Josai International University Bulletin Vol. 31, No. 7, March 2023 1-11

〈Research Article〉

Changes in Skin Adenosine Triphosphate after Antisepsis with and without Skin Wiping

Motoko Kitada[1)], Minoru Kabashima[1)], Satoko Hoshino[1)], Kazuko Tateno[1)], Yayoi Saito[2)]

1) Faculty of Nursing, Josai International University
2) Faculty of Nursing, Shumei University

Abstract

Optimization of skin antisepsis is essential for preventing infection related to a peripheral venous catheter (PVC). Alcohol is a widely used disinfectant and its efficacy is influenced by organic substances on the skin. PVCs are most often inserted in the forearm, which is believed to be covered with organic substances because this area has frequent contact with environmental surfaces. Wiping off non-visible organic substances prior to antisepsis may serve to optimize skin cleanliness. In this study, experimental sites on the forearm were disinfected with alcohol, preceded by skin wiping (two-step procedure) or no skin wiping (one-step procedure). Transparent dressings were applied to the sites immediately after antisepsis and kept in place for 72 hours. Adenosine triphosphate (ATP) bioluminescence using the total adenylate test (A3: ATP + adenosine diphosphate [ADP] + adenosine monophosphate[AMP]) and pH were measured before and after intervention to evaluate skin cleanliness. We analyzed the data of 23 participants. ATP was dramatically decreased immediately after antisepsis in both procedures; however, ATP was significantly lower in the two-step procedure than in the one-step procedure ($p<.0001$).

At 72 hours after antisepsis, ATP values were below 1,000 relative light unit (RLUs) in both procedures, with no significant difference. No difference in pH was found. Considering the threshold of 2,000 RLU in the evaluation of hand cleanliness, wiping the skin before antisepsis at the time of catheter insertion is unlikely to contribute to the prevention of catheter-related infection.

Key words: adenosine triphosphate, alcohol, bed baths, catheter-associated infection, skin

1. Introduction

Peripheral venous catheters (PVC) are the most frequently used invasive devices in hospitals. It is estimated that up to 70% of patients require a peripheral venous line during their hospital stay (Zingg &

Pittet, 2009). A frequent complication with PVCs is infection, and skin florae are believed to be a main source of pathogens in catheter-related infections (Mermel, 2011). Skin preparation with an antiseptic (70% alcohol, tincture of iodine, or an iodophor or chlorhexidine gluconate) before PVC insertion is recommended for the prevention of infections (O'Grady et al., 2011). Alcohol is widely used to disinfect the PVC insertion site in clinical settings throughout Japan.

Alcohol denatures protein and is known to modulate lipid bilayer properties (Ingólfsson & Andersen, 2011). Therefore, the efficacy of alcohol in reducing bacterial counts is not only influenced by proteinaceous material on the skin (Boyce & Pittet, 2002), but also affected by the presence of lipids. It is considered that when the skin is visibly soiled or contaminated, the skin should be cleaned before application of an antiseptic solution (Boyce & Pittet, 2002).

A PVC is commonly inserted in the forearm, which has frequent contact with environmental surfaces, and the dorsal side of the forearm is rich in hairs, pores, and sebaceous glands. This means that forearm skin is covered with organic substances, some of which are invisible, and these can interfere with the efficacy of alcohol.

Bed baths are often used to clean the skin of patients, especially for those who are bedridden. It is reported that bed baths can reduce sebum as well as adenosine triphosphate (ATP) on the skin (Hashimoto & Saeki, 2003), making bed baths an effective way to clear organic residues as well as microorganisms from the skin. Although US Centers for Disease Control and Prevention guidelines do not outline a procedure for skin cleaning before the disinfection of skin that is not visibly soiled, as part of skin preparation before PVC insertion. Japanese nurses often perform bed baths before starting a PVC, with the expectation that this can help to reduce the risk of infection, especially in patients who are bedridden. Although the antimicrobial activity of alcohol has been widely studied (Harrington & Walker, 1903; Larson & Morton, 1991; Price, 1939), to our knowledge, there are no studies examining whether skin cleaning prior to the application of alcohol—even on skin that is not visibly soiled—can be beneficial to skin cleanliness. We therefore conducted the present study to compare skin cleanliness between two experimental sites; one site was disinfected with 83% alcohol preceded by skin wiping and the other was disinfected with alcohol only. In the present study, we used ATP as an indicator to evaluate skin cleanliness.

The presence of ATP indicates the presence of biological secretions, microorganisms, and organic residue. ATP bioluminescence testing is based on a reaction that occurs naturally in the North American firefly, *Photinus pyralis*. Catalyzed by the enzyme luciferase, this reaction uses chemical energy contained in the ATP molecule to drive the oxidative decarboxilation of luciferin, with the resultant production of light. Light output, and ATP content, are measured in relative light units (RLUs) using a luminometer (Aycicek et al., 2006). The ATP level in this test is not always correlated with the viable bacteria count. However, Aycicek et al. (2006) and Bakke et al. (2018) showed that low levels of ATP were correlated with the number of viable bacteria, although they found that low viable bacteria counts,

or no viable bacteria—were detected in some samples showing high ATP levels. ATP bioluminescence testing has been widely used in sanitation and hygiene monitoring (Tršan et al., 2020; Sogin et al., 2020; Bakke & Suzuki, 2018) and for the evaluation of skin cleanliness (Ishii et al., 2019; Konya et al., 2020).

Peripheral catheters are normally kept under the dressing and are not replaced at least for 72 hours unless clinically indicated (O'Grady, 2011). In this study, we compared skin cleanliness, with and without skin wiping, immediately after antisepsis and at 72 hours after antisepsis, once the skin had been covered with the dressing. We hypothesized that wiping the skin before antisepsis would maximize the effect of alcohol and result in cleaner skin with reduced proteinaceous material in comparison with antisepsis alone and that skin cleanliness could be maintained for 72 hours under a dressing, which would contribute to the inhibition of bacterial growth and catheter-related infection.

2. Methods

2.1 Study design and participants

In this study, we used a within-participant, cross-sectional, quasi-experimental design. Healthy adults aged 18 years and older were recruited from an educational institute in Chiba, Japan. Those who were allergic to alcohol, sensitive to adhesive medical tape, or who had skin abnormalities at the experimental site were excluded from the study. This study was approved by the ethics review board of the authors' affiliated university (reference no. 04X200014, 2 December 2020). Written informed consent was obtained from all participants. Upon study inclusion, participants were instructed to take a bath before midnight and to refrain from shaving the left forearm on the day before the intervention. Participants were also asked not to take a bath or apply any substances to the experimental site on the day of the intervention.

2.2 Procedures

We selected two experimental areas the size of the dressing that was applied later (6×7 cm) on the left side of the forearm in each participant. Each site was disinfected with 83% (v/v) ethanol preceded by skin wiping (two-step procedure) or with no skin wiping (one-step procedure). We alternated the procedure (one- or two-step) between the central and distal sites among participants (Figure 1). All procedures were performed by one trained researcher to standardize the procedure.

In the one-step procedure, the researcher disinfected the site with 83% (v/v) alcohol (Alwety®one2 ethanol, Osaki Medical, Japan) by wiping in a single direction, followed by air drying. In the two-step procedure, the site was first cleaned twice with a disposable rayon and polyester washcloth saturated with warm (40℃) water, with scrubbing in a single direction and a different washcloth surface used each time; the site was then disinfected with 83% (v/v) alcohol. After outcome measurement (described below), the sites were immediately dressed with a semi-permeable transparent dressing

(9534HP, Tegaderm™, 3M, MN, USA), which was kept in place for 72 hours. Participants were required to adhere to certain restrictions during the following 72 hours: the experimental sites were to be kept away from water and covered with a waterproof dressing before showering, and participants were to refrain from performing any exercise or activities that might cause perspiration.

2.3 Outcome measures

The total adenylate test (A3; ATP + adenosine diphosphate [ADP] + adenosine monophosphate[AMP]) was used to evaluate cleanliness of the skin. ATP, which is the universal energy-carrying molecule produced in living cells, can be unstable and decomposes into ADP and AMP via enzymatic reaction, heating, and pH. In the A3 monitoring test, ADP and AMP are converted back into ATP by pyruvate kinase and pyruvate orthophosphate dikinase; ATP levels are determined using the firefly luciferase–luciferin reaction (Sakakibara et al., 2003). The A3 test has higher detection sensitivities than the conventional ATP test for organic substances associated with health care settings (Bakke et al., 2019).

In addition to total adenylate, skin pH was measured as an environmental factor that influences microbial growth. The skin surface normally has a mildly acidic pH (4.5–5.75), with functions in cutaneous antimicrobial defense (Schmid-Wendtner & Korting, 2006). Most bacteria prefer ambient pH that is neutral to slightly alkaline for bacterial adhesion, growth, and invasion, and microbial metabolites further increase the skin pH (Rippke et al., 2018).

To collect samples for testing of skin total adenylate, the skin surface in a specific area at the experimental site (Figure 1) was wiped three times using a dedicated swab, following our previous study (Kitada et al., 2022). ATP was reacted as a reagent, and RLUs were quantified using the Lumitester

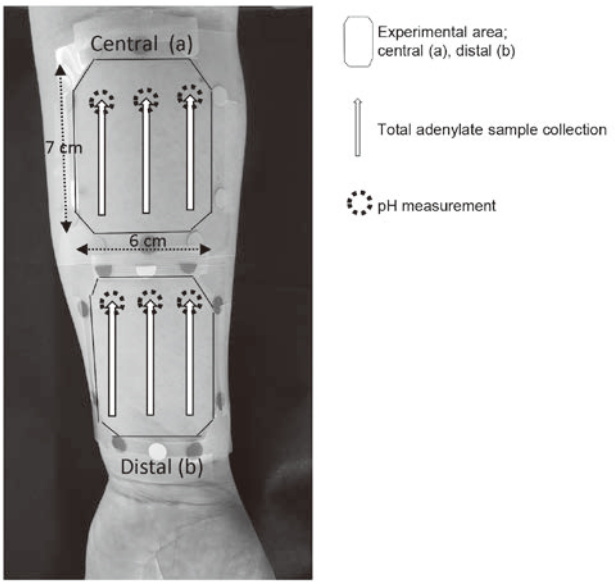

Figure 1 Experimental areas and measurements

Smart (Kikkoman Biochemifa Company, Japan). ATP levels were measured three times: before the procedure (baseline), immediately after antisepsis, and 72 hours after antisepsis. Skin pH was measured before the procedure and 72 hours later. Skin pH was measured using the Skin-pH-meter PH 905 (Courage+Khazaka electronic GmbH, Koln, Germany) at a specific area on the skin (Figure 1) in an air-conditioned room (21.6±1.7℃, 49.5%±7.1% relative humidity). Skin pH measurement was performed in triplicate, and the average of three measurements was used for the analysis.

2．4 Statistical analysis

The data are expressed as mean with standard deviation (*SD*) when normally distributed. In the case of a skewed distribution, median with interquartile range are reported. To assess statistical significance, we used the paired-samples *t*-test or Wilcoxon signed-rank test. A p-value ≤.05 was considered statistically significant. All statistical analyses were performed using JMP®11 (SAS Institute Japan Ltd., Tokyo, Japan).

3．Results

Between January and April 2021, we included 24 women aged 19–22 years in this study. Participants followed all instructions and restrictions on the day prior to, on the day of, and 72 hours after the experimental procedures. All experimental sites were visibly clean and free of any visible changes to the skin, and no loosening nor breaks in the transparent dressing were observed during the study. We excluded one participant with outlier data at baseline and analyzed the data of 23 participants.

Time-course ATP changes are presented in Figure 2. In the one-step procedure, ATP levels at baseline, immediately after antisepsis, and 72 hours after antisepsis were 2223 (1130) RLU, 424 (338–

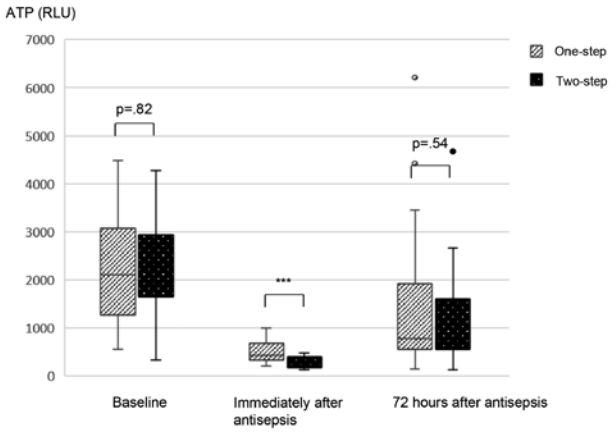

Figure 2 Time- course ATP changes

ATP (RLU) at baseline, immediately after antisepsis, and 72 hours after antisepsis in the one-step and two-step procedure (*n*=23)
*** *p*<.0001, Wilcoxon signed-rank.
ATP, adenosine triphosphate; RLU, relative light unit.

691) RLU, and 772 (563–1926) RLU, respectively. In the two-step procedure, ATP levels were 2169 (971) RLU, 222 (173–394) RLU, and 978 (554–1610) RLU, respectively. Baseline ATP levels showed no significant difference between procedures (p=.82). Although ATP levels were dramatically decreased immediately after antisepsis in both procedures, the ATP level was significantly lower in the two-step procedure (p<.0001). ATP increased over time after antisepsis in both procedures and there was no significant difference between the procedures at 72 hours.

Time-course pH changes are shown in Table 1. No significant differences were found between procedures both at baseline and at 72 hours after antisepsis (*p*=.59, *p*=.89, respectively). Skin pH was significantly decreased at 72 hours after antisepsis compared with the pH at baseline in both procedures (*p*<.0001).

Table 1 Time-course pH changes†

	One-step procedure	Two-step procedure	*p**
Baseline, mean (*SD*)	5.64 (0.42)	5.62 (0.41)	0.59
72 hours after antisepsis, mean (*SD*)	4.80 (0.18)	4.80 (0.22)	0.89

† pH at baseline and 72 hours after antisepsis in one-step and two-step procedure (*n*=23).
* Paired-samples *t*-test.
SD, standard deviation.

4. Discussion

In this study, we found that skin ATP was significantly decreased using the two-step procedure, indicating that wiping the skin before antisepsis with alcohol reduced organic residues and microorganisms on the skin. However, ATP levels under the dressing site were not significantly different 72 hours after antisepsis, with and without skin wiping, contrary to our expectations. The manufacturer instructions for the A3 test state that a skin sample can be considered “clean” if the ATP level is below 2,000 RLU after handwashing (Kikkoman Biochemifa Company, Operation manual, accessed 1 October 2022). Considering this value, at 72 hours after antisepsis, the skin could be evaluated as equally “clean” using both procedures because ATP levels were below 1,000 RLU at this time point. Although the ATP increased over time after antisepsis, increased ATP does not necessarily indicate bacterial growth on the skin because ATP is produced during normal cellular activity (Bonora et al., 2012). Additionally, skin pH was significantly decreased 72 hours after antisepsis in comparison with baseline pH, indicating that bacterial growth on the skin under the dressing was unlikely. We presumed that acidification of the skin was caused by the intrinsic pH of the dressing base.

ATP was significantly decreased immediately after antisepsis in the two-step procedure; however, wiping the skin before antisepsis did not affect cleanliness of the skin at 72 hours. Thus, the

question remains as to whether the temporal reduction in skin ATP at the time of catheter insertion (i.e., immediately after antisepsis) serves any role in controlling infection.

Bacteria on the skin are divided into two categories, transient and resident (Price, 1938). Transient florae colonize the superficial layers of the skin and are believed to be related to hospital-acquired infection whereas resident florae are attached to deeper layers of the skin, making them less likely to be associated with infection (Boyce et al., 2002). When considering infection control, it may be necessary to determine not only bacterial counts but also the types of flora targeted in antisepsis.

Maximal barrier precautions (surgical hand antisepsis and wearing a gown, cap, mask, and sterile gloves) during central venous catheter insertion contribute to reducing the risk of serious catheter-related infection (Lorente, 2019; Kinoshita et al., 2019; Mermel, 2000). It is considered that maximal barrier precautions are an effective way to prevent the ingress of surrounding superficial pathogenic transient flora along the catheter at the time of insertion, resulting in reduced infection risk. However, it is possible that the temporal reduction in ATP shown in our two-step procedure was a result of additional reduction of organic residues and microorganisms, possibly resident flora, which are less likely to be associated with infection.

A previous study showed that scrubbing the skin with an antiseptic detergent before application of povidone iodine/ethanol or chlorhexidine/isopropyl alcohol was not associated with a significant difference in catheter colonization and catheter-related infection among patients with a peripheral arterial catheter or central venous catheter (Mimoz et al., 2015). Taken together, it seems reasonable that wiping the skin before antisepsis with alcohol would cause no changes in the incidence of future catheter-related infection.

Nurses commonly perform bed baths before PVC insertion, with the expectation that this procedure reduces the risk of catheter-related infection, especially in patients who are bedridden. Considering our results, unless the skin is visibly soiled, it is unlikely that bed baths at the time of catheter insertion contribute to preventing catheter-related infection. Importantly, however, several studies have indicated that daily bathing with a 2% chlorhexidine-impregnated washcloth reduces the incidence of primary bloodstream infection among patients in the intensive care unit (Climo et al., 2013, Bleasdale et al., 2007). It should be noted that daily bed baths during infusion therapy would prevent the migration of transient flora under the dressing and contribute to reducing the risk of catheter-related infection.

5. Study limitations

Our study has several limitations. First, we could not measure bacterial counts on the skin using culture methods. Although ATP bioluminescence can reflect the bacterial count (Sogin et al., 2020),

culturing may have provided additional important information in this study. Second, we performed our experiments among participants with healthy, intact skin. Therefore, our results, especially those regarding skin cleanliness under the dressing, are not applicable to patients with a catheter. Third, although we hypothesized that wiping the skin before antisepsis would maximize the effect of alcohol and result in cleaner skin, we could not confirm whether the efficacy of alcohol was enhanced by wiping the skin. The decrease in ATP with the two-step procedure might be the result of removing ATP via skin wiping but may not be owing to enhanced alcohol efficacy. Additionally, ATP samples at 72 hours after antisepsis were collected from the skin after the dressing had been stripped off. It was impossible to prevent the dressing adhesive stripping off the stratum corneum to some degree. Thus, differences in the skin layers from which skin ATP samples were taken could affect our results. Finally, the environment and daily activities of participants might have affected our results; however, the impact of these should be limited because participants were required to adhere to restrictions during the experimental period and both the one-step and two-step procedures were performed at the same time on the left arm of each participant.

6. Conclusions

Although antisepsis with 83% alcohol preceded by skin wiping significantly decreased skin ATP levels in comparison with antisepsis only, ATP and skin pH under the dressing were not significantly different 72 hours after antisepsis. Our results showed that the effect of wiping the skin before antisepsis was temporary and did not help in maintaining cleanliness of the skin under the dressing site. We conclude that the temporal reduction in ATP in our study was possibly caused by the additional reduction of resident flora and is unlikely to be associated with reduced infection risk.

Acknowledgments

The authors would like to thank all participants for their cooperation and participation in our study. We also thank Dr. Shigeo Yamamura for his support in statistical analysis. This study was supported by a Josai International University research grant. We thank Analisa Avila, MPH, ELS, of Edanz (https://jp.edanz.com/ac) for editing a draft of this manuscript.

Disclosure

The authors declare no conflicts of interest.

Contributors

M.K. designed the study, was responsible for data collection and data analysis, and wrote the manuscript.

M.K, S.H., and K.T. made substantial contributions to data collection and were involved in drafting the manuscript. Y.S. was involved in drafting and critically reviewing the manuscript. All authors read and approved the final draft.

References

Aycicek, H., Oguz, U., Karci, K. (2006). Comparison of results of ATP bioluminescence and traditional hygiene swabbing methods for the determination of surface cleanliness at a hospital kitchen. *International Journal of Hygiene and Environmental Health, 209(2)*, 203-6. doi: 10.1016/j.ijheh.2005.09.007

Bakke, M., Sato, N., Suzuki, S., Kirihara, E., Watanabe, & M., Mikami, S. (2018). Sou Adenireito (ATP+ADP+AMP) wo Shihyou tosuru Shinki na Seijyoudo Kensa (A3 Hou) no Kaihatsu [Development of a Novel Hygiene Monitoring System Based on the Detection of Total Adenylate (ATP+ADP+AMP)]. *Japanese Society of Medical Instrumentation, 88(3)*, 357-363. doi: 10.4286/jjmi.88.357

Bakke, M., & Suzuki, S. (2018). Development of a novel hygiene monitoring system based on the detection of total adenylate (ATP+ADP+AMP). *Journal of Food Protection*, *81*(5), 729-737. https://doi.org/10.4315/0362-028X.JFP-17-432

Bakke, M., Suzuki, S., Kirihara, E., & Mikami, S. (2019). Evaluation of the total adenylate (ATP + ADP + AMP) test for cleaning verification in healthcare settings. *Journal of Preventive Medicine and Hygiene*, *60(2)*, E140-E146. doi: 10.15167/2421-4248/jpmh2019.60.2.1122

Bleasdale, S. C., Trick, W. E., Gonzalez, I. M., Lyles, R. D., Hayden, M. K., & Weinstein R.A. (2007). Effectiveness of chlorhexidine bathing to reduce catheter-associated bloodstream infections in medical intensive care unit patients. *Archives of Internal Medicine*, *167*(19), 2073-2079. https://doi.org/10.1001/archinte.167.19.2073

Bonora, M., Patergnani, S., Rimessi, A., De Marchi, E., Suski, JM., Bononi, A., ... Pinton, P. (2012). ATP synthesis and storage. *Purinergic Signalling*, *8(3),* 343-57. doi: 10.1007/s11302-012-9305-8

Boyce, J. M., & Pittet, D. Healthcare Infection Control Practices Advisory Committee; HICPAC/SHEA/APIC/IDSA Hand Hygiene Task Force. (2002). Guideline for Hand Hygiene in Health-Care Settings. Recommendations of the Healthcare Infection Control Practices Advisory Committee and the HICPAC/SHEA/APIC/IDSA Hand Hygiene Task Force. *Morbidity and Mortality Weekly Report Recommendations and Reports*, *51*(RR-16), 1-45, quiz CE1-4.

Climo, M. W., Yokoe, D. S., Warren, D. K., Perl, T. M., Bolon, M., Herwaldt, L. A., ... Wong, E.S. (2013). Effect of daily chlorhexidine bathing on hospital-acquired infection. *The New England Journal of Medicine*, *368*(6), 533-542. https://doi.org/10.1056/NEJMoa1113849

Harrington, C., & Walker, H. (1903). The germicidal action of alcohol. *Boston Medical and Surgical Journal, 148*, 548–52.

Hashimoto, M., & Saeki, Y. (2003). Hifu no Suibunryou, Yubunryou, pH narabini Seijyoudo karamita Seishiki no Kuoka [The effects of bed bath on stratum corneum hydration, skin lipid, skin surface pH and ATP bioluminescence – in comparison with tub bath in healthy females]. *Japanese Journal of Nursing Art and Science*, *2*(1), 61-68. https://doi.org/10.18892/jsnas.2.1_61

Ingólfsson, H. I., & Andersen, O. S. (2011). Alcohol's effects on lipid bilayer properties. *Biophysical Journal, 101(4)*, 47-55. doi: 10.1016/j.bpj.2011.07.013

Ishii, K., Nakada, H., Kobayashi, H., & Kawashima, K. (2019). Dispouzaburu Taoru wo mochiita Bubun Seishiki ga Koureisha no Hifu ni ataeru Eikyou [Effect of partial bed bath using a disposable towel on the elderly]. *Japanese Journal of Nursing Art and Science*, *18*, 17-25. https://doi.org/10.18892/jsnas.18.0_17

Kikkoman Biochemifa Company. Unyou Manyual (Kijyunchi Settei nado) [Operation manual (e.g., standard value setting)]. Retrieved from https://biochemifa.kikkoman.co.jp/kit/atp/method/guide/

Kinoshita, D., Hada, S., Fujita, R., Matsunaga, N., Sakaki, H., & Ohki, Y. (2019). Maximal sterile barrier precautions independently contribute to decreased central line-associated bloodstream infection in very low birth weight infants: A prospective multicenter observational study. *American Journal of Infection Control*, *47*(11), 1365-1369. https://doi.org/10.1016/j.ajic.2019.05.006

Kitada, M., Kabashima, M., Hoshino, S., Tateno, K., & Saito, Y. (2022). Onto-seishiki niyoru Hifu Seijyoudo no Kentou -Arukoulu wo Mochiita Shoudoku tono Hikaku-[Comparison of Skin Cleanliness after Wiping with Warm Water versus after Antisepsis with Alcohol]. *Japanese Society of Nursing Art and Science*, *21.* (Accepted Oct. 14, 2022).

Konya, I., Yamaguchi, S., Sugimura, N., Matsuno, C., & Yano, R. (2020). Effects of differences in wiping pressure applied by nurses during daily bed baths on skin barrier function, cleanliness, and subjective evaluations. *Japan Journal of Nursing Science*, *17*(3), e12316. https://doi.org/10.1111/jjns.12316

Larson, E. L., & Morton, H. E. (1991). Alcohols. In Block, S. S. (ed), Disinfection, sterilization and preservation (4th ed.), PA: Lea and Febiger.

Lorente, L. (2019). Antiseptic measures during the insertion and manipulation of vascular catheters. *Medicina Intensiva* (Engl Ed), *43*(Suppl 1), 39-43. https://doi.org/10.1016/j.medin.2018.09.012

Mermel, L. A. (2000). Prevention of intravascular catheter-related infections. *Annals of Internal Medicine*, *132*(5), 391-402. https://doi.org/10.7326/0003-4819-132-5-200003070-00009

Mermel, L. A. (2011). What is the predominant source of intravascular catheter infections? *Clinical Infectious Diseases*, *52*(2), 211-212. https://doi.org/10.1093/cid/ciq108

Mimoz, O., Lucet, J. C., Kerforne, T., Pascal, J., Souweine, B., Goudet, V., ... Timsit, J.F.; CLEAN trial investigators. (2015). Skin antisepsis with chlorhexidine-alcohol versus povidone iodine-alcohol, with and without skin scrubbing, for prevention of intravascular-catheter-related infection (CLEAN): an open-label, multicentre, randomised, controlled, two-by-two factorial trial. *The Lancet*, *386*(10008), 2069-2077. https://doi.org/10.1016/S0140-6736(15)00244-5

O'Grady, N. P., Alexander, M., Burns, L. A., Dellinger, E. P., Garland, J., Heard, S. O., ... Saint, S.; Healthcare Infection Control Practices Advisory Committee (HICPAC). (2011). Guidelines for the prevention of intravascular catheter-related infections. *Clinical Infectious Disease*, *52*(9), e162-e193. https://doi.org/10.1093/cid/cir257

Price, P. B. (1938). The bacteriology of normal skin; a new quantitative test applied to a study of the bacterial flora and the disinfectant action of mechanical cleansing. *The Journal of Infectious Diseases*, *63*, 301-318. https://doi.org/10.1093/infdis/63.3.301

Price, P. B. (1939). Ethyl alcohol as a germicide. *Arch Surg, 38,* 528–42.

Rippke, F., Berardesca, E., & Weber, T. M. (2018). pH and Microbial Infections. Current *Problems in Dermatology, 54,* 87-94. doi: 10.1159/000489522

Sakakibara, T., Murakami, S., & Imai, K. (2003). Enumeration of bacterial cell numbers by amplified firefly bioluminescence without cultivation. *Analytical Biochemistry, 312(1)*, 48-56. doi: 10.1016/s0003-2697(02)00427-x

Schmid-Wendtner, M. H., & Korting, H. C. (2006). The pH of the skin surface and its impact on the barrier function. *Skin Pharmacology and Physiology, 19(6)*, 296-302. doi: 10.1159/000094670

Sogin, J. H., Lopez, V. G., Yordem, B., Lingle, C. K., David, J. M., Cobo, M., & Worobo, R. W. (2020). Implementation of ATP and microbial indicator testing for hygiene monitoring in a tofu production facility improves product quality and hygienic conditions of food contact surfaces: a case study. *Applied Environmental Microbiology*, *87*(5), e02278-e02320. https://doi.org/10.1128/AEM.02278-20

Tršan, M., Vehovc, M., Seme, K., & Srčič, S. (2020). Evaluation of ATP bioluminescence for monitoring surface hygiene in a hospital pharmacy cleanroom. *Journal Microbiological Methods*, *168*, 105785. https://doi.org/10.1016/j.mimet.2019.105785

Zingg, W., & Pittet, D. (2009). Peripheral venous catheters: an under-evaluated problem. *International Journal of Antimicrobial Agents, 34* (Suppl 4), S38-S42. https://doi.org/10.1016/S0924-8579(09)70565-5