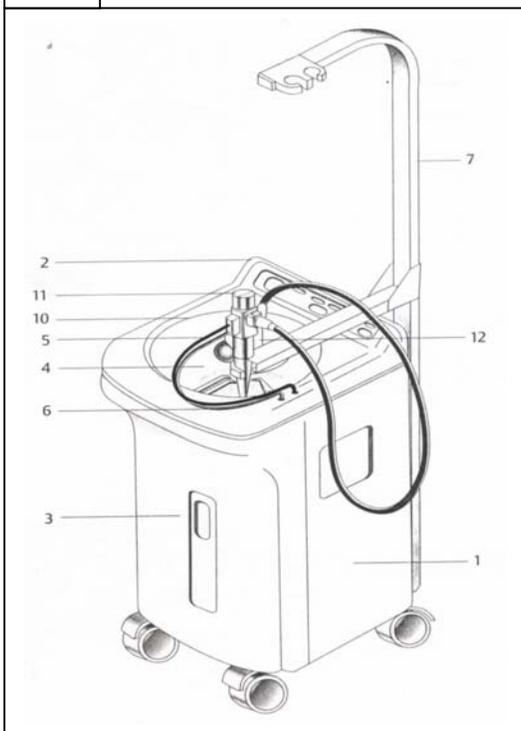


Figure 1 The WM-1 endoscope cleaning and disinfection machine. The device measures 495 mm (length) x 400 mm (width) x 790mm (height), and weighs 66kg. This is the prototype. In the second version, all the parts of the endoscope, including the control box and umbilical section, will be immersed in WM-1 washing machine, including tank for water, generator for acidic electrolytic water (AEW), and pumps for washing

- 2 Control panel
- 3 Water level indicator
- 4 Sink for endoscope
- 5 AEW suction inlet
- 6 AEW air/water channel inlet
- 7 Hanger for the endoscope
- 10 The endoscope
- 11 Switches or eyepiece
- 12 Connector for light source

Fig.2

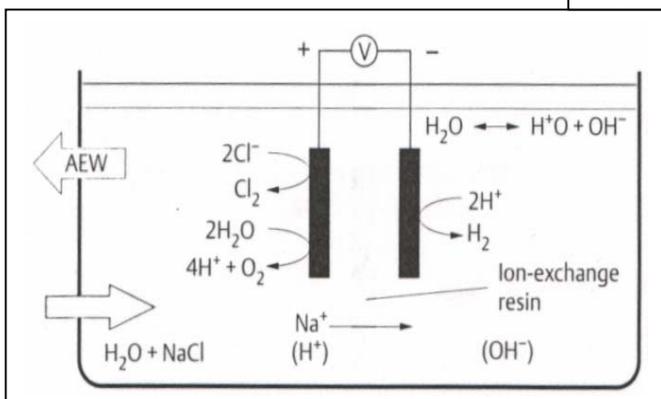


Figure 2 Generating acidic electrolytic water (AEW) in the WM1. The generator consists of a tank, two electrodes for electrolysis of the water, and ion-exchange resin. The electrodes in the WM-1 are made of platinum-coated titanium. The anode of the generator produces Cl_2 , oxygen, and H^+ . Around the anode, Cl_2 reacts with water to produce $HClO$ and HCl . $HClO$ can oxidize organic substances, including bacteria and viruses, while HCl lowers the pH of the solution. The disinfectant effects of the anolyte appear to be due mainly to the oxidative nature of $HClO$; however, the acidic nature of the solution prevents $HClO$ from undergoing rapid degradation. The oxidizing ability (redox potential) of AEW is enhanced by the low pH of the solution [123]. Thus, Cl_2 , $HClO$, and a low pH in the AEW all contribute to the inactivation of pathogens

Materials and Methods

Investigation of the Effects

Effects of the WM-1 on artificial microbial contamination. Methicillin-sensitive *Staphylococcus aureus* (MSSA), *Escherichia coli*, *Pseudomonas cepacia*, methicillin-resistant *Staphylococcus aureus* (MRSA), *Klebsiella pneumoniae*, *Stenotrophomonas maltophilia*, coagulase-negative *Staphylococcus* spp. (CNS), *Serratia marcescens*, *Acinetobacter baumannii*, *Enterococcus faecalis*, and *Pseudomonas aeruginosa* were obtained from clinical isolates and grown on blood agar (Eiken, Tokyo, Japan). *Cryptococcus neoformans*, *Aspergillus fumigatus*, and *Candida albicans* were grown on Sabouraud's agar (Nissui, Tokyo, Japan). *Clostridium difficile* was grown anaerobically on Brucella agar (Kyokuto, Osaka, Japan), and *H. pylori* was grown on Dent's agar (Oxoid-Kanto Chemical, Tokyo, Japan) in a microaerophilic environment. Mixtures of spores and vegetative forms of *Clostridium difficile* and vegetative cells of other bacteria and fungi were suspended in distilled water at densities of 10^7 - 10^8 colony-forming units per milliliter (CFU/ml).

A video endoscope (GIF-Q200, Olympus, Hachioji, Japan) was immersed in the suspension and contaminated with pathogens. The biopsy channel of the endoscope was also contaminated by flushing 1 ml of the suspension through the biopsy port, as the biopsy port is usually exposed to gastric juice, blood, and biopsy tissue. The insertion portion of the endoscope was placed horizontally to disperse bacteria along the entire channel from the biopsy port to the distal tip. Recovery of the pathogens from the endoscope was then examined. In brief, the endoscope was immersed in sterile water, and wiped with sterile gauze, and the biopsy channel was rinsed with 40ml, of sterile saline. Both the gauze and rinse samples were collected into a sterile container and mixed for 30 seconds. A 5-ml aliquot of the rinse was diluted tenfold, 100-fold and 1000-fold, and 100 μ l of the rinse and diluted samples were seeded onto appropriate agar plates. Bacterial contamination of the endoscope was confirmed by growth of the bacterial colonies after 48 hours of incubation (detection limit $> 4 \times 10^2$ CFU/ml).

The endoscope was then contaminated with the pathogens in the same manner, and immersed and washed for seven minutes in the WM-1. The machine not only allows immersion of the insertion tube in AEW, but also irrigates the biopsy channel, the aeration channel, and the aspiration channel of the endoscope with AEW. The endoscope was wiped with sterile gauze, and the biopsy channel was rinsed with 40 ml of sterile saline. Both the gauze and the rinse were collected into a sterile container and mixed for 30 seconds. The AEW in the WM-1 was also analyzed for bacterial contamination. A 5-ml aliquot of the rinse was diluted, 100 μ l of which was seeded onto appropriate agar plates. The bacterial colonies were identified and counted after 48 hours of incubation at 37 °C. When the colonies of fungi could not be isolated and counted, only their growth was recorded.

Adenovirus types 1, 2, and 3, and poliovirus type 3, 10^4 - 10^6 plaque-forming units per milliliter (PFU/ml) were prepared. The endoscope was immersed in the virus suspension, and the biopsy channel was also contaminated. The endoscope was washed in the clean WM-1 generator for seven minutes and then wiped with sterile gauze. The biopsy channel was rinsed with 40 ml of saline. Viral activity in the rinse and gauze, the AEW in the generator, and in the original suspension was measured by cell lysis of the MRC-5 fetal lung cell line, the Hep-2 human cancer cell line, and the MA104 kidney cell-line. In brief, 0.1 ml of the rinse diluents were neutralized with sodium hydroxide to pH 7.0 and seeded onto MRC-5, Hep-2, and MA104 cells that were incubated for two months in Eagle minimum essential medium (Nissui) supplemented with 5% fetal bovine serum and 100U/ml penicillin, 100 μ g/ml streptomycin, 0.5 μ g/ml amphotericin B, and 18 μ g/ml tyrosine. The activity was assessed by the lysis of the target cells.

Effects of WM-1 on microbial contamination acquired during upper gastrointestinal endoscopy. A clean GIFQ200 video endoscope (Olympus, Hachioji, Japan) was used in the endoscopic unit for upper gastrointestinal screening of 30 patients (three patients per day). *H. pylori* infection was examined using a rapid urease test (CLO test, Kokusai Shiyaku Co., Osaka, Japan), histology, culture, and polymerase chain reaction (PCR) against the *H. pylori* urease A gene (*ureA*), as described below. Infection with the hepatitis C virus (HCV) was examined using serum antibody against HCV prior to the endoscopy. None of the patients were infected with hepatitis B virus. After endoscopic examination, the endoscope was briefly wiped with sterile gauze, and the forceps and aspiration channels were rinsed with 40ml of saline. The gauze and saline were collected into a sterile container. A 10-ml aliquot of saline was transferred into a sterile tube and stored at 4 °C for assessment of bacterial contamination. The samples were diluted to 10¹-10⁶ with sterile saline, plated onto a blood agar plate (0.1 ml/plate), and incubated at 37 °C for 48 hours. Another aliquot was frozen at -20 °C for PCR for *H. pylori ureA* [26] and RT-PCR for HCV RNA [27]. The samples were also seeded onto Dent's agar, and stored in a microaerophilic bag (Campy-pouch, Beckton-Dickinson, Cockeysville, Maryland, USA). The endoscope was then soaked in AEW for five minutes. Thereafter, the endoscope was wiped with gauze, and the forceps channel was rinsed with 40 ml saline. The gauze and the saline were centrifuged and used for a second microbiological assessment.

Contamination of the endoscope with blood was examined using Haemastix (Bayer-Sankyo, Tokyo, Japan). The sensitivity of the kit was 0.015 mg hemoglobin per 100 ml of the sample.

The redox potential of the AEW was monitored using an Ag/AgCl electrode, and the pH was monitored using a glass electrode (HM-14, Toa, Tokyo, Japan) before and after each washing and disinfection cycle in the WM-1.

Investigation of Possible Side Effects

For patients (animals, cytotoxicity, and mutagenicity). Fifteen male Japanese white rabbits were used for the primary irritation test for skin and eyes using the methods described by Draize et al. [28], with minor modification. In brief, four observation areas were made by shaving the dorsal skin in each of six animals. Two of the areas were damaged by scratching the epidermis. The damaged and intact areas were exposed to sterile gauze containing either AEW (0.5 ml) or the same volume of sterile water as a control for four hours in six rabbits. The sites were assessed for irritation four, 24 and 48 hours after exposure to the AEW. To test eye irritation, one of the eyes was exposed to 0.1 ml of AEW in each of the nine remaining animals. In three animals, both eyes were rinsed with 300 ml of sterile water 30 seconds after exposure to AEW. The eyes of the remaining six animals remained untreated for 24 hours. The opacity of the cornea, edema, responses of the pupils to light, and reddishness and edema in the conjunctivae were assessed one, 24, 48, and 72 hours, and four and seven days after exposure to AEW or sterile water.

AEW was administered to male and female ICR strain mice at a dose of 50 ml/kg through orogastric tubes after four hours of fasting. As a control, distilled water was given to some animals. The condition of the animals was checked every day for two weeks. Their weight was also checked before and one and two weeks after orogastric administration of AEW. The mice were then sacrificed, and the organs examined. These animal studies were approved by the institution's ethics committee.

The effects of AEW on the cells were assessed with a plaque-forming assay using V79 Chinese hamster fibroblasts (Riken Cell Bank, Tsukuba, Japan). In brief, the AEW was filtered, mixed with concentrated Eagle's minimum essential medium with Earle's salt, and supplemented with 1 mM pyruvate and 5 % fetal bovine serum (M05). The cells were trypsinized and seeded onto 35-mm dishes. After six hours of preincubation, V79 cells were incubated for seven days in 0%, 25%, and 50% mixtures of AEW in M05 medium. Zinc dibutyldithiocarbamate and zinc diethylthiocarbamate were used as positive controls.

The mutagenicity of the AEW was examined using a modified Ames test with *Salmonella typhimurium* TA100, TA98, TA1535, TA 1537 strains, and the *E. coli* WP2 uvrA. strain. In brief, 100, 200, 300, 400, and 500 μ l of AEW were mixed with or without a postmitochondrial fraction mixture prepared from rat liver that potentiates carcinogen (S9 mix, Kikkoman, Chiba, Japan). The bacteria were preincubated with the mixture for 20 minutes and mixed with top agar, which consists of Bacto agar (Difco, Detroit, Michigan, USA), NaCl and 0.05 mM L-histidine and D-biotin for *S. typhimurium*, or 0.05 mM L-tryptophan for *E. coli*. The bacteria were seeded onto minimum glucose agar and back-mutated colonies were counted after 48 hours (incubation). A mutagen was considered to produce mutant colonies in a dose-dependent manner of more than twice the number of colonies as the negative control (distilled water). Mutagens, 9-aminoacridine (9-AA), N-ethyl-N-nitro-N-nitrosoguanidine (ENNG), 2-(2-furyl)-3-(5-nitro-2-furyl)acrylamide (AF-2), and 2-aminoanthracene (2-AA) were used as positive controls.

Effects of WM-1 on endoscopes. During the above in vitro and in vivo studies, the macroscopic appearance of the endoscope was examined after each use. A fiberoptic from Fujinon was connected to the WM-1 device, disinfected in AEW for seven minutes, and left in the machine for 30 minutes. This cycle was repeated ten times a day for eight days. The functioning and gross appearance of the endoscope were checked every day.

Results:

Investigation of the Effects

Effects of the WM-1 on artificial microbial contamination. *MSSA*, *Escherichia coli*, *Pseudomonas cepacia*, *MRSA*, *Klebsiella pneumoniae*, *Stenotrophomonas maltophilia*, *CNS*, *Serratia marcescens*, *Acinetobacter baumannii*, *Enterococcus faecalis*, and *P. aeruginosa* grew on blood agar from the samples from the endoscope that was artificially inoculated with the bacteria and rinsed with untreated water. *Cryptococcus neoformans*, *Aspergillus flavus*, *Candida albicans*, and *H. pylori* were also recovered from the endoscope rinsed with untreated water. None of the bacteria or fungi grew from the endoscope after treatment with AEW, or from the solution in the WM-1 (Table 1). These results were not due to dilution of the sample by water.

The samples taken from the endoscope that was artificially contaminated with the viruses but not processed in the WM-1 maintained lytic activities in cells. Recovery from the untreated endoscope was 1.0×10^1 in adenovirus type 1 and type 2, 3.2×10^1 in adenovirus type 3, and 3.2×10^1 in poliovirus type 3. All of the viruses, however, were completely inactivated after exposure to AEW in the WM-1. Simple dilution of the viruses with distilled water did not inactivate the viruses (data not shown).

Effects of WM-1 on microbial contamination acquired during upper gastrointestinal endoscopy. Seven bacterial species grew from samples from the endoscope immediately after 19 of 30 examinations (63.3%, Table 2). It should be noted that all of the bacteria concerned commonly exist within the oral cavity. Some of them are pathogenic, including *Staphylococcus aureus*, which can cause opportunistic infection and food poisoning. None of the bacteria were detected from the endoscope after exposure to the AEW.

Twenty-three of 30 patients (76.7%) were infected with *H. pylori*. The bacteria did not grow on Dent's agar from the endoscope before or after exposure of the endoscope to the AEW. PCR revealed that the endoscope was contaminated with the *H. pylori* genome immediately after 16 of 23 examinations in the *H. pylori*-positive human subjects (69.6%). After the endoscope was exposed to the AEW, PCR did not detect the *H. pylori ureA* gene (Table 3).

Of the 30 patients who underwent endoscopy, five were seropositive for the HCV antibody (16.7%). HCV RNA was not detected from the endoscope immediately after the endoscopic examinations, although occult blood was detected in two of the five HCV-positive cases. Blood was also detected in 14 of the 30

cases before the endoscope was exposed to the AEW After washing in AEW in the WM-1, hemoglobin was not detected in the endoscope.

Table 1: Disinfection of an artificially inoculated endoscope using acidic electrolytic water (AEW) in the WM-1 device. The numbers of bacteria are shown as colony-forming units (CFUs). Recovery of *Aspergillus fumigatus* is expressed as "grown," since colonies of the fungus could not be isolated. The detection limit of the culture ($> 4 \times 10^2$ CFU/ml) indicates a $> 5 \log^{10}$ reduction in several pathogens, including *Cryptococcus neoformans*, *Serratia marcescens*, and *Helicobacter pylori*.

Bacteria examined	Agar used	Recovery from untreated endoscope	Recovery after treatment	
			Endoscope	Cleantop
MSSA	Blood agar	2.0×10^7	n.d.	n.d.
<i>Escherichia coli</i>	Blood agar	3.5×10^7	n.d.	n.d.
<i>Pseudomonas cepacia</i>	Blood agar	1.3×10^7	n.d.	n.d.
<i>Cryptococcus neoformans</i>	Sabouraud's agar	4.1×10^7	n.d.	n.d.
MRSA	Blood agar	3.9×10^7	n.d.	n.d.
<i>Klebsiella pneumoniae</i>	Blood agar	3.5×10^7	n.d.	n.d.
<i>Stenotrophomonas maltophilia</i>	Blood agar	3.3×10^7	n.d.	n.d.
<i>Clostridium difficile</i>	Brucella agar	2.0×10^7	n.d.	n.d.
CNS	Blood agar	1.8×10^7	n.d.	n.d.
<i>Serratia marcescens</i>	Blood agar	5.0×10^7	n.d.	n.d.
<i>Acinetobacter baumannii</i>	Blood agar	4.5×10^7	n.d.	n.d.
<i>Aspergillus fumigatus</i>	Sabouraud's agar	Grown	n.d.	n.d.
<i>Enterococcus faecalis</i>	Blood agar	1.7×10^7	n.d.	n.d.
<i>Pseudomonas aeruginosa</i>	Blood agar	3.5×10^7	n.d.	n.d.
<i>Candida Albicans</i>	Sabouraud's agar	5.0×10^7	n.d.	n.d.
<i>Helicobacter pylori</i>	Dent's agar	9.3×10^7	n.d.	n.d.

n.d.: not detected. CNS: coagulase-negative *Staphylococcus* spp.; MRSA: methicillin-resistant *Staphylococcus aureus*; MSSA: methicillin-sensitive *Staphylococcus aureus*.

Table 2 : Bacterial contamination in an upper gastrointestinal endoscope before and after treatment in the WM-1. Data are shown as the numbers of positive cases per total number of cases, and the number of colonies grown from the sample. Bacterial titers after the application of acidic electrolytic water (AEW) are omitted, since none of the bacteria were detected after five minutes of rinsing and immersion in the WM-1. *Helicobacter pylori* was not cultured or isolated from any samples

Bacteria isolated	Before treatment (positive cases)	Bacterial titre (CFU/ml)	After treatment (positive cases)
<i>Neisseria</i> spp	12/30	$20 - 4.0 \times 10^3$	0/30
<i>Streptococcus</i> spp	16/30	$90 - 1.5 \times 10^4$	0/30
<i>Staphylococcus</i> spp	2/30	$20 - 2.3 \times 10^2$	0/30
<i>Staphylococcus aureus</i>	1/30	40	0/30
<i>Staphylococcus epidermis</i>	1/30	10	0/30
<i>Corynebacterium</i> spp	3/30	$70 - 2.0 \times 10^3$	0/30
<i>Micrococcus</i> spp	1/30	1.2×10^2	0/30

Table3 Endoscopic contamination with *Helicobacter pylori*, as assessed by bacterial culture and polymerase chain reaction (PCR) for the *H. pylori* ureA gene, hepatitis C virus (HCV) RNA, and blood after endoscopic examination. *H. pylori* contamination was examined in 23 *H. pylori*-infected individuals. HCV RNA was examined in samples from the instrument after endoscopy in HCV-seropositive individuals (n = 5). Occult blood was tested in 30 cases.

	Before treatment	After treatment
<i>H. pylori</i> (culture)	0/23	0/23
<i>H. pylori</i> ureA gene (PCR)	16/23	0/23
HCV RNA (RT- PCR)	0/5	0/5
Occult blood	14/30	0/30

AEW in the WM-1 maintained a redox potential greater than 1000mV and a pH lower than 2.7. The WM-1 was therefore able to maintain the oxidative and acidic property of AEW even after the endoscope was cleaned immediately after clinical use.

Possible Side Effects

For patients (animals, cytotoxicity, and mutagenicity)

None of the skin areas in the rabbits were erythematous or edematous four, 24, and 48 hours after exposure to the AEW. The cornea, pupils, and conjunctivae showed no signs of irritation one, 24, 48, and 72 hours, and four and seven days after exposure to the AEW. AEW was therefore classified as a nonirritant.

The mice tolerated 50 ml/kg of the AEW as well as the same volume of distilled water. There were no significant differences in the weight of the animals throughout the experiment. There were no observable abnormalities in stomach, intestine, lung, heart, kidneys, and muscles two weeks after AEW administration.

AEW did not inhibit V79 cells from forming colonies, whereas zinc dibutyldithiocarbamate at (1.96 µg/ml) and zinc diethyldithiocarbamate (0.007 µg/ml) inhibited colony formation by 50%.

Distilled water, the negative control, did not induce formation of the back-mutated colonies. AF-2, ENNG, and 2-AA induced base transitions in *Salmonella typhimurium* TA100, TA1535 or *E. coli* WP uvrA. AF-2, 9-AA, and 2-AA induced frame-shift mutations in *S. typhimurium* TA98 and TA1537. AEW, however, did not induce mutation in either the *S. typhimurium* strains or the *E. coli*.

For endoscopes. The macroscopic appearance of the Olympus endoscope did not change following 30 cycles of disinfection with AEW. Neither adhesive substances nor stains were observed on the surface of the endoscope. No faults occurred with the endoscope during the trial. Neither the appearance nor the functioning of the Fujinon fiberscope changed during repeated applications of AEW (ten times a day for eight days).

Discussion:

Gastrointestinal endoscopes are highly contaminated with microorganisms, some of which are pathogenic if transferred to a susceptible host. These results demonstrate that over 60% of upper gastrointestinal endoscopes are contaminated with bacteria immediately after use. Although it has been reported to occur infrequently [9], endoscopy-related transmission of infection must be eliminated. In particular, *H. pylori* can be transmitted via endoscopes, and causes acute gastritis with achlorhydria, duodenal ulcer recurrence, chronic gastritis, and gastric malignancies [17-21]. In this study, we found an intact *ureA* genome of *H. pylori* in nearly 70 % of the instruments after examination of *H. pylori*-infected subjects. Although positive results in PCR do not necessarily mean that a viable organism exists, it does indicate the existence of an intact organism and suggests a potential for bacterial contamination. Since HCV RNA was not detected from the endoscopes after endoscopy in six HCV-positive patients, the risk for transmission of HCV through endoscopes appears to be low even before exposure to the AEW. Occult blood tests frequently showed contamination of the instruments with blood, however, before the endoscope was processed in the WM-1.

Methods such as manual washing and suction of a disinfectant with the endoscope have been in use during repeated endoscopic examinations, and are carried out after each examination. These methods, however, are reportedly ineffective in eliminating various pathogens. Electrolysis is also an effective means of disinfecting water [25]. AEW has a strong oxidizing ability and has excess H^+ , Cl_2 and $HClO$, all of which are potent factors for disinfection. Okubo et al. [23] have reported that bacteria do not survive in an environment with a redox potential greater than 900 mV and a pH lower than 3. The ability of AEW to achieve general disinfection was also reported by Iwasaki et al. [22] and Masuda et al. [24]. The present study investigated the usefulness of an AEW generator for disinfecting endoscopes.

High-level disinfection should normally be carried out after thorough cleaning to remove microorganisms and organic matter, followed by chemical disinfection. In this study, the authors used the WM-1 without any prior cleaning procedures, in order to assess the equipment's ability to achieve

disinfection. Despite the lack of prior cleaning, all of the examined bacteria, including the spore form of *Clostridium*, were destroyed after five minutes' immersion in AEW. AEW also successfully inactivated several viruses after artificial application. No clinical isolates were recovered from the upper gastrointestinal endoscopes after five minutes' immersion in AEW. The present data indicate that the machine successfully disinfects endoscopic instruments, even without prior cleaning. The results also suggest that contamination of the endoscope with the intact *H. pylori* genome and blood was unlikely after treatment in the WM-1. In the present study, however, we were unable to examine all possible pathogens, and those not studied include mycobacteria and the hepatitis B virus. For example, Birnie et al. [29] reported a case of type B viral hepatitis acquired during endoscopy from an instrument processed in a conventional manner, although the risk for endoscopic transmission appears to be low [30,31]. Mycobacteria such as *M. terrae* should also be used as a test organism in further studies, to investigate the ability of the apparatus to achieve high-level disinfection of bronchoscopes.

The present study also showed that AEW does not irritate skin and eyes. Mice tolerated the solution for two weeks. In vitro studies also indicate that the AEW generated by the WM-1 is neither toxic to cultured cells nor mutagenic. AEW thus appears to be safe for both patients and endoscopists, and it does not damage endoscopes. By contrast, conventional disinfectants such as glutaraldehyde solution can cause irritation in the eyes and respiratory system if the disinfectant is used inappropriately [81]. Furthermore, high-level disinfection with glutaraldehyde solution requires an exposure period of more than 30 minutes [14]. Although ten minutes of exposure in 2% glutaraldehyde is recommended for reprocessing gastrointestinal endoscopes, most international working parties recommend 310 hours for complete sterilization. Detergents with disinfectant properties, such as hyamine, have been used after routine endoscopy [141], but are reportedly ineffective in eliminating *H. pylori* [201]. AEW is therefore a very promising means of disinfecting gastrointestinal endoscopes.

In this study, the performance of the WM-1 and AEW was not directly compared with that of a conventional endoscope disinfectant such as the EW-30 (Olympus). Disinfection is defined as at least a 5 log₁₀ reduction in test organisms in vitro using a prescribed test method; 2-3.5% glutaraldehyde solution satisfies this criterion, and is therefore an effective disinfectant. However, glutaraldehyde solution requires long exposure periods for disinfection, and it also irritates human tissue. An ordinary washing and disinfecting machine consumes 550-1200 Wh of electricity (costing ¥8.65 per kWh in Osaka, Japan), 22L of glutaraldehyde solution that is reusable for two or four weeks after activation (¥28950 yen every four weeks), detergent (¥121 every four weeks) and a large amount of tap water (12 L/ min, ¥250 per kL in Osaka) to rinse out the instrument and prevent risks from the disinfectant. We estimate that an endoscopic unit performing ten endoscopic procedures every eight hours per day, five days a week, in Osaka, Japan, consumes 840 L of water (¥210 per day) and 4000 Wh of electricity (¥74.6 per day). It therefore costs ¥1738.2 per day (\$14.73 per day) to run the disinfection machine, including the disinfectant (¥1447.5 per day) and the detergent (¥6.1 per day). The endoscope, and its forceps channels in particular, need to be cleaned with a brush before disinfection, so that staff costs in the endoscopic unit are also involved. By contrast, the WM-1 consumes only 10L of tap water (¥2.5 in Osaka, Japan), 5 g of NaCl (¥6.7) and 17-550Wh of electricity (i.e., 801 Wh of electricity in the same endoscopic unit in the above example; ¥15) per day. Mechanical cleaning and brushing of the endoscopic channels needs to be carried out separately prior to the disinfection with the WM-1, as well as with the ordinary endoscope washer and disinfectant. Therefore, under these conditions, running this machine costs ¥24.2 per day (\$0.21 per day). Although the WM-1 is a prototype disinfecting machine using AEW and is not yet commercially available we estimate that the cost of a commercial version will be \$10000-12000 US dollars. This is less expensive than a conventional endoscope washing machine (approximately \$20000 and \$15000 dollars for the Olympus OER and EW-30, respectively). A conventional washing machine for endoscopes requires a permanent connection for water intake and drainage. Consequently, it has to permanently occupy a space (0.5 m² in the case of the Olympus OER and EW-30) in the endoscopic unit near a faucet. On the other hand, AEW does not irritate tissues, damage cells, or cause mutation, and the device therefore does not need to be rinsed out thoroughly with water after each disinfection cycle. The WM-1 occupies less than 0.2 m² of floor space, does not require a

permanent connection for water intake and drainage, and can be removed after use. Cleaning and disinfection with AEW using the WM-1 device thus appears to be an effective, space-saving, and economical alternative to conventional disinfectants, at least after upper gastrointestinal endoscopy. The full disinfecting potential of the apparatus and its long-term compatibility for endoscopes remain to be examined.

Conclusion:

In upper gastrointestinal endoscopes, which have a high rate of contamination with bacteria and *H. pylori* DNA, this study demonstrated that the WM-1, a novel endoscope washing and disinfecting apparatus, eliminates various bacteria and viruses after five minutes. This method addresses many of the problems with the current approaches to endoscope processing, such as the toxicity of liquid chemical germicides, long exposure times, and the high costs associated with their use.

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