⟨*Original Article* ⟩

A Novel Electrolyzed Sodium Chloride Solution
for the Disinfection of Dried HIV-1

Jun-ichi KITANO[1,2], Takehiro KOHNO[2], Kouichi SANO[2], Chizuko MORITA,

Masaki YAMAGUCHI[3], Toyoyuki MAEDA[3] and Nobuhiko TANIGAWA[1]

[1] *Department of Surgery, Osaka Medical College,*

Takatsuki-shi, Osaka 569-8686, Japan

[2] *Department of Microbiology, Osaka Medical College,*

Takatsuki-shi, Osaka 569-8686, Japan

[3] *Department of Medical Instrument, Kaigen Co., Ltd., Osaka 541-0045, Japan*

ABSTRACT

Electrolyzed products of a sodium chloride solution contain free residual chlorine and have been proved to be effective for disinfection. Electrolyzed strong acid water containing a low sodium chloride concentration (ESW-L) is prepared by the electrolysis of a solution containing a low sodium chloride concentration (0.1% or less). Although ESW-L has been confirmed to be an effective disinfectant, disinfective efficacy against dried HIV-1 and a target of ESW-L against HIV-1 have not been clarified. In this study, we attempted to demonstrate the efficacy of ESW-L against dried HIV-1 which relatively resists disinfection and to analyze disinfection target. We demonstrated that ESW-L inactivated the infectivity of dried HIV-1. In the analysis of the mechanism of disinfection, although the HIV-1 structural protein, p24 within the virus particle, was not inactivated by ESW-L, the enzymatic activity of reverse transcriptase (RT) and genomic RNA within the particle, however, were inactivated after the treatment with ESW-L. These findings suggest that the enzymatic activity of RT and genomic RNA are the target of ESW-L.

INTRODUCTION

Glutaraldehyde is a strong disinfectant and is used daily in clinical settings. Since the disinfectant is toxic to humans and the environment, we are searching for a substitute. Chlorination is another method of disinfection which has been shown to be effective against blood-borne pathogens, such as human immunodeficiency virus (HIV) (Bloomfield et al., 1990; Katner et al., 1988; Rutala et al., 1997; Shapshak et al., 1994)

and hepatitis B virus (HBV) both *in vitro* (Bond et al., 1977; Nath et al., 1982; Sehulster et al., 1981) and *in vivo* (Bond et al., 1983; Kobayashi et al., 1984). Electrolyzed products in a sodium chloride solution contain free residual chlorine and have been shown to be effective for disinfection. In Japan, in the 1990s, electrolyzed sodium chloride solutions containing high free-chlorine levels were examined from the viewpoint of the feasibility of their clinical application. At present, two kinds of electrolyzed solution are available,

namely, electrolyzed strong acid water (ESW) and electrolyzed weak acid water (EWW). ESW is prepared from sodium chloride solution. To obtain ESW, a sodium chloride solution is electrolyzed with positive and negative electrodes in wells separated by a cationic membrane. ESW is obtained by electrolysis of salt solutions at high concentrations in the well of the positive electrode (Kumon, 1997).

ESW is effective against many microbes (Abe et al., 1994; Iwasawa and Nakamura, 1996; Iwasawa et al., 1993) including *Bacillus subtilis* (Sakashita et al., 2002) and *Mycobacterium tuberculosis* (Iwasawa and Nakamura, 1993). Another electrolyzed solution of sodium chloride, EWW, obtained by electrolysis of solutions containing high sodium chloride concentrations in a single well without a cationic membrane, has been applied for disinfection. EWW has been demonstrated to be effective against various bacteria (Yoh et al., 1994; Wu et al., 1996) and blood-borne viruses (Kakimoto et al., 1997). Iwasawa and Nakamura (1996) demonstrated that EWW is suitable for stable disinfection. Both EWW and ESW, however, corrode instruments and facilities in hospitals owing to their high salt concentrations. To minimize its corrosive effect, ESW was prepared by the electrolysis of a solution containing a low sodium chloride concentration (0.1% or less) and was confirmed to be effective as a disinfectant (ESW-L) (Iwasawa and Nakamura, 1996). ESW-L is suitable for application in clinical settings. ESW-L is well established as a bactericidal disinfectant (Tsuji et al., 1999; Kiura et al., 2002) and its inactivation potential against the infectivity of blood-borne viruses in suspension has been demonstrated (Morita et al., 2000; Tagawa et al., 2000).

Hanson et al. (1989) demonstrated that HIV-1 had a residual infectivity of the same value after having been dried and immersed in buffered saline. They also demonstrated that disinfectants effective against wet HIV-1 cannot be assumed to be equally effective against dried HIV-1. Although Morita et al. (2000) stated that ESW-L has an inactivation potential against the infectivity of HIV-1 particles in suspension, they did not demonstrate the efficacy of ESW-L against dried HIV-1. They also did not demonstrate the disinfection mechanism of ESW-L against HIV-1. In this study, we attempted to demonstrate the efficacy of ESW-L against HIV-1 and in detail its targets.

Materials and Methods

2.1. Electrolyzed strong acid water containing sodium chloride at a low concentration (ESW-L)

ESW-L was prepared in an electrolysis apparatus (CLEANTOP WM-1, Kaigen Co., Ltd., Osaka, Japan). The apparatus consisted of two wells separated by a cationic membrane (Nafion 450, Dupont, New York, USA), with the positive and negative electrode in the other well electrode installed in one well. Ten liters of 0.05% NaCl in tap water was electrolyzed for 45 min at room temperature using a 3 A current. ESW-L, whose ORP, pH, free-chlorine concentration and temperature were 1,053 mV, 2.34, 4.20 ppm and 27.5℃ respectively, was obtained from the well with the positive electrode. Alkaline water, whose ORP, pH and available-chlorine concentration were -680 mV, 11.45 and 0 ppm, respectively, was obtained from the well with the negative electrode. ORP, pH and available-chlorine concentration were measured with an ORP meter (D-14, Horiba, Kyoto, Japan), a pH meter (D-14, Horiba) and a free-chlorine meter (Hach, Colorado, USA), respectively.

2.2. Infectivity assay of dried-HIV-1-infected cells

The micro-carrier test (Shimakoshi, 1995) was performed to determine the effect of ESW-L on infectivity of dried-HIV-1-infected cells. In brief, 5 μl of the HIV-1-infected Molt-4 cell suspension at 2×10^7 cells/ml was dried on a well of a microplate at room temperature for 2 h in a biosafety cabinet. The well was treated with 100 μl of ESW-L at room temperature for the designated time. To neutralize ESW-L 100 μl of 3% bovine serum albumin solution and 100 μl of alkaline water were added to the well and washed 3 times with 200 μl of 150 mM phosphate-buffered saline (pH 7.2). Uninfected Molt-4 cells were inoculated into the well at a density of 1×10^4 cells/well, and cultured for 2 weeks in 200 μl of the RPMI-1640 medium supplemented with 10% fetal bovine serum (FBS). The culture supernatant was harvested for the measurement of HIV-1 reverse transcriptase activity using a non-radioisotopic RT assay kit (Asahi Chemical Industry, Shizuoka, Japan) (Nakano et al., 1994; Sano et al., 1995).

2.3. Detection of reverse transcriptase activity

Twenty-five microliters of 1,000 μU/ml HIV-1

rRT (Saitoh et al., 1990) solution in 0.05 M Tris-HCl buffer (pH 7.8) and the same volume of HIV-1 suspension containing approximately 102.6 TCID50/50 μl of the virus in RPMI-1640 supplemented with 10% FBS were mixed separately with 175 μl of ESW-L. The mixture were then allowed to stand at room temperature for the designated time. The residual free chlorine in and pH of the mixtures were neutralized by adding 180 μl of 3% BSA and 125 μl of alkaline water. RT activities in the final RT solution and virus suspension were measured with a nonradioisotopic RT assay kit.

2.4. Detection of HIV-1 p24 antigenicity

To evaluate the efficacy of ESW-L on antigenicity of structural protein of HIV-1, we used HIV-1 p24 assay kit (Retro-tek HIV-1 p24 antigen ELISA, ZeptoMerix Co., NY, USA) as a quantitative assay. Twenty-five microliters of the HIV-1 p24 protein solution from the HIV-1 p24 assay kit was mixed with 175 μl of ESW-L, and 25 μl of HIV-1 suspension containing approximately 102.6 $\text{TCID}_{50}/50 \mu$l of the virus in RPMI-1640 supplemented with 10% FBS was mixed with 175 μl of ESW-L. These mixtures were allowed to stand at room temperature for the designated time, and were analyzed for p24 antigen using the kit.

2.5. Detection of HIV-1 gene

Twenty-five microliters of the HIV-1 suspension containing approximately 1×104 $\text{TCID}_{50}/50 \mu$l of the virus in RPMI-1640 supplemented with 10% FBS was mixed with 175 μl of ESW-L. The viral RNA was amplified by RT-PCR using the GeneAmp EZ rTh RNA kit (BioRad, USA) with the following primer pairs: M667/AA55 for the LTR region, SK-38/SK-424 for the gag region, mpol-1/mpol-2 for the pol region and DM-151/DM-152 for pAW 109 RNA as a standard RNA of the kit. RT-PCR products were electrophoresed, stained with 0.5 μg/ml etidium bromide solution and observed under an ultraviolet transilluminator. The size marker DNA (AmpliSize Molecular Ruler, BioRad, USA) suspension was mixed with the same volume of ESW-L, and electrophoresed on a polyacrylamide gel. Resulting bands were compared with those of the DNA mixed with unelectrolyzed sodium chloride.

Results

3.1. Effect of ESW-L on infectivity of dried-HIV-1-infected cells

To examine whether ESW-L inactivates the infectivity of dried HIV-1-infected cells, virus in the culture supernatant obtained following co-cultivation of uninfected cells and ESW-L-treated HIV-1-infected cells was measured as a reproduced RT activity. HIV-1 in the culture supernatant decreased in a time-dependent manner from the time of contact with ESW-L (Fig. 1). The activity reached undetectable levels within 5 min of contact time. In contrast, HIV-1 in the culture supernatant obtained following co-cultivation of HIV-1-infected cells and unelectrolyzed sodium-chloride-treated HIV-1-infected cells did not exhibit any decrease in the reproduction of the virus. These data indicate that ESW-L is able to inactivate the infectivity of dried-HIV-1-infected cells.
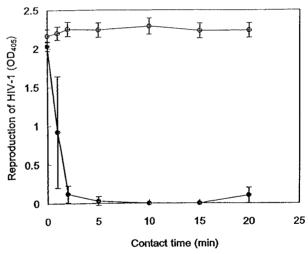

Fig. 1 Inactivation of infectivity of dried-HIV-1-infected cells
Dried-HIV-1-infected cells on a microplate well were treated with ESW-L for the designated time and cultured with uninfected cells for 14 days, and the reproduced RT activity of the culture supernatant was measured (closed circles). The dried-HIV-1-infected cells were treated with unelectrolyzed 0.05% sodium chloride solution, cultured with uninfected cells, and examined for their reproduction of RT activity in the same manner (open circles).

3.2. Effect of ESW-L on the antigenicity of HIV-1 structural protein

To clarify which viral component was inactivated by the electrolyzed products, the direct effect of ESW-L on the antigenicity of HIV-1 p24 was examined. The antigenicity of the HIV-1 p24 protein decreased in a time-dependent manner and was completely inactivated (**Fig. 2a**). To assure whether the structual protein in the virus particle is inactivated by ESW-L, an HIV-1 suspension was mixed with ESW-L, and the antigenicity of the p24 structural protein was measured by the HIV-1 p24 antigen capture enzyme immunoassay. The p24 antigenicity in HIV-1 virus particles decreased but was not completely inactivated (**Fig. 2b**). The treatment with the unelectrolyzed sodium chloride solution did not cause significant reduction in the p24 antigenicities (**Figs.2 a,b**).
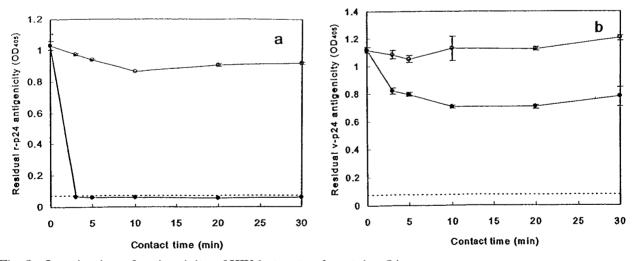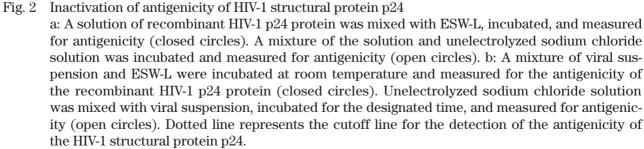
3.3. Effect of ESW-L on RT activity

To examine whether ESW-L inactivates a viral enzyme, reverse transcriptase (RT) activity in a mixture of HIV-1 rRT and ESW-L was measured. RT activity decreased below the background level within 3 min (**Fig. 3a**). The kinetics of the decrease was almost same as that observed in the experiments using recombinant HIV-1 p24 solu-tion. Since RT activity in the mixture may not be inactivated, we further examined the direct effect of ESW-L on RT molecules in the virus particles. RT activity in the mixture of the virus and ESW-L decreased in a time-dependent manner (**Fig. 3b**).
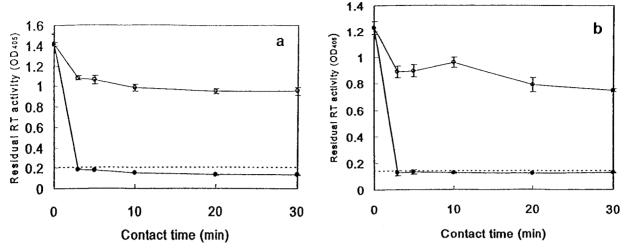
3.4. Effect of ESW-L on nucleic acids and HIV-1 gene

To clarify whether ESW-L destroys nucleic acids, the DNA marker solution was mixed with ESW-L and loaded on a gel. Compared with the DNA size marker without any treatment (**Fig. 4, lane a**), the intensity of the signal of the DNA ladder slightly decreased following mixing with unelectrolyzed sodium chloride solution (**Fig. 4, lane b**) and completely disappeared following treatment with ESW-L (**Fig. 4, lane c**). The ampli-con pAW109 without any treatment revealed strong band by RT-PCR (**Fig. 4, lane d**) An RNA solution of pAW 109 was mixed with the unelec-trolyzed sodium chloride solution (**Fig. 4, lane e**) or ESW-L (**Fig. 4, lane f**), and amplified by RT-PCR. These data indicate that ESW-L destroys both DNA and RNA.

In order to confirm whether ESW-L destroys genomic RNA in viral particles, a mixture of viral suspension and ESW-L was examined by RT-PCR

Fig. 2   Inactivation of antigenicity of HIV-1 structural protein p24
a: A solution of recombinant HIV-1 p24 protein was mixed with ESW-L, incubated, and measured for antigenicity (closed circles). A mixture of the solution and unelectrolyzed sodium chloride solution was incubated and measured for antigenicity (open circles). b: A mixture of viral sus-pension and ESW-L were incubated at room temperature and measured for the antigenicity of the recombinant HIV-1 p24 protein (closed circles). Unelectrolyzed sodium chloride solution was mixed with viral suspension, incubated for the designated time, and measured for antigenic-ity (open circles). Dotted line represents the cutoff line for the detection of the antigenicity of the HIV-1 structural protein p24.

Fig. 3 Inactivation of enzymatic activity of HIV-1 RT
a: Recombinant HIV-1 RT solution was treated with ESW-L (closed circles) or unelectrolyzed sodium chloride solution (open circles), and measured for RT activity. b: Viral solution was treated with ESW-L at room temperature, and measured for RT activity (closed circles). The viral solution was mixed with unelectrolyzed sodium chloride solution, incubated at room temperature, and measured for RT activity (open circles). Dotted line represents the cutoff line for the detection of HIV-1 RT activity in the culture supernatant.
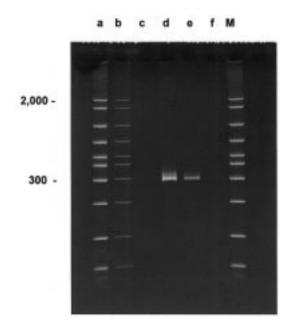

Fig. 4 Destruction of nucleic acid by ESW-L
The DNA marker solution was mixed with unelectrolyzed sodium chloride solution (lane b) or ESW-L (lane c) and compared with untreated DNA marker solution (lane a) on the gel. An RNA solution of pAW 109 was mixed with unelectrolyzed sodium chloride solution (lane e) or ESW-L (lane f), and amplified by RT-PCR. The RNA was simply amplified pAW 109 without any treatment (lane d). Lane M indicated marker for bp.
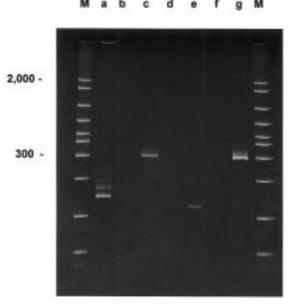

Fig. 5 Destruction of genomic RNA in viral particles by ESW-L
The HIV-1 suspension was mixed with ESW-L, and amplified for LTR (lane b), pol (lane d) and gag (lane f) genes. The mixture with unelectrolyzed sodium chloride solution was examined in the same manner for LTR (lane a), pol (lane c) and gag (lane e) genes. The positive-control pAW109 RNA was well amplified in the assay (lane g). Lane M indicated marker for bp.

analysis for genes of LTR, pol, and gag regions. The genes of LTR, pol, and gag were amplified from the viral suspension mixed with the unelectrolyzed sodium chloride solution (Fig. 5, lanes a, c and e), but not from that mixed with ESW-L (Fig. 5, lanes b, d and f) were amplified by RT-PCR. The positive-control pAW109 RNA was well amplified in the assay (Fig. 5, lane g). Lane M indicated marker for bp.

Discussion

Hanson et al. (1989) demonstrated that dried HIV is relatively resistant against disinfectants compared with wet HIV. In this study, we successfully demonstrated that ESW-L inactivated the infectivity of dried HIV-1. In the analysis of the mechanism of disinfection, the enzymatic activity of RT and genomic RNA in the virus, however, was degenerated after the treatment with ESW-L; ESW-L directly destroyed the RT molecules and the RNA within the virus particles. These findings suggest that the viral RT molecules and the viral RNA in HIV-1 are targets of ESW-L. Kiura et al. (2002) demonstrated that the destruction of nucleic acids by ESW-L is not always necessary to kill bacteria. In the case of HIV-1, since the virion is much smaller than bacteria, ESW-L probably penetrated its envelope, reached the viral RNA easily and destroyed the nucleic acid. The viral protein p24, however, is less subject to disinfectants. The findings indicated that the inactivation of viral protein within the particles is not always necessary to inactivate the infectivity of HIV-1. The findings also suggest that degeneration of structural protein is not suitable to evaluate the efficacy of disinfectants.

For the disinfection of endoscopes in a clinical setting, aldehydes have been used (Hanson 1990; Jeng et al., 1987; Reynolds et al., 1992; Rutala et al., 1991; Working Party of the British Society of Gastroenterology Endoscopy Committee, 1998). Although glutaraldehyde has been throughly evaluated as a suitable chemical for the disinfection of blood-borne pathogenic viruses (Meester et al., 1995; Russell, 1994) such as HBV (Bond et al., 1983; Kobayashi et al., 1984) and HIV (Hanson 1990), insufficient rinsing of the chemical may cause bloody diarrhea and abdominal cramps (Durante et al., 1992). The chemical exhibited cytotoxic and genotoxic potentials in cultured human cells (St-Clair et al., 1991; Sun et al., 1990). These reports indicated that a new disinfectant with low toxicity to humans is required.

There are several disinfectants that are not as toxic as glutaraldehyde. These include peracetic acid, hydrogen peroxidase, and orthophthalaldehyde. Such disinfectants, though effective, still be corrodent to medical apparatus or irritant to human (Alvarado et al., 2000). ESW-L does not irritate skin and eyes and damage endoscopes (Tsuji et al., 1999). Since ESW-L is a novel electrolyzed sodium chloride solution, only four articles have been published as regards its application to bacteria and fungi (Tsuji et al., 1999; Kiura et al., 2002), and to blood-borne viruses (Morita et al., 2000; Tagawa et al., 2000). The disinfection spectrum almost covers relatively insensitive microorganisms, such as *Mycobacteria*, *Bacillus* spores and "hepadnaviruses". Although it was of a big problem that infectivity remains in dried HIV-infected cells which attached to medical instruments, we clarify ESW-L can inactivate the infectivity of dried HIV. ESW-L may be a promising substance for disinfection.

REFERENCES

ABE S, MIYA Y, OKUDA R: Inactivation of hepatitis B virus by high oxidation potential water. J Conserv Dent 37: 1616-1623, 1994 (in Japanese)

ALVARADO CJ, REICHELDERFER M, The 1997, 1998, and 1999 APIC Guidelines Committees: APIC guideline for infection prevention and control in flexible endoscopy. Am J Infect Control 28: 138-155, 2000

BLOOMFIELD SF, SMITH-BURCHNELL CA, DALGLEISH AG: Evaluation of hypochlorite-releasing disinfectants against the human immunodeficiency virus (HIV). J Hosp Infect 15: 273-278, 1990

BOND WW, PETERSEN NJ, FAVERO MS: Viral hepatitis B. Aspects of environmental control. H. L. S 14: 235-252, 1977

BOND WW, FAVERO MS, PETERSEN NJ, EBERT JW: Inactivation of hepatitis B virus by intermediate-to-high-level disinfectant chemicals. J Clin Microbiol 18: 535-538, 1983

DURANTE L, ZULTY JC, ISRAEL E, POWERS PJ, RUSSELL RG, QIZILBASH AH, MORRIS JG Jr: Investigation

of an outbreak of bloody diarrhea: association with endoscopic cleaning solution and demonstration of lesions in an animal model. Am J Med 92: 476-480, 1992

HANSON PJ: AIDS: practising safe endoscopy. Baillieres Clin Gastroenterol 4: 477-494, 1990

HANSON PJ, GOR D, JEFFRIES DJ, COLLINS JV: 1989. Chemical inactivation of HIV on surfaces. BMJ 298: 862-864, 1989

IWASAWA A and NAKAMURA Y: Bactericidal effect of acidic electrolyzed water-comparison of chemical acidic sodium hydrochloride (NaOCl) solution. J Jpn Assoc Infect Dis 70: 915-922, 1996 (in Japanese)

IWASAWA A and NAKAMURA Y: Antimicrobial activity of aqua oxidized water. Clin Bacteriol 20: 469-473, 1993 (in Japanese)

IWASAWA A, NAKAMURA Y, OKADA J, MIZUNO T: Antiviral activity of acid water. Clin Bacteriol 20: 231-236, 1993 (in Japanese)

JENG HW, FEIGAL RJ, MESSER HH: Comparison of the cytotoxicity of formocresol, formaldehyde, cresol, and glutaraldehyde using human pulp fibroblast cultures. Pediatr Dent 9: 295-300, 1987

KAKIMOTO K, HAYASHI K, NAKANO T, SANO K, SIMOKAWA T, NAKAI M: Effects of electrolytic products of sodium chloride on HIV-1 infectivity and HBs immunoreactivity. Environ Infections 12: 1-4, 1997 (in Japanese).

KANTER HP, BUCKLEY RL, SMITH MU, HENDERSON AM: Endoscopic cleaning and disinfection procedures for preventing iatrogenic spread of human immunodeficiency virus. J Fam Pract 27: 271-276, 1988

KIURA H, SANO K, MORIMATSU S, NAKANO T, MORITA C, YAMAGUCHI M, MAEDA T, KATSUOKA Y: Bactericidal activity of electrolyzed acid water from solution containing sodium chloride at low concentration, in comparison with that at high concentration. J Microbiol Methods 49: 285-293, 2002

KOBAYASHI H, TSUZUKI M, KOSHIMIZU K, TOYAMA H, YOSHIHARA N, SHIKATA T, ABE K, MIZUNO K, OTOMO N, ODA T: Susceptibility of hepatitis B virus to disinfectants or heat. J Clin Microbiol 20: 214-216, 1984

KUMON K: What is functional water? Artif Organs Jan 21: 2-4, 1997

MEESTER HHM, VAN LOENEN AC, DE GOEDE PNFC, KOELEMAN JGM: Glutaraldehyde: current status and uses. Infect Control Hosp Epidemiol 16: 621-622, 1995

MORITA C, SANO K, MORIMATSU S, KIURA H, GOTO T, KOHNO T, HONG W, MIYOSHI H, IWASAWA A,

NAKAMURA Y, TAGAWA M, YOKOSUKA O, SAISHO H, MAEDA Y, KATSUOKA Y: Disinfective potential of electrolyzed solutions containing sodium chloride at low concentration. J Virol Methods 85: 163-174, 2000

NAKANO T, SANO K, ODAWARA F, SAITOH Y, OTAKE T, NAKAMURA T, HAYASHI K, MISAKI H, NAKAI M: Improvement and evaluation of new non-radioisotopic reverese transcriptase assay. J Jpn Assoc Infect Dis 68: 923-931, 1994

NATH N, FANG CT, DODD RY: Inactivation of DNA-polymerase associated with hepatitis B virus. J Med Virol 10: 131-140, 1982

REYNOLDS CD, RHINEHART E, DREYER P, GOLD-MANN DA: Variability in reprocessing policies and procedures for flexible fiberoptic endoscopes in Massachusetts hospitals. Am J Infect Control 20: 283-290, 1992

RUSSEL AD: Glutaraldehyde: current status and uses. Infect Control Hosp Epidemiol 15: 724-733, 1994

RUTALA WA, CLONTZ EP, WEBER DJ, HOFFMANN KK: Disinfection practices for endoscopes and other semicritical items. Infect Cont Hosp Epidemiol 12: 282-288, 1991

RUTALA WA and WEBER DJ: Uses of inorganic hypochlorite (bleach) in health-care facilities. Clin Microbiol Rev 10: 597-610, 1997

SAITOH A, IWASAKI H, NAKATA A, ADACHI A, SHINA-GAWA H: Overexpression of human immunodeficiency virus type 1 reverse transcriptase in Escherichia coli and purification of the enzyme. Microbiol Immunol 34: 509-521, 1990

SAKASHITA M, IWASAWA A, NAKAMURA Y: Antimicrobial effects and efficacy on habitually hand-washing of strong acidic electrolyzed water-a comparative study of alcoholic antiseptics and soap and tap water. J Jpn Assoc Infect Dis 76: 373-377, 2002

SANO K, ODAWARA F, NAKANO T, MORIMATSU S, NAKAMURA T, SAITOH Y, JIANG Y, MISAKI H, SAKAI Y, NAKAI M: Comparable sensitivities for detection of HIV-1 reverse transcriptase (RT) and other polymerases by RT assays requiring no radioisotopic materials. J Virol Methods 53: 235-244, 1995

SEHULSTER LM, HOLLINGER FB, DREESMAN GR, MELNICK JL: Immunological and biophysical alteration of hepatitis B virus antigens by sodium hypochlorite disinfection. Appl Environ Microbiol 42: 762-767, 1981

SHAPSHAK P, MCCOY CB, SHAH SM, PAGE JB, RIVERS JE, WEATHERBY NL, CHITWOOD DD, MASH DC: Preliminary laboratory studies of inactivation of HIV-1 in needles and syringes contain-

ing infected blood using undiluted household bleach. J Acquir Immune Defic Syndr 7: 754-759, 1994

Shimakoshi Y: Micro-carrier-test: evaluating disinfectants for HIV. J Jpn Assoc Infect Dis 69: 1151-1158, 1995

St. Clair MB, Bermudez E, Gross EA, Butterworth BE, Recio: Evaluation of the genotoxic potential of glutaraldehyde. Environ Mol Mutagen 18: 113-119, 1991

Sun HW, Feigal RJ, Messer HH: Cytotoxicity of glutaraldehyde and formaldehyde in relation to time of exposure and concentration. Pediatr Dent 12: 303-307, 1990

Tagawa M, Yamaguchi T, Yokosuka O, Matsutani S, Maeda T, Saisho H: Inactivation of hepadnavirus by electrolyzed acid water. J Antimicrob Chemother 46: 363-368, 2000

Tsuji S, Kawano S, Oshita M, Ohmae A, Shinomura Y, Miyazaki Y, Hiraoka S, Matsuzawa Y, Kamada T, Hori M, Maeda T: Endoscope disinfection using acidic electrolytic water. Endoscopy. 31: 528-535, 1999

Yoh M, Akiyama Y, Shimokawa T, Honda T: 1994. Flow water hand washing with electrolytic product of sodium chloride. Environ Infect 9: 20-23, 1994 (in Japanese)

WORKING PARTY OF THE BRITISH SOCIETY OF GASTROENTEROLOGY ENDOSCOPY COMMITTEE: Cleaning and disinfection of equipment for gastrointestinal endoscopy. Book, Gut 42: 585-93, 1998

Wu H, Morimatsu H, Shimakoshi Y, Nakano T, Kawabe K, Kakimoto K, Handa A, Nakai M, Shimokawa T: Disinfectional effect of electrolytic product of sodium chloride against Helicobacter pylori. Environ Infect 11: 203-206, 1996 (in Japanese)