Digestive Endoscopy (2003) **15**, 19–24

ORIGINAL ARTICLE

ENDOSCOPE CONTAMINATION FROM HBV- AND HCV-POSITIVE PATIENTS AND EVALUATION OF A CLEANING/DISINFECTING METHOD USING STRONGLY ACIDIC ELECTROLYZED WATER

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Background: It is well known that strongly acidic electrolyzed water (SAEW) has a potent bactericidal effect. We examined residual viruses on endoscopes that were used in hepatitis B virus (HBV)-positive and hepatitis C virus (HCV)-positive patients and evaluated the effectiveness of SAEW in cleaning/disinfecting the endoscopes.
Methods: A random sample of endoscopes used in 109 endoscopies on HBV-positive patients and 107 endoscopies on HCV-positive patients, who underwent upper gastrointestinal endoscopy for various reasons was taken to determine the degree of HBV and HCV contamination. Samples were taken using 10 mL of physiological saline injected through the forceps channel of each endoscope and collected at the distal end to be assayed using polymerase chain reaction (PCR). After examination, each endoscope was treated with air aspiration, then 200 mL of tap water that contained an enzyme detergent was absorbed, and SAEW was aspirated after cleaning with a brush. After each procedure, PCR was used for comparison and to identify any residual viruses.
Results: In saline collected after air aspiration, viruses were detected in 39/109 endoscopes used in HBV patients and in 20/107 endoscopes used in HCV patients. In the saline aspirated with tap water containing an enzyme detergent, HBV was detected in 12/109 endoscopes and HCV was detected in 6/107 endoscopes. However, neither HBV nor HCV was detected after the endoscopes were cleaned manually with a brush and disinfected with SAEW.
Conclusion: Endoscopes contaminated with HBV and HCV are effectively cleaned and disinfected by SAEW.

Key words: disinfection, endoscope, hepatitis B virus, hepatitis C virus.

INTRODUCTION

The rapid increase in the application of digestive endoscopy has brought about the re-use of endoscopes in different patients within a short period of time in many endoscopy centers. Under such circumstances, the cleaning and disinfecting of endoscopes must be carried out with meticulous care, and many guidelines have been recommended.[1,2] However, there has been one surprising report of trans-endoscope infection, even after glutaraldehyde (GA) was used in an automatic endoscope reprocessor.[3] This report was significant in that it made clear that GA is not a perfect means of disinfecting, and that manual cleaning of the endoscope is important prior to the use of a disinfectant. The reason that the guidelines for cleaning and disinfecting endoscopes are not being followed strictly is that GA is time-consuming and troublesome for the processing of enough endoscopes to complete the required number of examinations. Furthermore, the use of GA is gradually being limited because of the adverse effects this chemical has on the environment.[4]

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Received 8 April 2002; accepted 2 September 2002.

Strongly acidic electrolyzed water (SAEW) has a potent bactericidal effect,[5] rapidly killing bacteria within a few seconds. The main sterilizing effect of SAEW has been identified recently as due to the hydroxy radicals contained in SAEW. The highest quantity of hydroxy radicals is found at the strongly acidic level of pH 2.7, at which hydroxy radicals have a quick, potent sterilizing effect in the presence of chlorine and oxidation reduction potential.[6] Although SAEW has a strong effect on HBV and HCV, there have been few studies into disinfecting clinically contaminated endoscopes using SAEW.

MATERIALS AND METHODS

Contamination was examined on upper gastrointestinal endoscopes used in patients who had been positive for viremia prior to endoscopy between December 1997 and December 1999. Endoscopes used in 109 endoscopies in 93 HBV-antibody-positive patients and in 107 endoscopies on 93 HCV-antibody-positive patients were randomly chosen and examined. Immediately after the examination, the endoscopes were air aspirated, and 10 mL of physiological saline was injected through the biopsy channel and collected at the distal end. Then, 200 mL of tap water with 10 mL of enzyme detergent (Endozyme; Ruhof, USA) was aspirated from the tip of the endoscope. After this, the above proce-

dure was repeated. Next, the endsocope was cleaned manually using a brush, as recommended by the Japan Gastroenterological Endoscopy Technicians Society.[7] The air and water buttons and the forceps cap were removed from the scope body, which was then immersed in SAEW for 10 s after the manual cleaning, and the endoscope was also immersed in a SAEW tub for 10 s. The endoscope was then connected to a light source, the buttons and the forceps cap were reattached, and 50 mL of SAEW was aspirated from the distal end of the endoscope. After air aspiration, 10 mL of physiological saline was injected into the biopsy channel and collected to determine HBV and HCV contamination using polymerase chain reaction (PCR). The collected liquid was preserved separately at a temperature of –80°C until an assay could be performed. The samples were placed in random order after each cleaning step, so as to prevent any possibility of prediction.

For HBV assay, DNA was obtained from each step of saline collection using the ISOQICK kit (ORCA Research, Bothel, WA, USA). The DNA pellet was dissolved in 20 μL of EDTA. The DNA was amplified in the S region of HBV, which has a high homology between various subtypes. The primer set used to detect HBV has been described previously.[8]

The following protocol was used to carry out PCR. Taq polymerase 0.5 U (Promega, Madison, WI, USA) and anti-Taq antibody 0.5 U (Clontech, Palo Alto, CA, USA) were mixed at room temperature for 5 min. Then, 3 μL of DNA was used in a total volume of 20 μL containing Taq reaction buffer, 0.2 mmol/L dNTP, 1.5 mmol/L $MgCl_2$, 20 pmol of each primer, and 0.5 U Taq DNA polymerase added to 0.5 U anti-Taq antibody. The PCR cycle was performed in a Gene Amp PCR system 9600 (Perkin Elmer; Applied Biosystems, Fostercity, CA, USA) with the following temperature profile: denaturation at 95°C, primer annealing at 55°C, and primer extension at 72°C, each for 30 s. After an initial denaturation (95°C for 105 s), the cycle was repeated 35 times, followed by a final extension step of 7 min at 72°C. A second PCR was then performed on 3 μL of the product of the first PCR, then the product of the second PCR was electrophoresed on a 2% NuSieve 3:1 (FMC BioProduct, Rocklond, ME, USA) agarose gel, stained with ethidium bromide at 0.5 μg/mL, and visualized by UV transillumination. The size of the PCR product was estimated from the relative migration of a 100-bp ladder (Gibco BRL; Invitrogen, Carlsbad, CA, USA).

For the HCV assay, the total RNA in each 200 μL of saline was isolated by a modified acid guanidine thiocyanate phenol chloroform method using ISOGEN (NIPPON Gene, Tokyo, Japan). Briefly, 200 μL of saline was directly lyzed in denaturing solution, followed by chloroform extraction. The lysate was isopropanol precipitated. The resulting RNA pellet was rinsed in 70% ethanol, dried and finally dissolved in 20 μL of 0.1% diethylpyrocarbonate water.

For HCV detection, a reverse transcription nested PCR was performed using the following method: 5 μL of extracted total RNA was incubated at 70°C and chilled on ice. Then cDNA was synthesized for 120 min at 42°C with 50 U Molony murine leukemia virus transcriptase (Gibco BRL), 5 mmol/L random primer, 2.5 μmol/L oligo (dT)16, RNase inhibitor (Promega, Roche Diagnostics) at 1 U/μL, dATP, dTTP, dCTP and dGTP (Roche Diagnostics) each at 1 mmol/L and Taq reaction buffer (10 mmol/L Tris pH 9.0 50mmol/L KCl 0.1% v/v Triton X-100; Promega) supplemented with 5 mmol/L $MgCl_2$ in a total volume of 20 μL. After compression, the mixture was heated for 6 min at 95°C and diluted 20 μL with Taq buffer. For the first PCR amplification, 5 μL of this product was used in a total volume of 20 μL using the same temperature profile as was used in the HBV PCR. The second PCR was carried out using 3 μL of the product of the first PCR. The primer set used has been described previously.[9]

For the preliminary study, 200 μL of hepatitis B surface antigen (HBsAg)-positive sera and HCV-positive sera were exposed to 800 μL of saline, enzyme detergent and SAEW for 3 min at room temperature. Then the DNA or RNA was extracted from 200 μL of each sample.

The SAEW used in the present study was produced by a SAEW generator, Olympus ESW-45 (Olympus). The SAEW had a pH of 2.7, an oxidation-reduction potential of 1100 mV and a residual chlorine concentration of 50 p.p.m. The endoscopes used were the Olympus Videoendoscope GIF XQ-200 (Olympus).

RESULTS

Patient characteristics

Patients characteristics are shown in Table 1. The purpose of performing the endoscopies varied from a routine endoscopy to invasive treatment. Nine (8.3%) of the HBV-positive endoscopes were used for endoscopic injection sclerotherapy or endoscopic variceal ligation, as were four (3.7%) of the HCV-positive endoscopes. Three each of the of the HBV- and HCV-positive endoscopes were used in the treatment of bleeding gastric ulcers in HBV- and HCV-positive patients (2.75% and 2.80%, respectively); 28 endoscopes were used in biopsies on HBV-positive patients (25.7%), and 24 endoscopes were used in biopsies on HCV-positive patients (22.4%).

Polymerase chain reaction assays for hepatitis B virus and hepatitis C virus and the effect of strongly acidic electrolyzed water

DNA was extracted from 100 μL of blood from an HBsAg-positive patient to determine the detection limit of the assay

Table 1. Patient characteristics

	HBV-positive ($n = 93$)	HCV-positive ($n = 93$)
Age range (years)	22–85	22–89
Mean age (years)	52.0	61.4
Male : female	71 : 22	61 : 32
Reason for endoscopy		
Routine observation	69 (74.2%)	76 (81.7%)
Biopsy	28 (30.1%)	24 (25.8%)
Bleeding ulcer	3 (3.2%)	3 (3.2%)
Variceal treatment	9 (9.7%)	4 (4.3%)

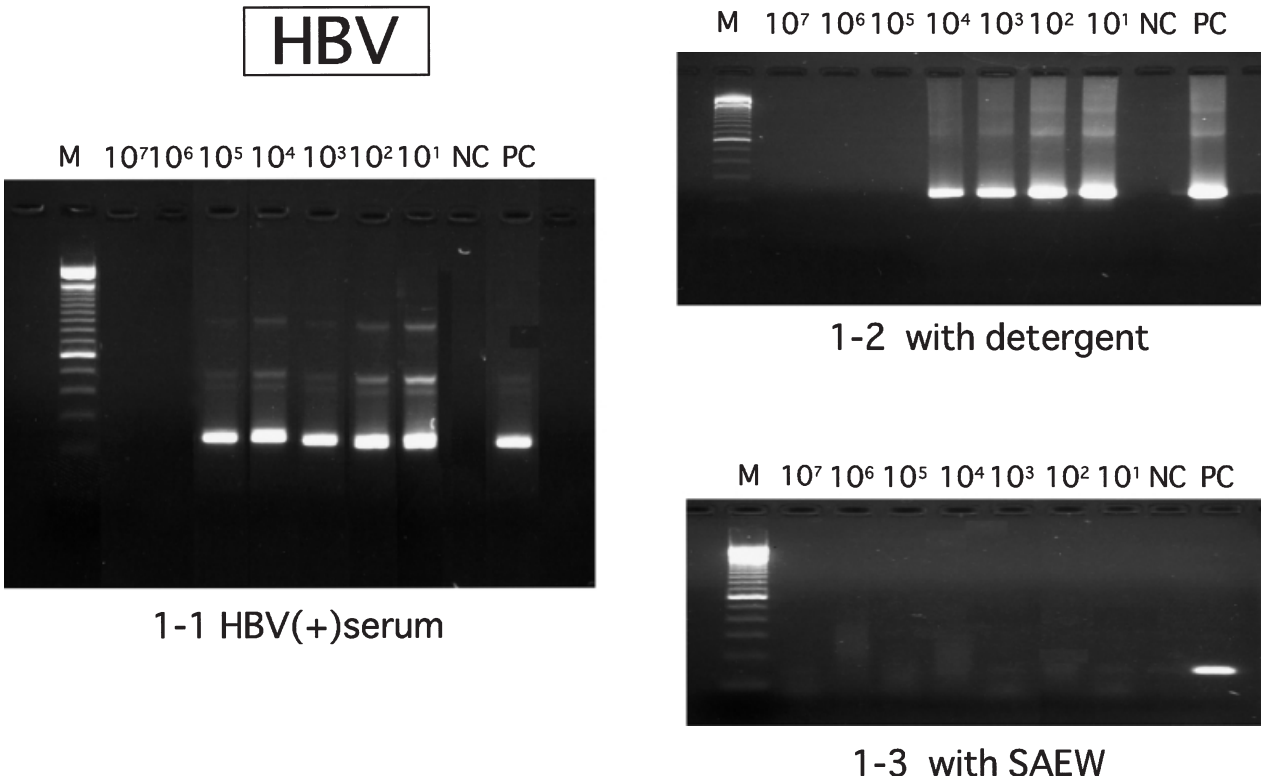

Fig. 1. Hepatitis B virus (HBV) polymerase chain reaction. (a) Detection limit of HBV, detectable in up to a 10–5 dilution. (b) Effect of detergent, with added detergent, virus detectability is kept at 10–4. (c) With strongly acidic electrolyzed water added, no HBV DNA can be detected. PC, positive control; NC, negative control; M, DNA marker.

method used. In the detection of HBV, the HBV-specific well-preserved S-range was amplified to obtain a PCR product of 233 bp. As shown in Fig. 1a, HBV was detected in up to a 10^{-5} dilution of HBV serum. After the addition of 900 µL of detergent, the detection limit was maintained at a 10^{-3} dilution (Fig. 1b). However, when 100 µL of HBV serum came into contact with 900 µL of SAEW, no HBV DNA was detected, even in the original solution (Fig. 1c).

As shown in Fig. 2a, HCV was detected in up to a 10^{-6} dilution. The detection limit when the serum came into contact with the detergent was a 10^{-3} dilution (Fig. 2b). However, when 100 µL of HCV serum came into contact with 900 µL of SAEW, no HCV RNA was detected, even in the original concentration (Fig. 2c).

Frequency of residual hepatitis B virus DNA and the effects of cleaning and disinfecting

Examinations of HBV-contaminated endoscopes after air aspiration showed residual HBV DNA on 39/109 (35.7%; Fig. 3). After rinsing with detergent containing tap water, HBV DNA was still detected on 12/109 endoscopes (11.0%). However, no HBV DNA was detected when the endoscopes were immersed in SAEW for 10 s after being cleaned manually and after 50 mL of SAEW was aspirated from the tip of the endoscope.

There were 40 endoscopes showing obvious blood contamination and of these, 28 were used in biopsies, nine in the treatment of bleeding from esophageal varices, and three in endoscopic hemostasis for bleeding peptic ulcers. Thirteen of the endoscopes used in biopsies and five used in the treatment of massive bleeding were HBV-positive after being air aspirated, and 13 of these were still HBV-positive after cleaning with detergent containing tap water. Out of the endoscopes used in routine endoscopy, not including biopsies, 14 were positive after air drying and seven were still positive after cleaning with tap water. When SAEW was used after the endoscopes were cleaned manually, all of those that had been HBV-positive were found to be HBV-negative. The remaining 98 endoscopes were also HBV-negative.

Frequency of residual hepatitis C virus RNA and the effects of cleaning and disinfecting

After air aspiration, HCV was detected on 20/107 endoscopes (18.7%). After rinsing with tap water that contained an enzyme detergent, six (5.6%) were found to be HCV-RNA-positive and the remaining 101 were found to be negative. No HCV RNA was detected after the endoscopes were cleaned manually and immersed in SAEW (Fig. 4). There were 31 endoscopes showing obvious blood contamination and of these, 24 were used in biopsies, four in the treatment of esophageal varices, and three in the treatment of bleeding from gastric ulcers. Out of 31 endoscopes, seven (20%) were HCV-positive after being air aspirated and two of them (5.7%) were still positive after being rinsed with tap water. Out of the 76 endoscopes used in routine endoscopy, 13 (17.8%) were HCV-positive after being air aspiration and

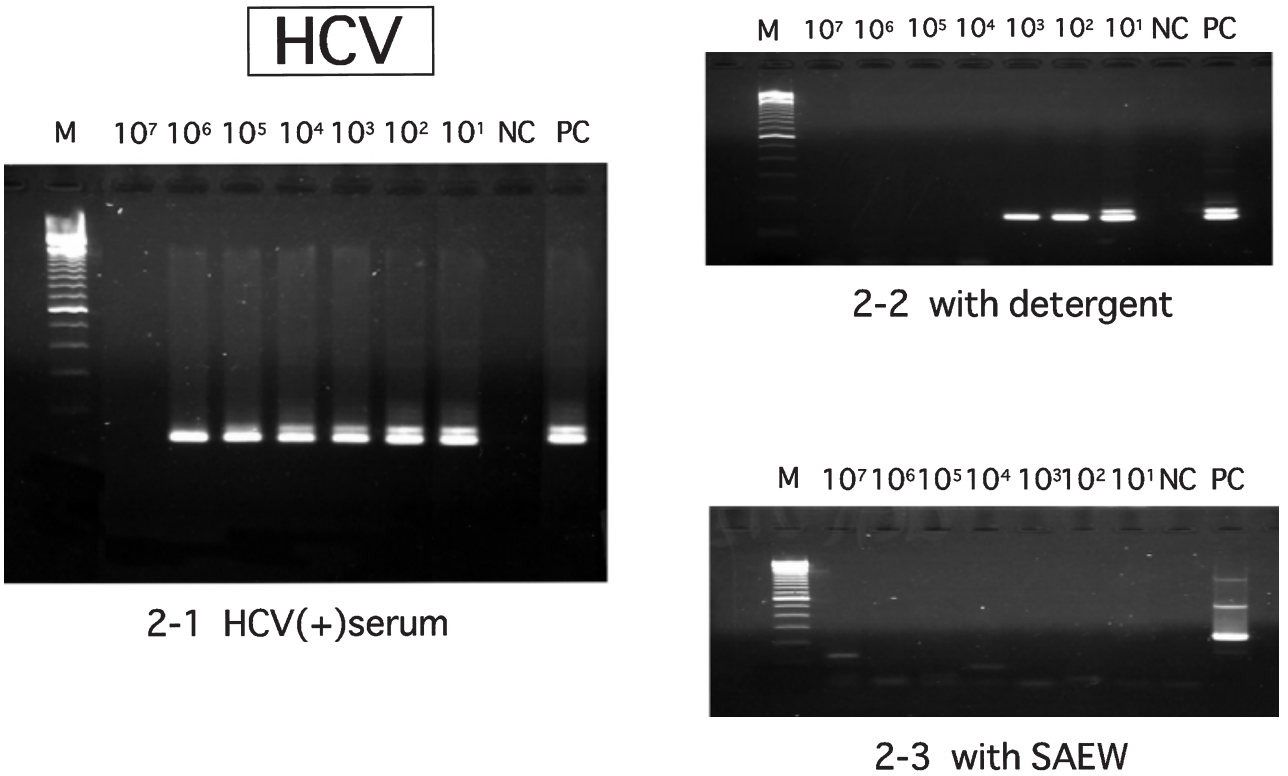

Fig. 2. Hepatitis C virus (HCV) polymerase chain reaction. (a) Detection limit of HCV, detectable in up to a 10–6 dilution. (b) Effect of detergent, with added detergent, virus detectability is kept at 10–5. (c) With strongly acidic electrolyzed water added, no HCV RNA can be detected. PC, positive control; NC, negative control; M, RNA marker.

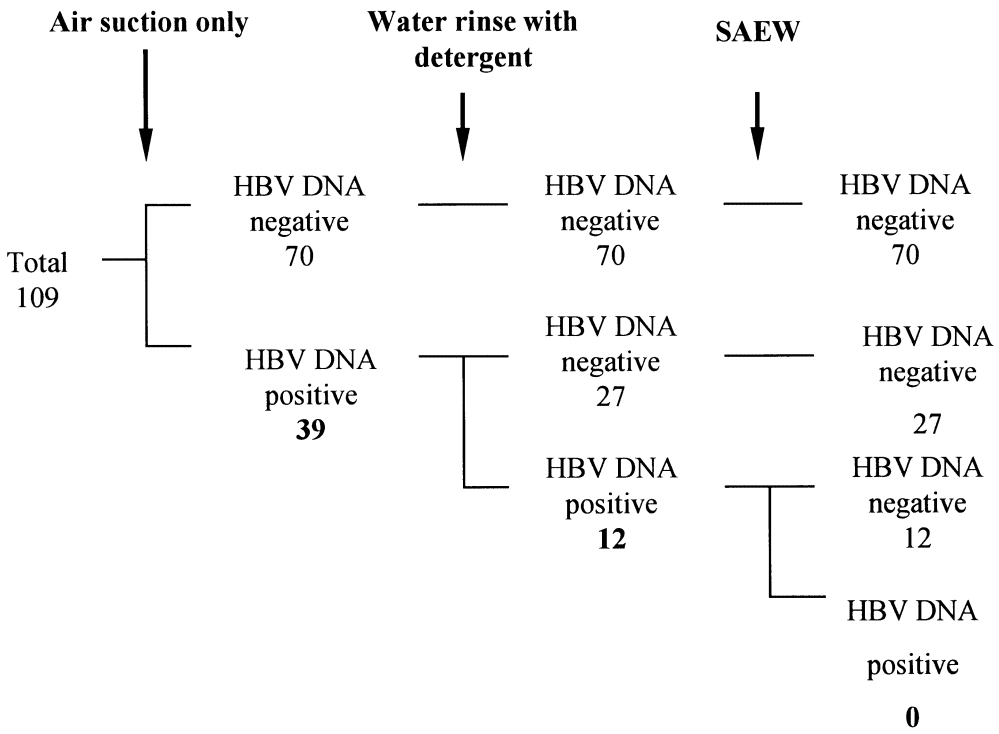

Fig. 3. Detection of hepatitis B virus (HBV) DNA on endoscopes used in HBV-positive patients after each unit has been cleaned.

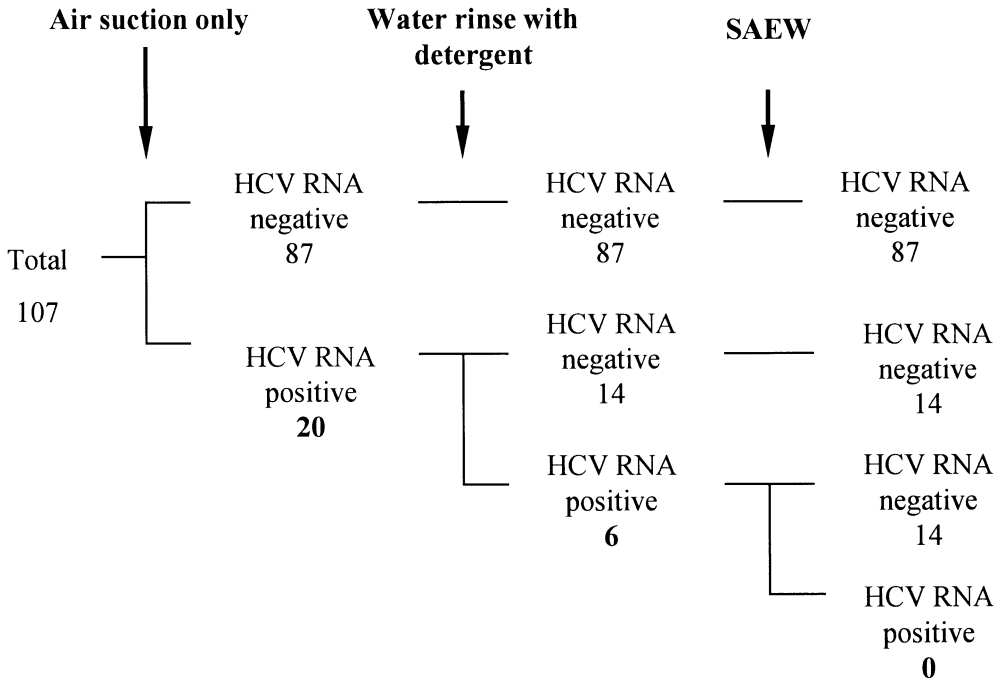

Fig. 4. Detection of hepatitis C virus (HCV) RNA on endoscopes used in HCV-positive patients after each unit has been cleaned.

four (5.4%) were still positive after tap water aspiration. All of them were found to be negative after cleaning and disinfecting with SAEW.

DISCUSSION

Infection through the use of endoscopes has been reported since 1974, for example, Spach *et al*. reported on 281 cases of infection through the use of common digestive endoscopes.[10] However, the actual frequency of occurrence has not been measured clearly. Various guidelines have been proposed for cleaning and disinfecting endoscopes, recommending the immersion of the endoscope in GA for 20–30 min because this has been considered to be an effective means of killing *Mycobacterium*. However, even with this method, it is difficult to completely kill the spores of *Clostridium difficile*. In addition, simple immersion in GA will facilitate the growth of microorganisms under the fixed soil and reduce the sterilizing effect. Therefore, the manual cleaning of endoscopes is highly recommended, and the cleaning and disinfecting of endoscopes with GA requires much time and attention.

It is known that bactericidal action of SAEW is very strong and that the effect is achieved in an extremely short periods of time.[11] For endoscopes contaminated with *Pseudomonas and* HBV-positive blood, SAEW has already proved to be a useful disinfectant.[12] Selkon reported on acidic electrolyzed water (Sterilox) for endoscope disinfection.[13] Sterilox is a weak acid with a pH of 6–7 and is a stable solution in terms of chlorine concentration, but contains less hydroxy radicals, which play a major part in the antimicrobial action. The present study used SAEW with a pH of 2.7, a residual chlorine concentration of 50 p.p.m., and an oxidation reduction potential of 1100 mV.

The effectiveness of SAEW in inactivating the herpes and Coxsackie viruses has been verified.[14] Harada *et al*. proved the virocidal effects of SAEW by infecting cultured cells with HIV virus.[15] Morita *et al*. also verified the strong action of SAEW on HIV and HBV using the PCR method.[16]

Chanzy reported experiments with HCV-contaminated endoscopes,[17] and concluded that routine manual cleaning alone could reduce HCV contamination to 10^5 or fewer, thus providing effective infection control. However, this must be evaluated in the presence of viscous saliva and blood.[18] The present study did not use experimental contamination, but there have been very few reports on clinically soiled endoscopes.

The percentage of endoscopes positive for HBV and HCV was not as high as expected after the forced air aspiration treatment. The method of assaying is sensitive enough to detect HBV and HCV in blood. However, strong acidic gastric juices and saliva containing amylase may have affected viral nucleic acid in the clinical situation. As to HCV assays in saliva, Couzigou *et al*. verified the presence of HCV antibodies in 62% of patients with chronic hepatitis C.[19] A strong correlation has been found between HBsAg in saliva and HBsAg in the blood in young HBV-positive women in Thailand.[20] The amount of HBV in the blood is high in young HBsAg carriers and the virus amount slowly reduces with age. The average age of the patients in the present study was over 50 years.

The present study found residual virus on 5–11% of endoscopes after they had been cleaned with water containing detergent. In addition, residual virus was found after routine endoscopies, where the endoscopes were not obviously contaminated with blood. Blood tests for HBV and HCV should be performed prior to endoscopies, but it is impossible to confirm the complete absence of any viral infection before the endoscopy, unless a blood test is carried out on the same day.

The antimicrobial effect of SAEW varies greatly depending upon the organic material present. Okubo *et al*. verified

by experiment that SAEW becomes ineffective when 0.25% of bovine serum albumin is added, but remains effective for 0.05%.[21] To remove such organic material, manual cleaning with brushes is essential.

In the same way as for GA, SAEW is less effective against *Mycobacterium* and spores of bacteria. However, digestive endoscopes do not need to be disinfected for atypical *Mycobacterium* and bacterial spores. The effectiveness of SAEW has been verified against *Pseudomonas*, *Helicobacter pylori* and *Escherichia coli*.[22] The effectiveness of SAEW against HBV and HCV has been established in the present study. Thus, SAEW provides a useful means of cleaning and disinfecting digestive endoscopes between patients. It is safe for the human body and for the environment, and SAEW is far less expensive to use than GA (US$0.05 or less per litre). We believe that cleaning and disinfecting endoscopes using SAEW should be performed widely in the future.

ACKNOWLEDGMENTS

The present study was supported by grant from the Inohana Alumni Association of Chiba University School of Medicine (No. 01019). We are very grateful to Dr Y Sato and Dr K Daito for consulting viral hepatitis patients.

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