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ORIGINAL ARTICLE

The efficacy and safety of electrolysed water against Covid-19 as alternative hand sanitiser

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ABSTRACT

Aim To explore the efficacy of electrolysed water against viruses and its safety to the skin.

Methods. Virus culture was carried out at level-3 Bio-Safety (BSL-3) facilities. The test material was prepared at room temperature mixed with one part virus suspension and one organic load. As an antiseptic and disinfectant control, 0.7% formaldehyde is used. Cytotoxic effects of electrolysed water are performed on Vero cells. In order to assess the safety of electrolysed water, a skin sensitivity test was conducted for electrolysed water exposure.

Results. Electrolysed water has a higher value of reduction factor than antiseptic and disinfectant control, formaldehyde, and is statistically different from control. Cytotoxicity test results on Vero cells showed that electrolysed water showed safety in Vero cell viability. As many as 58 participants who met the inclusion criteria took the electrolysed water sensitivity test to the skin. The sensitivity test showed that participants with reactions to the electrolysed water were all female, with a mean age of 32.6 years. The patch-test was positive in 3 of the 4 participants who reacted to the product.

Conclusion. Electrolysed water is effective as a new antiseptic and disinfectant against viruses and safe for human skin.

Keywords: Electrolysed water, efficacy, hand sanitiser, disinfectant, COVID-19.

INTRODUCTION

During the pandemic, the need for antiseptics and disinfectants increases to break the chain of virus spread and transmission. COVID-19 pandemic has become a significant health problem experienced by the world. This pandemic originated from discovering a novel virus from SARS-CoV (SARS coronavirus) in Wuhan, Hubei Province, China, at the end of December 2019 (1). Its infection spread very quickly and has become a significant pandemic throughout the world. Also, the virus can transmit from human to human via secretions from patients or carriers (2). The fact that makes the problems caused by this virus even more remarkable is that the virus can survive outside the host cell. It can survive in the air and where droplets or secretions stick for several hours to several days (3-4). This causes this virus' virulence to be very high and very difficult for all world citizens.

The socioeconomic impacts of this pandemic are very severe due to lockdown policies that have been implemented in all parts of the world. The world economy has stopped, economic activity was stagnant, factories and large companies have ceased to operate and fired their employees, which has led to high unemployment and poverty everywhere. Citizens of the world are quarantined in their homes and cities, resulting in emotional and psychological disorders due to limited social relationships (5-6).

Exploring and developing antiseptics and disinfectants can be one way to break the chain of the virus spreading (7). Antiseptics and disinfectants are expected to play a role in killing viruses that roam the air or stick to parts of the human body to prevent the further spread of the virus. Antiseptics and disinfectants commonly used, such as ethanol, glutaraldehyde, or sodium hypochlorite, have severe irritation levels or mucosa and eyes when used for persistent periods and direct contact with the mucosa or eyes (8,9). This shows that existing antiseptic and

disinfectants irritate the skin and mucous membranes (10). If used continuously and for a long time, it can cause skin problems (eczema and dermatitis) (11).

Electrolysed water results from bioengineering using the electrolysis process of sodium chloride to produce ions with the ability as an antiseptic and disinfectant without using a mixture of bleaching or ethanol (12). The absence of ethanol or bleaching makes this antiseptic and disinfectant have advantages in safety against the skin, mucosa, and eyes if used continuously (12). This study is the first research that explores the efficacy of electrolysed water against viruses and explores the safety of electrolysed water on human skin.

MATERIAL AND METHODS

Culture of viruses

SARS-CoV-2 isolates were obtained from sputum patients diagnosed with SARS infection at the Moh Hoesin General Hospital, Palembang, Indonesia. SARS-CoV-2 was grown on Vero cell culture (African green monkey kidney, ATCC num. CCL-81, Virginia, USA) (13). The medium used was minimum essential medium (MEM) without fetal calf serum (FCS), 100 ug/ml of streptomycin and 100 IU/mL of penicillin added. The virus culture is then stored at -80^{o}C. Determining cultures infected with the SARS-CoV-2 virus was determined if more than 50% of cell cultures were infected with viruses. The initial $\log_{10}$ virus titers were between 8.92 ± 0.25 and 9.4 ± 0.38. This virus culture was carried out based on WHO recommendations at level-3 Biosafety (BSL-3) facilities (13). The following test material was prepared at room temperature mixed with one part virus suspension and one organic load. The organic load used was 0.3% albumin, 10% FCS and 0.3% albumin with 0.3% sheep erythrocytes. After incubation, proceed with dilution with 1:10 ice-cold MEM. Next, a three-day incubation was carried out at 37^{o}C in a CO2 incubator. As an antiseptic and disinfectant control, 0.7%

formaldehyde is used (dilution 1:10 as a disinfectant control). Next, electrolysed water's cytotoxic effects are performed on suspended Vero cells in 96-well plates using MTT cell Proliferative Kit I (Roche, Basel, Switzerland). Cytotoxicity tests to assess electrolysed water's toxic effect on cells are carried out by testing electrolysed water against Vero cells without adding a virus.

Electrolysed water preparation

Electrolysed water is an antiseptic and disinfectant that was made by utilising electrophoresis technology from sodium chloride. Electrolysis was carried out on a solution of 10% sodium chloride (Sigma Aldrich, Singapore). Furthermore, ions that have the potential to be antiseptic and disinfectant will be produced. Electrolysed water used in this study was the concentration of 0.05%, 0.5%, and 5%.

Calculation of reduction factor (RF)

The reduction factor (RF) was calculated as the difference between infection titration before incubation (control titration) and infection titration after incubation with viruses and electrolysed water. Next, the $\log_{10}$ titer and standard deviation are calculated as the variance of RF.

Ethical approval

The procedure of this study complied with the principles of the Declaration of Helsinki. This study was approved by the Ethical Committee of the Faculty of Medicine, Universitas Sriwijaya, Palembang, Indonesia (No. 155/kptfkunsri-rsmh/2021).

Evaluation of electrolysed water safety

To assess the safety of electrolysed water, a skin sensitivity test was conducted for electrolysed water exposure in September 2021. The sensitivity test was conducted on the Palembang

primary care centre, where they often used hand sanitiser to educate the community. The inclusion criteria for participants were people aged 20-40 years and willing to participate in the study. Institutional review board approval was obtained, and all participants signed informed consent for the skin patch test. Participants completed an initial interview in which they reported their usual hand-hygiene practices and skin condition. Participants were instructed not to apply any products to the skin of the back of the hand for 72 hours before testing. Also, they were not allowed to ingest antihistamines or anti-inflammation drugs or wash the area during the test.

On the first day, 0.1 mL of electrolysed water was applied to clean the skin of the back of the hand and covered with nonstick plaster. The test site was assessed on the third day for erythema and papules, the same dressing reapplied, and on day four the site was reevaluated. Results were read by a dermatologist and scored as recommended by the International Contact Dermatitis Research Group using a rating of negative (-), 1+ if erythema was present; and 2+ if both erythema and vesicles/papules or blistering were present. [14]

Statistical analysis

Data were analysed using SPSS 25.0 (SPSS, Inc., Armonk, NY, United States) to assess each parameter's mean expression levels. The Fisher exact or Mann-Whitney test was used to compare selected variables between those with and without reactions, and a p-value of 0.05 was considered statistically significant.

RESULTS

Cytotoxicity test result

Table 1 shows that anolyte water has an RF value higher than the RF value of antiseptic and disinfectant control, formaldehyde, and is statistically significantly different. The higher RF values indicate the better efficacy of the test material to reduce viral load. Anolyte water with a higher concentration can effectively increase the RF value, which directly shows the ability of anolyte water as an antiseptic and disinfectant.

Cytotoxicity test results on Vero cells demonstrate that anolyte water was safe in Vero cell viability. Vero cell viability was above 90% (figure 1), which indicates that anolyte water with a concentration of 0.05% - 5% is safe against normal cells. The higher anolyte water concentration showed a slight decrease in Vero cell viability.

Sensitivity test result

Participants with reactions to the electrolysed water were all female with a mean age of 32.6 years (range: 21-40 years). There were no significant differences between those with or without a reaction in ages ($p = 0.11$) (Table 2). Lost to follow-up were 3 participants. The remaining 4 participants with reactions were patch tested to the electrolysed water. The patch test was positive in 3 of the 4 participants who reacted to the product. See Table 3 for a descriptive summary of the 4 participants who presented with a reaction and were available for patch testing.

DISCUSSION

Electrolysed water contains a variety of ions that function as antiseptics and disinfectants (7). Electrolysed water is rich in hypochlorite ions and sodium hydroxide ions, producing these ions from sodium chloride and water electrolysis. Hypochlorite ions have anti-bacterial, anti-viral, and anti-fungal effects (15,16) Ion hypochlorite can damage the walls of bacterial cells composed of complex proteins, lipids, and carbohydrates (17). These ions can

damage complex bacterial cell walls, especially virus protectors, only in simple proteins (16,17). The ability of hypochlorite ions is strengthened by sodium hydroxide ions that fight as a detergent that can accelerate the lysis of the viral protective wall (capsid) (15).

Electrolysed water has other advantages in terms of safety of use. Unlike the various antiseptic and disinfectants that have been there before, electrolysed water is safer for the skin than former antiseptic (18). Electrolysed water is rich in ions, where these ions are easily degraded when in contact with the skin (19). In contrast to chemicals found in antiseptic and disinfectants that have been there before, where the chemical contained in the antiseptic and disinfectant, not easily degraded to produce sufficient contact with skin, mucosa, and eyes, which in the end can trigger hypersensitivity reactions on the skin in the form of rashes or reddish spots that will cause damage to the skin barrier and will lead to further invasion and infection of various microorganisms on the skin (19). This is proven in various electrolyzed water safety tests on Vero cells and human skin applications. In conclusion, electrolysed water is effective as a new antiseptic and disinfectant against the virus and is safe for human skin.

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TRANSPARENCY DECLARATION

Conflict of interest: None to declare.

REFERENCES

1. Song, Z, Xu Y, Bao L, Zhang L, Yu P, Qu Y, Zhu H, Zhao W, Han Y, Qin C. From SARS to MERS, thrusting coronaviruses into the spotlight. Viruses 2019;11:59. https://dx.doi.org/10.3390/v11010059
2. Liu SL, Saif L. Emerging viruses without borders: The Wuhan coronavirus. Viruses 2020;12:130. https://dx.doi.org/10.3390/v12020130
3. Chan JF, Yuan S, Kok KH, To KK, Chu H, Yang J, Xing F, Liu J, Yip CC, Poon RW, et al. A familial cluster of pneumonia associated with the 2019 novel coronavirus indicating person-to-person transmission: a study of a family cluster. Lancet 2020;395: 514-23. https://doi.org/10.1016/S0140-6736(20)30154-9
4. Jin Y, Yang H, Ji W, Wu W, Chen S, Zhang W, Duan G. Virology, epidemiology, pathogenesis, and control of COVID-19. Viruses 2020;12:372. https://dx.doi.org/10.3390/v12040372
5. Wulandari P, Hidayat R. General anxiety disorder-related coronavirus disease-19 outbreak in Indonesia: a case report. Open Acc Maced J Med Sci 2020;8:36-8. https://doi.org/10.3889/oamjms.2020.4762
6. Yan P, Daliri EB, Oh DH. New clinical applications of electrolyzed water: a review. Microorganisms 2021; 9(1):136. https://doi.org/10.3390/microorganisms9010136
7. Jing JLJ, Yi TP, Bose RJC, McCarthy JR, Tharmalingam N, Madheswaran T. Hand sanitizers: a review on formulation aspects, adverse effects and regulations. Int J Environ Res Public Health. 2020. 17(9): 3326. https://doi.org/10.3390/ijerph17093326
8. Duarte I, Silveira JEPS, Hafner MF. Sensitive skin: review of an ascending concept. An Bras Dermatol 2017. 92 (4): 521-5. https://doi.org/10.1590/abd1806-4841.201756111

9. Olowatuyi SV, Agbele AT, Ogunrinde ME, Ayo ATV, Ayo AM, Fayoke AB, Funmilayo OM, Deborah AA. Alcohol-based hand sanitizers: review of efficacy and adverse effect. J Health Med Nursing. 2020; 81: 102-17. Available at: https://iiste.org/Journals/index.php/JHMN/article/view/54513. Accessed on 30/02/2021.
10. Athayde DR, Flores DRM, Silva JS, Silva MS, Genro ALG, Wagner R, Campagnol PCB, Menezes CR, Cichoski AJ. Characteristic and use of electrolyzed water in food industries. Int Food Res J. 2018; 25 (1): 12-6. Available at: http://ifrj.upm.edu.my/25%20(01)%202018/(2).pdf Accessed on 30/11/2021.
11. Kim HJ, Tango CN, Chelliah R, Oh DH. Sanitization efficacy of slightly acidic electrolyzed water again pure cultures of Eschericia coli, Salmonella enterica, Typhimurium, Staphylococcus aureus and Bacillus cereus spores, in comparison with different water hardness. Sci Rep 2019; 9: 4348. https://doi.org/10.1038/s41598-019-40846-6
12. Moorman E, Montazeri N, Jaykus LA. Efficacy of neutral electrolyzed water for inactivation of human norovirus. Appl Environ Microbiol. 2017; 83 (16): e00653-17. https://dx.doi.org/10.1128%2FAEM.00653-17
13. Sakuma C, Sekizuka T, Kuroda M, Kasai F, Saito K, Ikeda M, Yamaji T, Osada N, Hanada K. Novel endogenous simian retroviral integrations in Vero cells: implications for quality control of a human vaccine cell substrate. Sci Rep. 2018; 8(1): 644. https://doi.org/10.1038/s41598-017-18934-2
14. Eichenfield LF, Ahluwalia J, Waldman A, Borok J, Udkoff J, Boguniewicz M. Current guidelines for the evaluation and management of atopic dermatitis: a comparison of the joint task force practice parameter and American Academy of Dermatology guidelines,

J Allergy Clin Immunol. 2017;139 (4S): S49-57. https://doi.org/10.1016/j.jaci.2017.01.009

15. Block MS, Rowan BG. Hypochlorous acid: A review. J Oral Maxillofac Surg 2020; 78(9): 1461-6. https://dx.doi.org/10.1016/j.joms.2020.06.029
16. Sipahi H, Reis R, Dinc O, Kavaz T, Dimoglo A, Aydin A. In vitro biocompatibility study approaches to evaluate the safety profile of electrolyzed water for skin and eye. Human Exp Toxicol 2019; 38 (11): 1314-26. https://doi.org/10.1177/0960327119862333
17. Dewi FR, Stanley R, Powell SM, Burke CM. Application of electrolysed oxidising water as a sanitiser to extend the shelf-life of seafood products: a review. J Food Sci Technol 2017; 54 (5): 1321-32. https://dx.doi.org/10.1007/s13197-017-2577-9
18. Fadriquela A, Sajo MEJ, Bajgai J, Kim DH, Kin CS, Kim SK, Lee KJ. Effects of strong acidic electrolyzed water in wound healing via inflammatory and oxidative stress response. Oxid Med Cell Longev 2020; 2459826. https://doi.org/10.1155/2020/2459826
19. Novak-Bilic G, Vucic M, Japundzic I, Mestrovic-Stefekov J, Lugovic-Mihic L. Irritant and allergic contact dermatitis-skin lesion characteristics. Acta Clin Croat. 2018; 57 (4): 713-20. https://dx.doi.org/10.20471%2Facc.2018.57.04.13

TABLES

Table 1. Antiseptic and anolyte water disinfectant tests against viruses

Test material	Reduction factors (and standard deviation)		
	0,3% BSA	10% FCS	0,3% BSA and sheep erythrocytes
Electrolyzed water 0,05%	≥4,15 (0,12)*	≥4,15 (0,12)*	≥4,15 (0,12)*
Electrolyzed water 0,5%	≥4,45 (0,21)*	≥4,45 (0,21)*	≥4,45 (0,21)*
Electrolyzed water 5%	≥5,76 (0,35)*	≥5,76 (0,35)*	≥5,76 (0,35)*
Formaldehyde	≥3,25 (0,23)	≥3,25 (0,23)	≥3,25 (0,23)

* independent T test, $p<0,05$ versus formaldehyde; BSA: Bovine serum albumin, FCS: Fetal calf serum.

Table 2. Characteristics of participants with and without skin reactions

	Reactions (n=7)	Non reactions (n=51)	P values*
Age (mean)	32.6	39.0	0.11
History of itchy, sore hands	21.1%	2.9%	0.047

Table 3. Summary of skin reactions in participants.

Age, ethnicity	Times before reaction	Description	Allergies	Patch-test results description
25 y, Sumatran	Immediately on contact	Red, blotchy, itching, progressing to cracks.	History of eczema	Negative
31 y, Javanese	Immediately on contact	Fine white rash with red center, itching.	History of eczema and asthma	2+ with blisters
22 y, Sumatran	Immediately on contact	Itching, progressing to dry.	Amoxicillin	2+ with blisters
35 y, Sumatran	Immediately on contact	Itching progressing to excessive dryness; cracked.	History of eczema	1+, raised erythema, no blisters

Proofreading certificate (February 10, 2022)

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Certificate Proofreading Service Confirmation

Medical Association of Zenica-Doboj Canton Language Service provided comprehensive editing services for manuscript entitled *The efficacy and safety of electrolysed water against Covid-19 as alternative hand sanitiser* by Rachmat Hidayat and Patricia Wulandari.

The edit has achieved Grade A: priority publishing; no language polishing required after editing.

Should you require any additional information, please do not hesitate to contact me.

Sincerely,

Sven Kurbel, PhD

Medical Association of Zenica-Doboj Canton Language Service

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INTRODUCTION

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RESULTS

Cytotoxicity test result

Table 1 shows that anolyte water has an RF value higher than the RF value of antiseptic and disinfectant control, formaldehyde, and is statistically significantly different. The higher RF values indicate the better efficacy of the test material to reduce viral load. Anolyte water with a higher concentration can effectively increase the RF value, which directly shows the ability of anolyte water as an antiseptic and disinfectant.

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REFERENCES

1. Song, Z, Xu Y, Bao L, Zhang L, Yu P, Qu Y, Zhu H, Zhao W, Han Y, Qin C. From SARS to MERS, thrusting coronaviruses into the spotlight. Viruses 2019;11:59. https://dx.doi.org/10.3390/v11010059
2. Liu SL, Saif L. Emerging viruses without borders: The Wuhan coronavirus. Viruses 2020;12:130. https://dx.doi.org/10.3390/v12020130
3. Chan JF, Yuan S, Kok KH, To KK, Chu H, Yang J, Xing F, Liu J, Yip CC, Poon RW, et al. A familial cluster of pneumonia associated with the 2019 novel coronavirus indicating person-to-person transmission: a study of a family cluster. Lancet 2020;395: 514-23. https://doi.org/10.1016/S0140-6736(20)30154-9
4. Jin Y, Yang H, Ji W, Wu W, Chen S, Zhang W, Duan G. Virology, epidemiology, pathogenesis, and control of COVID-19. Viruses 2020;12:372. https://dx.doi.org/10.3390/v12040372
5. Wulandari P, Hidayat R. General anxiety disorder-related coronavirus disease-19 outbreak in Indonesia: a case report. Open Acc Maced J Med Sci 2020;8:36-8. https://doi.org/10.3889/oamjms.2020.4762
6. Yan P, Daliri EB, Oh DH. New clinical applications of electrolyzed water: a review. Microorganisms 2021; 9(1):136. https://doi.org/10.3390/microorganisms9010136
7. Jing JLJ, Yi TP, Bose RJC, McCarthy JR, Tharmalingam N, Madheswaran T. Hand sanitizers: a review on formulation aspects, adverse effects and regulations. Int J Environ Res Public Health. 2020. 17(9): 3326. https://doi.org/10.3390/ijerph17093326
8. Duarte I, Silveira JEPS, Hafner MF. Sensitive skin: review of an ascending concept. An Bras Dermatol 2017. 92 (4): 521-5. https://doi.org/10.1590/abd1806-4841.201756111

9. Olowatuyi SV, Agbele AT, Ogunrinde ME, Ayo ATV, Ayo AM, Fayoke AB, Funmilayo OM, Deborah AA. Alcohol-based hand sanitizers: review of efficacy and adverse effect. J Health Med Nursing. 2020; 81: 102-17. Available at: https://iiste.org/Journals/index.php/JHMN/article/view/54513. Accessed on 30/02/2021.
10. Athayde DR, Flores DRM, Silva JS, Silva MS, Genro ALG, Wagner R, Campagnol PCB, Menezes CR, Cichoski AJ. Characteristic and use of electrolyzed water in food industries. Int Food Res J. 2018; 25 (1): 12-6. Available at: http://ifrj.upm.edu.my/25%20(01)%202018/(2).pdf Accessed on 30/11/2021.
11. Kim HJ, Tango CN, Chelliah R, Oh DH. Sanitization efficacy of slightly acidic electrolyzed water again pure cultures of Eschericia coli, Salmonella enterica, Typhimurium, Staphylococcus aureus and Bacillus cereus spores, in comparison with different water hardness. Sci Rep 2019; 9: 4348. https://doi.org/10.1038/s41598-019-40846-6
12. Moorman E, Montazeri N, Jaykus LA. Efficacy of neutral electrolyzed water for inactivation of human norovirus. Appl Environ Microbiol. 2017; 83 (16): e00653-17. https://dx.doi.org/10.1128%2FAEM.00653-17
13. Sakuma C, Sekizuka T, Kuroda M, Kasai F, Saito K, Ikeda M, Yamaji T, Osada N, Hanada K. Novel endogenous simian retroviral integrations in Vero cells: implications for quality control of a human vaccine cell substrate. Sci Rep. 2018; 8(1): 644. https://doi.org/10.1038/s41598-017-18934-2
14. Eichenfield LF, Ahluwalia J, Waldman A, Borok J, Udkoff J, Boguniewicz M. Current guidelines for the evaluation and management of atopic dermatitis: a comparison of the joint task force practice parameter and American Academy of Dermatology guidelines,

J Allergy Clin Immunol. 2017;139 (4S): S49-57. https://doi.org/10.1016/j.jaci.2017.01.009

15. Block MS, Rowan BG. Hypochlorous acid: A review. J Oral Maxillofac Surg 2020; 78(9): 1461-6. https://dx.doi.org/10.1016/j.joms.2020.06.029
16. Sipahi H, Reis R, Dinc O, Kavaz T, Dimoglo A, Aydin A. In vitro biocompatibility study approaches to evaluate the safety profile of electrolyzed water for skin and eye. Human Exp Toxicol 2019; 38 (11): 1314-26. https://doi.org/10.1177/0960327119862333
17. Dewi FR, Stanley R, Powell SM, Burke CM. Application of electrolysed oxidising water as a sanitiser to extend the shelf-life of seafood products: a review. J Food Sci Technol 2017; 54 (5): 1321-32. https://dx.doi.org/10.1007/s13197-017-2577-9
18. Fadriquela A, Sajo MEJ, Bajgai J, Kim DH, Kin CS, Kim SK, Lee KJ. Effects of strong acidic electrolyzed water in wound healing via inflammatory and oxidative stress response. Oxid Med Cell Longev 2020; 2459826. https://doi.org/10.1155/2020/2459826
19. Novak-Bilic G, Vucic M, Japundzic I, Mestrovic-Stefekov J, Lugovic-Mihic L. Irritant and allergic contact dermatitis-skin lesion characteristics. Acta Clin Croat. 2018; 57 (4): 713-20. https://dx.doi.org/10.20471%2Facc.2018.57.04.13

TABLES

Table 1. Antiseptic and anolyte water disinfectant tests against viruses

Test material	Reduction factors (and standard deviation)		
	0,3% BSA	10% FCS	0,3% BSA and sheep erythrocytes
Electrolyzed water 0,05%	≥4,15 (0,12)*	≥4,15 (0,12)*	≥4,15 (0,12)*
Electrolyzed water 0,5%	≥4,45 (0,21)*	≥4,45 (0,21)*	≥4,45 (0,21)*
Electrolyzed water 5%	≥5,76 (0,35)*	≥5,76 (0,35)*	≥5,76 (0,35)*
Formaldehyde	≥3,25 (0,23)	≥3,25 (0,23)	≥3,25 (0,23)

* independent T test, $p<0,05$ versus formaldehyde; BSA: Bovine serum albumin, FCS: Fetal calf serum.

Table 2. Characteristics of participants with and without skin reactions

	Reactions (n=7)	Non reactions (n=51)	P values*
Age (mean)	32.6	39.0	0.11
History of itchy, sore hands	21.1%	2.9%	0.047

Table 3. Summary of skin reactions in participants.

Age, ethnicity	Times before reaction	Description	Allergies	Patch-test results description
25 y, Sumatran	Immediately on contact	Red, blotchy, itching, progressing to cracks.	History of eczema	Negative
31 y, Javanese	Immediately on contact	Fine white rash with red center, itching.	History of eczema and asthma	2+ with blisters
22 y, Sumatran	Immediately on contact	Itching, progressing to dry.	Amoxicillin	2+ with blisters
35 y, Sumatran	Immediately on contact	Itching progressing to excessive dryness; cracked.	History of eczema	1+, raised erythema, no blisters

Submission acknowledgement (March 15, 2022)

Medical Association of Zenica-Doboj Canton
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Zenica, 15 March 2022

ID MS: MG-1485/22
Title: The efficacy and safety of electrolysed water against Covid-19 as alternative hand sanitiser
Authors: 1) Rachmat Hidayat, 2) Patricia Wulandari

Dear dr. Hidayat,

Review of your manuscript is completed. I have received positive feedback from the reviewer for your work.
But, there is need for a revision.
In order to preparing the manuscript for publication I have preformatted (yellow highliter) your text and made some corrections (in red color). My comments wrote down in the blue color.
Please leave this corrections (including the colors) in the new version of your manuscript.
Your next corrections **according to reiwers and my comments** please wright down in **green** color and send us a new version of your manuscript in the next 10 days.

YOU SHOULD STRONGLY FOLLOW GUIDELINES FOR AUTHORS FOR MG (www.ljkzedo.ba; http://ljkzedo.ba/instructions-to-authors/) in ALL DETAILS!

Editor suggestions for improvement:

Whole text: New Times Roman (12, except the Title – 14); align text left, double-spaced;
No paragraphs should be used – corrected;
All statements and sentences should be citated with appropriate references.

Title page: see at the text;

Abstract (second page) (up to 250 words): see in the text!
Key words (3-5) (in alphabetical order): For the key words you should use **MeSH (Medical Subject Headings)** (http://www.nlm.nih.gov/mesh/meshhome.html); **do not use the words which are contained in the article title!**

Introduction: see in the text! Why you did decide to conduct this investigation on corona virus– there are some researches investigating electrolyzed water on other viruses, other microorganisms or for other use – references 10-13? Describe them!

Materials and methods: **see in the text! They should be described in three subheadings!**
I have rearrenged some parts of the text – check it!

Results: see in the text! Expand description of the results!!

Discussion: see in the text! Discussion should begin with short repetition of your main results! Then, you should comment your results with other's results!

References: Are not cited according to instructions to authors for Medicinski Glasnik – please make corrections! See my corrections and at: http://ljkzedo.ba/instructions-to-authors/

Tables: see in the text!

Best regards
Prof. Selma Uzunović
Editor-in-Chief
Medicinski Glasnik

Peer reviewer results (March 30, 2022)

REV 1

The efficacy and safety of electrolysed water against Covid-19 as alternative hand sanitiser

1. Is the question posed by authors new and well defined?
   Yes. It is new modality for hand sanitiser and well defined by author.
2. Are the methods appropriate and well described, and are sufficient details provided to replicate the work?
   Yes, but author can more explain about how to produce electrolysed water.
3. Are the data sound and well controlled?
   Yes.
4. Does the manuscript adhere to the relevant standards for reporting and data deposition? (see: Guidelines for authors, Medicinski Glasnik).
   Yes.
5. Are the discussion and conclusions well balanced and adequately supported by the data?
   Yes, but author can more explain biological plausibility how electrolysed water can kill the SARS COV2.
6. Do the title and abstract convey what has been found?
   Yes.
7. Is the writing acceptable?
   Yes
8. Please indicate how interesting you find the manuscript.
   The manuscript explains new modality for hand sanitiser. It is very interesting.
9. Quality of written English- Acceptable.
10. Statistical review: No, the manuscript does not need to be seen by a statistician.
11. Given your assessment of the manuscript, what do you advise should be next step?
    Accept after minor essential revisions (which the authors can be trusted to make).

Please indicate priority for publication (overall the paper rating) (+):

Excellent	Good	Fair	Poor
+			

REV 2

Review of the manuscript " The efficacy and safety of electrolysed water against Covid-19 as alternative hand sanitiser".

1. Is the question posed by authors new and well defined?
   Yes. It is new and well defined.
2. Are the methods appropriate and well described, and are sufficient details provided to replicate the work?
   Yes. Methods are appropriate and well described. There are sufficient details provided to replicate the work.
3. Are the data sound and well controlled?
   Yes. The data are well controlled.
4. Are the discussion and conclusions well balanced and adequately supported by the data?
   Yes. The discussion are well. Please improve the discussion by adding pathophysiology hypersensitivity related to sensitivity reaction of participants.
5. Do the title and abstract convey what has been found?
   Yes. The abstract is good enough and explains the findings of the study.
6. Is the writing acceptable?
   Yes. The writing is acceptable
7. Please indicate how interesting you find the manuscript.
   An article of outstanding merit and interest in its field.
8. Quality of written English- **Acceptable**.
9. Given your assessment of the manuscript, what do you advise should be next step?
   Accept after minor essential revisions (which the authors can be trusted to make).

Please indicate priority for publication (overall the paper rating) (+):

Excellent	Good	Fair	Poor
+			

Revision sent back to editor (April 3, 2022)

ORIGINAL ARTICLE

The efficacy and safety of electrolyzed water against Covid-19 as alternative hand sanitizer

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Running title: Hidayat et al. Electrolyzed water as hand sanitizer

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ABSTRACT

Aim To investigate the efficacy of electrolyzed water against viruses and its safety to the skin.

Methods Virus culture was carried out at level-3 Bio-Safety (BSL-3) facilities. The test material was prepared at room temperature mixed with one part virus suspension and one organic load. As an antiseptic and disinfectant control, 0.7% formaldehyde was used. Cytotoxic effects of electrolyzed water were performed on Vero cells. In order to assess the safety of electrolyzed water, a skin sensitivity test was conducted for electrolyzed water exposure.

Results. Electrolyzed water has a higher value of reduction factor than antiseptic and disinfectant control, formaldehyde, and it was statistically different from control. Cytotoxicity test results on Vero cells showed that electrolyzed water showed safety in Vero cell viability. As many as 58 participants who met the inclusion criteria took the electrolyzed water sensitivity test to the skin. The sensitivity test showed that participants with reactions to the electrolyzed water were all female, with a mean age of 32.6 years. The patch-test was positive in 3 of the 4 participants who reacted to the product.

Conclusion. Electrolyzed water is effective as a new antiseptic and disinfectant against viruses and safe for human skin.

Key words: cell survival, disinfectants, local anti-infective agents, Vero cells, viruses

INTRODUCTION

During the pandemic, the need for antiseptics and disinfectants increases to break the chain of virus spread and transmission. COVID-19 pandemic has become a significant health problem experienced by the world. This pandemic originated from discovering a novel virus from SARS-CoV (SARS coronavirus) in Wuhan, Hubei Province, China, at the end of December 2019 (1). Infection spread very quickly and has become a significant pandemic throughout the world. Also, the virus can transmit from human to human via secretions from patients or carriers (2). The fact that makes the problems caused by this virus even more remarkable is that the virus can survive outside the host cell. It can survive in the air and where droplets or secretions stick for several hours to several days (3-4). This causes this virus' virulence to be very high and very difficult for all world citizens.

The socioeconomic impacts of this pandemic are very severe due to lockdown policies that have been implemented in all parts of the world. The world economy has stopped, economic activity was stagnant, factories and large companies have ceased to operate and fired their employees, which has led to high unemployment and poverty everywhere. Citizens of the world are quarantined in their homes and cities, resulting in emotional and psychological disorders due to limited social relationships (5-6).

Exploring and developing antiseptics and disinfectants can be one way to break the chain of the virus spreading (7). Antiseptics and disinfectants are expected to play a role in killing viruses that roam the air or stick to parts of the human body to prevent the further spread of the virus. Antiseptics and disinfectants commonly used, such as ethanol, glutaraldehyde, or sodium hypochlorite, have severe irritation levels or mucosa and eyes when used for persistent periods and direct contact with the mucosa or eyes (8-9). This shows that existing antiseptic and disinfectants irritate the skin and mucous membranes (10). If used continuously and for a long time, it can cause skin problems (eczema and dermatitis) (11).

Electrolyzed water results from bioengineering using the electrolysis process of sodium chloride to produce ions with the ability as an antiseptic and disinfectant without using a mixture of bleaching or ethanol (12). The absence of ethanol or bleaching makes this antiseptic and disinfectant have advantages in safety against the skin, mucosa, and eyes if used continuously (12).

The coronavirus pandemic has become an emerging disease. The need for hand sanitizers that effectively reduce the spread of coronavirus and are safe to use are essential during pandemic. Electrolyzed water can be a safe and effective alternative to hand sanitizers in preventing the spread of coronavirus (6,12).

This study is the first research that explores the efficacy of electrolyzed water against coronaviruses and explores the safety of electrolyzed water on human skin.

MATERIAL AND METHODS

Material and study design

SARS-CoV-2 isolates were obtained from sputum of patients diagnosed with SARS infection at the Moh Hoesin General Hospital, Palembang, Indonesia during the period April to August 2021.

To assess the safety of electrolyzed water, a skin sensitivity test was conducted for electrolyzed water exposure in September 2021. Participants who took part in this study were all nurses who worked at the Palembang Primary Care Centre, where they often used hand sanitizer for daily service activities. The inclusion criteria for participants were nurses aged 20-40 years and willing to participate in the study. The participants were selected by total sampling. Institutional review board approval was obtained, and all participants signed informed consent for the skin patch test.

Participants completed an initial interview in which they reported their usual hand-hygiene practices and skin condition. Participants were instructed not to apply any products to the

skin of the back of the hand for 72 hours before testing. Also, they were not allowed to ingest antihistamines or anti-inflammation drugs or wash the area during the test.

The procedure of this study complied with the principles of the Declaration of Helsinki. This study was approved by the Ethical Committee of the Faculty of Medicine, Universitas Sriwijaya, Palembang, Indonesia (No. 155/kptfkunsri-rsmh/2021).

Methods

Culture of viruses. SARS-CoV-2 was grown on Vero cell culture (African green monkey kidney, ATCC num. CCL-81, Virginia, USA) (13). The medium used was minimum essential medium (MEM) without fetal calf serum (FCS), 100 ug/mL of streptomycin and 100 IU/mL of penicillin added. The virus culture is then stored at -80^{o}C. Determining cultures infected with the SARS-CoV-2 virus was determined if more than 50% of cell cultures were infected with viruses. The initial $\log_{10}$ virus titers were between 8.92 ± 0.25 and 9.4 ± 0.38. This virus culture was carried out based on WHO recommendations at level-3 Biosafety (BSL-3) facilities (13).

The following test material was prepared at room temperature mixed with one part virus suspension and one organic load. The organic load used was 0.3% albumin, 10% FCS and 0.3% albumin with 0.3% sheep erythrocytes. After incubation, proceed with dilution with 1:10 ice-cold MEM. Next, a three-day incubation was carried out at 37 ^{o}C in a CO_2 incubator. As an antiseptic and disinfectant control, 0.7% formaldehyde was used (dilution 1:10 as a disinfectant control). Next, electrolyzed water's cytotoxic effects were performed on suspended Vero cells in 96-well plates using MTT cell Proliferative Kit I (Roche, Basel, Switzerland). Cytotoxicity tests to assess electrolyzed water's toxic effect on cells were carried out by testing electrolyzed water against Vero cells without adding a virus.

Electrolyzed water preparation. Electrolyzed water is an antiseptic and disinfectant that was made by utilizing electrophoresis technology from sodium chloride. Electrolysis was

carried out on a solution of 10% sodium chloride (Sigma Aldrich, Singapore). Furthermore, ions that have the potential to be antiseptic and disinfectant will be produced. Electrolyzed water used in this study was the concentration of 0.05%, 0.5%, and 5%.

Calculation of reduction factor (RF). The reduction factor (RF) was calculated as the difference between infection titration before incubation (control titration) and infection titration after incubation with viruses and electrolyzed water. Next, the $\log_{10}$ titer and standard deviation are calculated as the variance of RF.

Evaluation of electrolyzed water safety. On the first day, 0.1 mL of electrolyzed water was applied to clean skin of the back of the hand and covered with nonstick plaster. The test site was assessed on the third day for erythema and papules, the same dressing reapplied, and on day four the site was reevaluated. Results were read by a dermatologist and scored as it is recommended by the International Contact Dermatitis Research Group using a rating of negative (-), 1+ if erythema was present, and 2+ if both erythema and vesicles/papules or blistering were present (14).

Statistical analysis. The assessment of each parameter's mean expression levels was made. The Fisher exact or Mann-Whitney test was used to compare selected variables between those with and without reactions. The $p=0.05$ was considered statistically significant.

RESULTS

Electrolyzed water had an reduction factors (RF) value higher than the RF value of antiseptic and disinfectant control, formaldehyde, and it was statistically significantly different ($p=0.01$). The higher RF values indicate the better efficacy of the test material to reduce viral load. Electrolyzed water with a higher concentration can effectively increase the RF value, which directly shows the ability of electrolyzed water as an antiseptic and disinfectant (Table 1).

Cytotoxicity test results on Vero cells demonstrated that anolyte water was safe in Vero cell viability. Vero cell viability was above 90%, which indicates that electrolyzed water with a concentration of 0.05%-5% was safe against normal cells. The higher electrolyzed water concentration showed a slight decrease in Vero cell viability.

Among 58 participants in skin test, 51 (87.9%) were had not any reactions. Seven (12.1%) were with reactions to the electrolyzed water and all were females with a mean age of 32.6 years (range: 21-40 years). There were no significant differences between those with or without a reaction in age (p=0.11) (Table 2).

Three people out of 7 participants who experienced reactions to electrolyzed water chose not to follow the study follow-up. The remaining four participants with reactions were patch tested to the electrolyzed water. The patch test was positive in three of the four participants who reacted to the electrolyzed water (Table 3).

DISCUSSION

Electrolyzed water with a higher concentration can increase the reduction factor value and show the ability of electrolyzed water as an antiseptic and disinfectant against coronaviruses. The results of this study are supported by research by Takeda, which states that the use of acidic electrolyzed water as a contact disinfectant can inactivate virucidal activity in SARS-CoV-2 (15).

Electrolyzed water contains a variety of ions that function as antiseptics and disinfectants (7). Electrolyzed water is rich in hypochlorite ions and sodium hydroxide ions, producing these ions from sodium chloride and water electrolysis. Hypochlorite ions have anti-bacterial, anti-viral, and anti-fungal effects (16,17). Ion hypochlorite can damage bacterial cell wall composed of complex proteins, lipids, and carbohydrates (18). The viral capsid consists of simpler proteins than the bacterial cell wall, so the hypochlorite ion can quickly destroy the

capsid. (16,17). The ability of hypochlorite ions is strengthened by sodium hydroxide ions that fight as a detergent and can accelerate the lysis of the viral protective wall (capsid) (16). Electrolyzed water has other advantages in terms of safety of use. In our study, electrolyzed water was found safer for the skin than former antiseptic, and in various concentration proved safe on Vero cells and human skin applications. Electrolyzed water is rich in ions, where these ions are easily degraded when come in contact with the skin (19). This is different from the chemical substances found in antiseptics in general, where these substances are generally not easily degraded. This slow degradation intends to make chemical contact with the skin and mucosa sufficiently. However, the side effect is that chemicals in antiseptics can generally trigger a hypersensitivity reaction on the skin. Inflammatory reactions in the skin will damage the skin barrier and facilitate the infection of microorganisms on the skin (20). In conclusion, electrolyzed water is effective as a new antiseptic and disinfectant against the virus and is safe for human skin.

ACKNOWLEDGEMENT

The authors would express their gratitude to Dr Mohammad Hoesin General Hospital, Palembang, Indonesia.

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TRANSPARENCY DECLARATION

Conflict of interest: None to declare.

REFERENCES

1. Song, Z, Xu Y, Bao L, Zhang L, Yu P, Qu Y, Zhu H, Zhao W, Han Y, Qin C. From SARS to MERS, thrusting coronaviruses into the spotlight. Viruses 2019; 11:59.
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Electrolyzed water 0.5%	≥4.45 (0.21)*	≥4.45 (0.21)*	≥4.45 (0.21)*
Electrolyzed water 5%	≥5.76 (0.35)*	≥5.76 (0.35)*	≥5.76 (0.35)*
Formaldehyde	≥3.25 (0.23)	≥3.25 (0.23)	≥3.25 (0.23)

*independent T test: $p<0.05$ versus formaldehyde;
BSA, bovine serum albumin; FCS, fetal calf serum

Table 2. Characteristics of 58 participants with and without skin reactions

Variable	**Reactions** (n=7)	**No reactions** (n=51)	**p**
Mean age (years)	32.6	39.0	0.11
History of itchy, sore hands (No, %)	2 (21.1)	2 (2.9)	0.047

Table 3. Summary of skin reactions in four participants

Age (years), ethnicity	Times before reaction	Description	Allergies	Patch-test results description
25, Sumatran	Immediately on contact	Red, blotchy, itching, progressing to cracks.	History of eczema	Negative
31, Javanese	Immediately on contact	Fine white rash with red center, itching.	History of eczema and asthma	2+ with blisters
22, Sumatran	Immediately on contact	Itching, progressing to dry.	Amoxicillin	2+ with blisters
35, Sumatran	Immediately on contact	Itching progressing to excessive dryness; cracked.	History of eczema	1+, raised erythema, no blisters

Editor decision (18 April, 2022)

Medicinski Glasnik, Editorial Board
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ID: MG 1485/22
Title: The efficacy and safety of electrolysed water against Covid-19 as alternative hand sanitiser
Authors: Rachmat Hidayat, Patricia Wulandari
Date: 18.04.2022.

Dear Rachmat Hidayat,

Your article **"The efficacy and safety of electrolysed water against Covid-19 as alternative hand sanitiser"** will be accepted for publication in Medicinski Glasnik based on Editor-in-Chief's decision.

Thank you for your interest in publishing in the journal *Medicinski Glasnik*. If you have any questions or concerns, please contact us.

Regards,
Prof. Selma Uzunović, MD, MA, PhD
Editor-in-Chief
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Letter of Acceptance (May 27, 2022)

Medicinski Glasnik, Editorial Board
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Date: 27.05.2022.

Dear Rachmat Hidayat,

Your manuscript **"The efficacy and safety of electrolysed water against Covid-19 as alternative hand sanitiser"** has been accepted for publication in Medicinski Glasnik Vol 19 No2 (August 2022).

You will receive shortly the proofreading of your MS.

Before that, I am asking the following:
In order to promote your paper published in the MG and increase its use as a reference in international scientific literature, please send us email addresses of all co-authors of your article, as well as e-mail addressess of corresponding authors of all articles cited in Reference section from your paper (for correspondence purposes). You can do it directly in the text of the final version of your paper that we are sending, next to the author's name for each stated reference.
Also, you can make your profile at ResearchGate (http://www.researchgate.net/) and add your publication(s) (as a PDF file).
Finally, please quote your paper and all other papers published in the *Medicinski Glasnik* journal as often as possible.

Best regard
Prof. Selma Uzunović, MD, MA, PhD
Editor-in-Chief
Medicinski Glasnik

ORIGINAL ARTICLE

The efficacy and safety of electrolyzed water against Covid-19 as an alternative hand sanitizer

Rachmat Hidayat[1], Patricia Wulandari[2]

[1]Department of Biology, Faculty of Medicine, Universitas Sriwijaya, [2]Cattleya Mental Health Centre; Palembang, Indonesia

ABSTRACT

Aim To investigate the efficacy of electrolyzed water against viruses and its safety to the skin.

Methods Virus culture was carried out at level-3 Bio-Safety (BSL-3) facilities. The test material was prepared at room temperature mixed with one part virus suspension and one organic load. As an antiseptic and disinfectant control, 0.7% formaldehyde was used. Cytotoxic effects of electrolyzed water were performed on Vero cells. In order to assess the safety of electrolyzed water, a skin sensitivity test was conducted for electrolyzed water exposure.

Results Electrolyzed water has a higher value of reduction factor than antiseptic and disinfectant control, formaldehyde, and it was statistically different from control. Cytotoxicity test results on Vero cells showed that electrolyzed water demonstrated safety in Vero cell viability. As many as 58 participants who met the inclusion criteria took electrolyzed water sensitivity test to the skin. The sensitivity test showed that participants with reactions to electrolyzed water were all female, with a mean age of 32.6 years. The patch-test was positive in 3 of 4 participants who reacted to the product.

Conclusion Electrolyzed water is effective as a new antiseptic and disinfectant against viruses and safe for human skin.

Key words: cell survival, disinfectants, local anti-infective agents, Vero cells, viruses

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The socioeconomic impacts of this pandemic are very severe due to lockdown policies that have been implemented in all parts of the world. The world economy has stopped, economic activity was stagnant, factories and large companies have ceased to operate and fired their employees, which has led to high unemployment and poverty everywhere. Citizens of the world are quarantined in their homes and cities, resulting in emotional and psychological disorders due to limited social relationships (5-6).

Exploring and developing antiseptics and disinfectants can be one way to break the chain of the virus spreading (7). Antiseptics and disinfectants are expected to play a role in killing viruses that roam the air or stick to parts of the human body to prevent the further spread of the virus. Antiseptics and disinfectants commonly used, such as ethanol, glutaraldehyde, or sodium hypochlorite, have severe irritation levels or mucosa and eyes when used for persistent periods and direct contact with the mucosa or eyes (8-9). This shows that existing antiseptic and disinfectants irritate the skin and mucous membranes (10). If used continuously and for a long time, it can cause skin problems (eczema and dermatitis) (11).

Electrolyzed water results from bioengineering using the electrolysis process of sodium chloride to produce ions with the ability as an antiseptic and disinfectant without using a mixture of bleaching or ethanol (12). The absence of ethanol or bleaching makes this antiseptic and disinfectant have advantages in safety against the skin, mucosa, and eyes if used continuously (12).

The coronavirus pandemic has become an emerging disease. The need for hand sanitizers that effectively reduce the spread of the coronavirus and are safe to use are essential during a pandemic. Electrolyzed water can be a safe and effective alternative to hand sanitizers in preventing the spread of the coronavirus (6,12).

This study is the first research that explores the efficacy of electrolyzed water against coronaviruses and explores the safety of electrolyzed water on human skin.

MATERIAL AND METHODS

Material and study design

SARS-CoV-2 isolates were obtained from sputum of patients diagnosed with SARS infection at the Moh Hoesin General Hospital, Palembang, Indonesia, during the period April to August 2021.

To assess the safety of electrolyzed water, a skin sensitivity test was conducted for electrolyzed water exposure in September 2021. Participants who took part in this study were all nurses 20-40 years old and willing to participate in the study who worked at the Palembang Primary Care Centre, where they often used hand sanitizer for daily service activities. Institutional review board approval was obtained, and all participants signed informed consent for the skin patch test.

Participants completed an initial interview in which they reported their usual hand-hygiene practices and skin condition. Participants were instructed not to apply any products to the skin of the back of the hand for 72 hours before testing. Also, they were not allowed to ingest antihistamines or anti-inflammation drugs or wash the area during the test.

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Electrolyzed water preparation. Electrolyzed water is an antiseptic and disinfectant that was made by utilizing electrophoresis technology from sodium chloride. Electrolysis was carried out on a solution of 10% sodium chloride (Sigma Aldrich, Singapore). Furthermore, ions that have the potential to be antiseptic and disinfectant will be produced. Electrolyzed water used in this study was the concentration of 0.05%, 0.5%, and 5%.

Calculation of reduction factor (RF). The reduction factor (RF) was calculated as the difference between infection titration before incubation (control titration) and infection titration after incubation with viruses and electrolyzed water. Next, the $\log_{10}$ titer and standard deviation are calculated as the variance of RF.

Evaluation of electrolyzed water safety. On the first day, 0.1 mL of electrolyzed water was applied to clean skin of the back of the hand and covered with nonstick plaster. The test site was assessed on the third day for erythema and papules, the same dressing reapplied, and on day four the site was reevaluated. Results were read by a dermatologist and scored as it is recommended by the International Contact Dermatitis Research Group using a rating of negative (-), 1+ if erythema was present, and 2+ if both erythema and vesicles/papules or blistering were present (14).

Statistical analysis. The assessment of each parameter's mean expression levels was made. The Fisher exact or Mann-Whitney test was used to compare selected variables between those with and without reactions. The p=0.05 was considered statistically significant.

RESULTS

Electrolyzed water had reduction factors (RF) value higher than the RF value of antiseptic and disinfectant control, formaldehyde, and it was statistically significantly different (p=0.01). The higher RF values indicate the better efficacy of the test material to reduce viral load. Electrolyzed water with a higher concentration can effectively increase the RF value, which directly shows the ability of electrolyzed water as an antiseptic and disinfectant (Table 1).

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Test material	Reduction factors (and standard deviation)		
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Electrolyzed water 0.05%	4.15 (0.12)*	4.15 (0.12)*	4.15 (0.12)*
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Electrolyzed water 5%	5.76 (0.35)*	5.76 (0.35)*	5.76 (0.35)*
Formaldehyde	3.25 (0.23)	3.25 (0.23)	3.25 (0.23)

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Among 58 participants in the skin test, 51 (87.9%) had no reactions. Seven (12.1%) had

reactions to electrolyzed water and all were females with a mean age of 32.6 years (range: 21-40 years). There were no significant differences between those with or without a reaction in age (p=0.11) (Table 2).

Table 2. Characteristics of 58 participants with and without skin reactions

Variable	Reactions (n=7)	No reactions (n=51)	p
Mean age (years)	32.6	39.0	0.11
History of itchy, sore hands (No, %)	2 (21.1)	2 (2.9)	0.047

Three of seven participants who showed reactions to electrolyzed water chose not to follow the study follow-up. The remaining four participants with reactions were patch tested to electrolyzed water. The patch test was positive in three of the four participants who reacted to electrolyzed water (Table 3).

DISCUSSION

Electrolyzed water with a higher concentration can increase the reduction factor value and show the ability of electrolyzed water as an antiseptic and disinfectant against coronaviruses. The results of this study are supported by research by Takeda, which states that the use of acidic electrolyzed water as a contact disinfectant can inactivate virucidal activity in SARS-CoV-2 (15).

Electrolyzed water contains a variety of ions that function as antiseptics and disinfectants (7). Electrolyzed water is rich in hypochlorite ions and sodium hydroxide ions, producing these ions from sodium chloride and water electrolysis. Hypochlorite ions have anti-bacterial, anti-viral, and anti-fungal effects (16,17). Ion hypochlorite can damage bacterial cell wall composed of complex proteins, lipids and carbohydrates (18). The viral capsid consists of simpler proteins than the bacterial cell wall, so the hypochlorite ion can quickly destroy the capsid. (16,17). The ability of hypochlorite ions is strengthened by sodium hydroxide ions that fight as a detergent and can accelerate the lysis of the viral protective wall (capsid) (16).

Electrolyzed water has other advantages in terms of safety of use. In our study, electrolyzed water was found safer for the skin than former antiseptic, and in various concentration proved safe on Vero cells and human skin applications. Electrolyzed water is rich in ions, where these ions are easily degraded when they come in contact with the skin (19). This is different from chemical substances found in antiseptics in general, where these substances are generally not easily degraded. This slow degradation intends to make chemical contact with the skin and mucosa sufficiently. However, the side effect is that chemicals in antiseptics can generally trigger a hypersensitivity reaction on the skin. Inflammatory reactions in the skin will damage the skin barrier and facilitate the infection of microorganisms on the skin (20).

In conclusion, electrolyzed water is effective as a new antiseptic and disinfectant against the virus and is safe for human skin.

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No specific funding was received for this study.

TRANSPARENCY DECLARATION

Conflict of interest: None to declare.

Table 3. Summary of skin reactions in four participants

Age (years), ethnicity	Time before reaction	Description	Allergies	Patch-test results description
25, Sumatran	Immediately on contact	Red, blotchy, itching, progressing to cracks.	History of eczema	Negative
31, Javanese	Immediately on contact	Fine white rash with red centre, itching.	History of eczema and asthma	2+ with blisters
22, Sumatran	Immediately on contact	Itching, progressing to dry.	Amoxicillin	2+ with blisters
35, Sumatran	Immediately on contact	Itching progressing to excessive dryness; cracked.	History of eczema	1+, raised erythema, no blisters

REFERENCES

1. Song, Z, Xu Y, Bao L, Zhang L, Yu P, Qu Y, Zhu H, Zhao W, Han Y, Qin C. From SARS to MERS, thrusting coronaviruses into the spotlight. Viruses 2019; 11:59.
2. Liu SL, Saif L. Emerging viruses without borders: The Wuhan coronavirus. Viruses 2020; 12:130.
3. Chan JF, Yuan S, Kok KH, To KK, Chu H, Yang J, Xing F, Liu J, Yip CC, Poon RW, et al. A familial cluster of pneumonia associated with the 2019 novel coronavirus indicating person-to-person transmission: a study of a family cluster. Lancet 2020; 395:514-23.
4. Jin Y, Yang H, Ji W, Wu W, Chen S, Zhang W, Duan G. Virology, epidemiology, pathogenesis, and control of COVID-19. Viruses 2020; 12:372.
5. Wulandari P, Hidayat R. General anxiety disorder-related coronavirus disease-19 outbreak in Indonesia: a case report. Open Access Maced J Med Sci 2020; 8:36-8.
6. Yan P, Daliri EB, Oh DH. New clinical applications of electrolyzed water: a review. Microorganisms 2021; 9:136.
7. Jing JLJ, Yi TP, Bose RJC, McCarthy JR, Tharmalingam N, Madheswaran T. Hand sanitizers: a review on formulation aspects, adverse effects and regulations. Int J Environ Res Public Health 2020; 17:3326.
8. Duarte I, Silveira JEPS, Hafner MF. Sensitive skin: review of an ascending concept. An Bras Dermatol 2017; 92:521-5.
9. Olowatuyi SV, Agbele AT, Ogunrinde ME, Ayo ATV, Ayo AM, Fayoke AB, Funmilayo OM, Deborah AA. Alcohol-based hand sanitizers: review of efficacy and adverse effect. J Health Med Nursing 2020; 81:102-17.
10. Athayde DR, Flores DRM, Silva JS, Silva MS, Genro ALG, Wagner R, Campagnol PCB, Menezes CR, Cichoski AJ. Characteristic and use of electrolyzed water in food industries. Int Food Res J 2018; 25:12-6.
11. Kim HJ, Tango CN, Chelliah R, Oh DH. Sanitization efficacy of slightly acidic electrolyzed water again pure cultures of Escheriscia coli, Salmonella enterica, Typhimurium, Staphylococcus aureus and Bacillus cereus spores, in comparison with different water hardness. Sci Rep 2019; 9:4348.-
12. Moorman E, Montazeri N, Jaykus LA. Efficacy of neutral electrolyzed water for inactivation of human norovirus. Appl Environ Microbiol 2017; 8:e00653-17.
13. Sakuma C, Sekizuka T, Kuroda M, Kasai F, Saito K, Ikeda M, Yamaji T, Osada N, Hanada K. Novel endogenous simian retroviral integrations in Vero cells: implications for quality control of a human vaccine cell substrate. Sci Rep 2018; 8:644.
14. Eichenfield LF, Ahluwalia J, Waldman A, Borok J, Udkoff J, Boguniewicz M. Current guidelines for the evaluation and management of atopic dermatitis: a comparison of the joint task force practice parameter and American Academy of Dermatology guidelines. J Allergy Clin Immunol 2017; 139:S49-57.
15. Takeda Y, Uchiumi H, Matsuda S, Ogawa H. Acidic electrolyzed water potently inactivates SARS-CoV-2 depending on the amount of free available chlorine contacting with the virus. Biochem Biophys Res Commun. 2020; 530:1-3.
16. Block MS, Rowan BG. Hypochlorous acid: A review. J Oral Maxillofac Surg 2020; 78:1461-6.
17. Sipahi H, Reis R, Dinc O, Kavaz T, Dimoglo A, Aydin A. In vitro biocompatibility study approaches to evaluate the safety profile of electrolyzed water for skin and eye. Human Exp Toxicol 2019; 38:1314-26.
18. Dewi FR, Stanley R, Powell SM, Burke CM. Application of electrolysed oxidising water as a sanitiser to extend the shelf-life of seafood products: a review. J Food Sci Technol 2017; 54:1321-32.
19. Fadriquela A, Sajo MEJ, Bajgai J, Kim DH, Kin CS, Kim SK, Lee KJ. Effects of strong acidic electrolyzed water in wound healing via inflammatory and oxidative stress response. Oxid Med Cell Longev 2020; 2459826.
20. Novak-Bilic G, Vucic M, Japundzic I, Mestrovic-Stefekov J, Lugovic-Mihic L. Irritant and allergic contact dermatitis-skin lesion characteristics. Acta Clin Croat 2018; 57:713-20.

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Mean age (years)	32.6	39.0	0.11
History of itchy, sore hands (No, %)	2 (21.1)	2 (2.9)	0.047

Three of seven participants who showed reactions to electrolyzed water chose not to follow the study follow-up. The remaining four participants with reactions were patch tested to electrolyzed water. The patch test was positive in three of the four participants who reacted to electrolyzed water (Table 3).

DISCUSSION

Electrolyzed water with a higher concentration can increase the reduction factor value and show the ability of electrolyzed water as an antiseptic and disinfectant against coronaviruses. The results of this study are supported by research by Takeda, which states that the use of acidic electrolyzed water as a contact disinfectant can inactivate virucidal activity in SARS-CoV-2 (15).

Electrolyzed water contains a variety of ions that function as antiseptics and disinfectants (7). Electrolyzed water is rich in hypochlorite ions and sodium hydroxide ions, producing these ions from sodium chloride and water electrolysis. Hypochlorite ions have anti-bacterial, anti-viral, and anti-fungal effects (16,17). Ion hypochlorite can damage bacterial cell wall composed of complex proteins, lipids and carbohydrates (18). The viral capsid consists of simpler proteins than the bacterial cell wall, so the hypochlorite ion can quickly destroy the capsid. (16,17). The ability of hypochlorite ions is strengthened by sodium hydroxide ions that fight as a detergent and can accelerate the lysis of the viral protective wall (capsid) (16).

Electrolyzed water has other advantages in terms of safety of use. In our study, electrolyzed water was found safer for the skin than former antiseptic, and in various concentration proved safe on Vero cells and human skin applications. Electrolyzed water is rich in ions, where these ions are easily degraded when they come in contact with the skin (19). This is different from chemical substances found in antiseptics in general, where these substances are generally not easily degraded. This slow degradation intends to make chemical contact with the skin and mucosa sufficiently. However, the side effect is that chemicals in antiseptics can generally trigger a hypersensitivity reaction on the skin. Inflammatory reactions in the skin will damage the skin barrier and facilitate the infection of microorganisms on the skin (20).

In conclusion, electrolyzed water is effective as a new antiseptic and disinfectant against the virus and is safe for human skin.

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TRANSPARENCY DECLARATION

Conflict of interest: None to declare.

Table 3. Summary of skin reactions in four participants

Age (years), ethnicity	Time before reaction	Description	Allergies	Patch-test results description
25, Sumatran	Immediately on contact	Red, blotchy, itching, progressing to cracks.	History of eczema	Negative
31, Javanese	Immediately on contact	Fine white rash with red centre, itching.	History of eczema and asthma	2+ with blisters
22, Sumatran	Immediately on contact	Itching, progressing to dry.	Amoxicillin	2+ with blisters
35, Sumatran	Immediately on contact	Itching progressing to excessive dryness; cracked.	History of eczema	1+, raised erythema, no blisters

REFERENCES

1. Song, Z, Xu Y, Bao L, Zhang L, Yu P, Qu Y, Zhu H, Zhao W, Han Y, Qin C. From SARS to MERS, thrusting coronaviruses into the spotlight. Viruses 2019; 11:59.
2. Liu SL, Saif L. Emerging viruses without borders: The Wuhan coronavirus. Viruses 2020; 12:130.
3. Chan JF, Yuan S, Kok KH, To KK, Chu H, Yang J, Xing F, Liu J, Yip CC, Poon RW, et al. A familial cluster of pneumonia associated with the 2019 novel coronavirus indicating person-to-person transmission: a study of a family cluster. Lancet 2020; 395:514-23.
4. Jin Y, Yang H, Ji W, Wu W, Chen S, Zhang W, Duan G. Virology, epidemiology, pathogenesis, and control of COVID-19. Viruses 2020; 12:372.
5. Wulandari P, Hidayat R. General anxiety disorder-related coronavirus disease-19 outbreak in Indonesia: a case report. Open Access Maced J Med Sci 2020; 8:36-8.
6. Yan P, Daliri EB, Oh DH. New clinical applications of electrolyzed water: a review. Microorganisms 2021; 9:136.
7. Jing JLJ, Yi TP, Bose RJC, McCarthy JR, Tharmalingam N, Madheswaran T. Hand sanitizers: a review on formulation aspects, adverse effects and regulations. Int J Environ Res Public Health 2020; 17:3326.
8. Duarte I, Silveira JEPS, Hafner MF. Sensitive skin: review of an ascending concept. An Bras Dermatol 2017; 92:521-5.
9. Olowatuyi SV, Agbele AT, Ogunrinde ME, Ayo ATV, Ayo AM, Fayoke AB, Funmilayo OM, Deborah AA. Alcohol-based hand sanitizers: review of efficacy and adverse effect. J Health Med Nursing 2020; 81:102-17.
10. Athayde DR, Flores DRM, Silva JS, Silva MS, Genro ALG, Wagner R, Campagnol PCB, Menezes CR, Cichoski AJ. Characteristic and use of electrolyzed water in food industries. Int Food Res J 2018; 25:12-6.
11. Kim HJ, Tango CN, Chelliah R, Oh DH. Sanitization efficacy of slightly acidic electrolyzed water again pure cultures of Escheriscia coli, Salmonella enterica, Typhimurium, Staphylococcus aureus and Bacillus cereus spores, in comparison with different water hardness. Sci Rep 2019; 9:4348.-
12. Moorman E, Montazeri N, Jaykus LA. Efficacy of neutral electrolyzed water for inactivation of human norovirus. Appl Environ Microbiol 2017; 8:e00653-17.
13. Sakuma C, Sekizuka T, Kuroda M, Kasai F, Saito K, Ikeda M, Yamaji T, Osada N, Hanada K. Novel endogenous simian retroviral integrations in Vero cells: implications for quality control of a human vaccine cell substrate. Sci Rep 2018; 8:644.
14. Eichenfield LF, Ahluwalia J, Waldman A, Borok J, Udkoff J, Boguniewicz M. Current guidelines for the evaluation and management of atopic dermatitis: a comparison of the joint task force practice parameter and American Academy of Dermatology guidelines. J Allergy Clin Immunol 2017; 139:S49-57.
15. Takeda Y, Uchiumi H, Matsuda S, Ogawa H. Acidic electrolyzed water potently inactivates SARS-CoV-2 depending on the amount of free available chlorine contacting with the virus. Biochem Biophys Res Commun. 2020; 530:1-3.
16. Block MS, Rowan BG. Hypochlorous acid: A review. J Oral Maxillofac Surg 2020; 78:1461-6.
17. Sipahi H, Reis R, Dinc O, Kavaz T, Dimoglo A, Aydin A. In vitro biocompatibility study approaches to evaluate the safety profile of electrolyzed water for skin and eye. Human Exp Toxicol 2019; 38:1314-26.
18. Dewi FR, Stanley R, Powell SM, Burke CM. Application of electrolysed oxidising water as a sanitiser to extend the shelf-life of seafood products: a review. J Food Sci Technol 2017; 54:1321-32.
19. Fadriquela A, Sajo MEJ, Bajgai J, Kim DH, Kin CS, Kim SK, Lee KJ. Effects of strong acidic electrolyzed water in wound healing via inflammatory and oxidative stress response. Oxid Med Cell Longev 2020; 2459826.
20. Novak-Bilic G, Vucic M, Japundzic I, Mestrovic-Stefekov J, Lugovic-Mihic L. Irritant and allergic contact dermatitis-skin lesion characteristics. Acta Clin Croat 2018; 57:713-20.