UNDERSTANDING OF HAND RUB PRODUCT EFFECTIVENESS: INFLUENCE OF DIFFERENT CULTURE SAMPLING TECHNIQUES
POROZUMENIE ÚČINNOSTI RUČNÉHO VÝROBKU: VPLYV RÔZNYCH TECHNÍK ODBERU KULTÚRY

RIADINI Dwita[1], WARDHANI Puspa[2], AKBAR Kurnia Ardiansyah[3]

[1] *Clinical Pathology Specialization Programme, Faculty of Medicine, Airlangga University-Dr. Soetomo Hospital, Surabaya, Indonesia*
[2] *Department of Clinical Pathology, Faculty of Medicine, Airlangga University-Dr. Soetomo Hospital, Surabaya, Indonesia*
[3] *Faculty of Public Health, Jember University, Krajan Timur, Sumbersari, Kec. Sumbersari, Jember Regency, East Java,Indonesia*

ABSTRACT
Background: Hand hygiene is the most effective way to reduce the spread of pathogens and the incidence of healthcare-associated infections (HAIs) in health care facilities. The effectiveness of the hand rub in eliminating microorganisms from the surface of hands was assessed through hand culture. Two hand culture sampling techniques that are often used to assess the effectiveness of handwashing are hand-gloves and swab technique.
Purpose: This study aims to compare the culture sampling technique in assessing the effectiveness of handwashing using alcohol-based hand rub products.
Methods: An experimental study conducted at Dr. Soetomo Hospital, Surabaya, with 20 resident and nurse respondents. Handwashing was using an alcohol-based hand rub according to the WHO recommended method. Hand culture samples were taken, before and after handwashing, using hand-gloves and swab techniques. The data were analysed using Wilcoxon Rank test, ANOVA test and Linear regression.
Results: This study showed a significant difference before and after handwashing using hand rub in eliminating microorganisms on the hands (Sig = 0.000). However, the effectiveness of using three alcohol-based hand rub products (chlorhexidine gluconate, n-propanol, and hydrogen peroxide) in eliminating microorganisms is the same. This study also shows no significant difference between the application of three hand rub products in all hand culture sampling techniques used (Sig = 0.126). There are differences in the use of culture sampling techniques to assess hand culture results (Sig = 0.000).
Conclusion: The use of alcohol-based hand rub products with the combination of chlorhexidine gluconate, n-propanol, and hydrogen peroxide has the same effectiveness in eliminating microbes on the hands surface. The hand-gloves technique better describes the elimination of microorganisms after handwashing. Although it cannot replace the hand-gloves technique, the swab technique may be used as an alternative technique in peripheral health care facilities.

Key words: Culture sampling techniques. Healthcare associated infections. Swab technique. Hand-gloves. Hand rub

ABSTRAKT
Východiská: Hygiena rúk je najúčinnejším spôsobom znižovania šírenia patogénov a výskytu infekcií spojených so zdravotnou starostlivosťou (HAI) v zdravotníckych zariadeniach. Účinnosť trenia rúk pri eliminácii mikroorganizmov z povrchu rúk sa hodnotila pomocou kultivácie rúk. Dve techniky odberu vzoriek kultúry rúk, ktoré sa často používajú na hodnotenie účinnosti umývania rúk, sú rukavice a tampón.
Účel: Cieľom tejto štúdie je porovnať techniku vzorkovania kultúry pri hodnotení účinnosti umývania rúk s použitím prostriedkov na ručné trenie na báze alkoholu.
Metódy: Experimentálna štúdia uskutočnená v nemocnici Dr. Soetomo Hospital v Surabaji s 20 obyvateľmi a zdravotnými sestrami. Pri umývaní rúk sa používal krém na ruky na báze alkoholu podľa metódy odporúčanej WHO. Vzorky ručnej kultúry sa odoberali pred a po umývaní rúk pomocou ručných rukavíc a tampónovej techniky. Dáta sa analyzovali pomocou Wilcoxonovho Rank testu, ANOVA testu a lineárnej regresie.
Výsledky: Táto štúdia preukázala významný rozdiel pred a po umytí rúk pomocou ručného trenia v eliminácii mikroorganizmov na rukách (Sig = 0,000). Účinnosť použitia troch produktov na ručné trenie na báze alkoholu (chlórhexidín glukonát, n-propanol a peroxid vodíka) pri eliminácii mikroorganizmov je však rovnaká. Táto štúdia tiež nepreukázala žiadny významný rozdiel medzi aplikáciou troch produktov ručného trenia vo všetkých použitých technikách odberu vzoriek kultivácie rúk (Sig = 0,126). Existujú rozdiely v použití techník vzorkovania kultúry na hodnotenie výsledkov kultivácie v ruke (Sig = 0,000).
Záver: Používanie produktov na ručné trenie na báze alkoholu s kombináciou chlórhexidín glukonátu, n-propanolu a peroxidu vodíka má rovnakú účinnosť pri eliminácii mikróbov na povrchu rúk. Technika ručných rukavíc lepšie popisuje elimináciu mikroorganizmov po umývaní rúk. Aj keď nemôže nahradiť techniku ručných rukavíc, môže byť tampónová technika použitá ako alternatívna technika v periférnych zdravotníckych zariadeniach.

Kľúčové slová: Techniky odberu vzoriek kultúry. Infekcie spojené so zdravotnou starostlivosťou. Technika výterov. Ručné rukavice. Trenie ruky

INTRODUCTION

Maintaining hand hygiene is one of the most effective efforts to reduce the spread of pathogens and reduce the incidence of healthcare-associated infections (HAIs) in health care facilities (Pincock

et al., 2012; Rosenthal, 2013; Ridley, 2020) Hand hygiene can be done using soap or alcohol-based hand rub (Kingston et al., 2017; Widmer et al., 2007). Assessment of the efficacy of hand hygiene products (e.g., soap or hand rub) has been widely applied to evaluate the effectiveness of the product in removing pathogens from hand surfaces (Zapka et al., 2017; Müller et al., 2020). The results of the evaluation were a comparison of the total colony count before and after the application of the product.

Various sampling techniques can affect the interpretation of the colony count in culture. The two most commonly used methods are the hand-gloves technique or the sterile bag technique and the swab technique (Foo et al., 2009; Koçak Tufan et al., 2012). Based on FDA guidelines, the hand-gloves technique was used as a reference in culture sampling to assess the effectiveness of handwashing in the operating room. This technique is complicated and takes a long time so it is considered impractical (Hansen & Knøchel, 2003). The swab technique is another method of collecting culture samples that are commonly used to assess microorganisms on hand surfaces (Visalachy et al., 2016). This technique is an easy procedure and is considered to be an alternative to the use of the hand-gloves technique because it allows the quantification of microorganisms with a proportion of the surface area of the hand (Larson et al., 1980). However, until now, the effect of these two sampling techniques on assessing the success of hand washing is still unknown.

The World Health Organization (WHO) recommends hand rub formulations containing ethanol/isopropyl alcohol, glycerol, and hydrogen peroxide as an alternative handwashing liquid besides soap (WHO, 2006; Pradhan et al., 2020). propanol is better at eliminating skin microorganisms (Kampf & Ostermeyer, 2005; Hennig et al., 2017). Alcohol-based hand rubs in combination with n-propanol also appear to be superior to chlorhexidine gluconate (Kampf & Kramer, 2004; Zandiyeh & Roshanaei, 2015).

OBJECTIVE

This study aims to compare culture sampling techniques in assessing the success of handwashing using an alcohol-based hand rub product with a combination of chlorhexidine gluconate, n-propanol, and hydrogen peroxide.

RESEARCH SAMPLE, METHODOLOGY

The design of this research is experimental research which has been approved by the ethics committee of RSUD Dr. Soetomo. The study was conducted on residents and nurses in charge of infection prevention and control (PPI) Dr. Hospital. Soetomo Surabaya in the period September to October 2019. Sampling was carried out by consecutive sampling. The total sample until the end of the study was 20 people, with the inclusion criteria of clean hands and short nails who did not use antibacterial substances and was willing to participate until the end of the study. Pregnant women, people with skin diseases, and the presence of wounds or abrasions on the hands, as well as allergies to hand rub materials, were the exclusion criteria for this study (Wilkinson et al., 2017; Turgeon, 2017; Lucero & Dryden, 2019).

Culture sampling on each subject will be carried out using 2 techniques, namely hands-gloves and swabs, on different days. Sampling and culture implementation are carried out at intervals of not more than 30 minutes.

Each research subject will take a culture sample on the dominant hand (Girou et al., 2002), before and after washing hands using several types of hand rub. Each research subject was instructed to wash hands using a hand rub according to the method recommended by WHO (Pittet et al., 2009). The hand rub used was alcohol-based with a combination of chlorhexidine gluconate, n-propanol, and hydrogen peroxide.

Swab Technique: A cotton swab is applied/wiped on the surface of the dominant hand, around the nails, and between the fingers, then the cotton swab is placed in a sterile tube containing 2 mL of Tryptic Soy Broth (TSB). Before culture, the cotton swab is removed by pressing it against the tube wall (Favero et al., 1968; Ganime et al., 2015).

Hand-gloves Technique: The dominant hand (palms, fingers especially around the nails and between the fingers, excluding the back of the hand), is dipped and rubbed on the walls of a sterile polyethylene bag containing 100 mL of TSB for 1 minute (Hansen & Knøchel, 2003; Larson et al., 1980).

Spread-plating method: Before culture, both specimens in tubes and sterile bags containing TSB were homogenized for five seconds. The culture was made without dilution by taking 0.1 mL of liquid, then spread on Tryptic Soy Agar (TSA) petri

dish media (Ehrenkranz & Alfonso, 1991; Sanders, 2012).

Quantification of Culture Results: The number of bacteria in the petri dish was calculated after incubation for 18-24 hours at a temperature of 35-37ºC. Calculations were carried out by 2 observers. Calculation results are presented as colony forming units (CFU) which are calculated per ml (Ehrenkranz & Alfonso, 1991).

Differences in colony counts between observers and the significance of differences in colony count before and after hand rub application were analysed using the Wilcoxon rank test (Demšar, 2006). The difference in effectiveness between several hand rub products using 2 sampling techniques was analysed using the ANOVA test and followed by the Newman-Keuls post hoc test with Sig. < 0.005 (Weissgerber et al., 2018). Regression analysis was used to find the relationship between colony counts, which were taken using swab and hand-gloves sampling techniques (Stapor, 2020)

RESULTS

This study was followed by 20 subjects who participated in the entire series of studies, consisting of 12 residents and 8 PPI nurses. A total of 12 cultures were obtained from various treatments that were the same for each subject. The results of hand surface culture were observed by 2 observers, where the Wilcoxon rank test difference test showed no significant difference between the colony counts of the first and second observers (Sig. > 0.005) (Table 1).

Comparative analysis of hand culture colony counts before and after hand rub application found a significant difference in the number of microorganisms on the surface of the hands (Sig = 0.000) (Table 2). The use of the three alcohol-based hand rub products (chlorhexidine gluconate, n-propanol and hydrogen peroxide) in eliminating microorganisms has the same effectiveness. This study also showed that there was no significant difference between the application of the three hand rub products in all hand culture sampling techniques used in the study (Sig = 0.126) (Table 3).

Analysis of the application of hand-gloves and swab techniques to the assessment of hand culture results obtained a significant difference (Sig = 0.000) (Table 4).

Both before and after hand rub application, the hand-gloves technique gave a higher colony count than the swab technique, this can be observed in (Table 1). From these results, the researchers tried to perform a linear regression analysis, obtained a constant difference between the application of hand-gloves and swab techniques to the assessment of hand culture results. If Y is the result of the colony count using the hand-gloves technique, and X is the result of the colony count using the swab technique, then the equation Y = 6123.7 + 6.8 X is obtained.

Table 1. Differences in colony counts between observers

Sampling technique	Beforehand rub			After hand rub		
	P-1 Min-max (mean)	**P-2 Min-max (mean)**	***Sig.***	**P-1 Min-max (mean)**	**P-2 Min-max (mean)**	***Sig.***
Swab (n = 20)	100-2 640 (685)	100-2 660 (698)	0.041	0-280 (38)	0-300 (42)	0.052
Hand-gloves (n = 20)	2000-146 000 (36 533)	2000-140.000 (36 167)	0.212	0-46.000 (6 383)	0-42 000 (6 267)	0.059

Description: The first observer (P-1), the second observer (P-2). Count the colonies in CFU (colony forming units)

Table 2. Significance of differences in colony count before and after hand rub application based on sampling technique

Comparison of colony count	*Z score*	*Sig.*
Observer 1		
Swab, after vs beforehand rub	-6.736	0.000
Hand-gloves, after vs before hand rub	-6.737	0.000
Observer 2		
Swab, after vs beforehand rub	-6.736	0.000
Hand-gloves, after vs before hand rub	-6.737	0.000

Table 3. Effectiveness of application of various hand rubs cultured using swabs and hand-gloves

Treatment	Hand rub Type		Mean different (a-b)	*Sig.*	*Sig.* Whole Group
	(a)	(b)			
Beforehand rub (Swab)	CG	HP	147	0.693	0.126
	CG	NP	21	0.992	
	NP	HP	126	0.763	
After hand rub (Swab)	CG	HP	-8	0.890	
	CG	NP	-19	0.524	
	NP	HP	11	0.803	
Beforehand rub (Hand-gloves)	CG	HP	15.350	0,222	
	CG	NP	2.400	0,953	
	NP	HP	12.950	0.339	
After hand rub (Hand-gloves)	CG	HP	5.150	0,085	
	CG	NP	1.050	0,898	
	NP	HP	4.100	0,204	

Description: Chlorhexidine-gluconate (CG), n-propanol (NP), hydrogen peroxide (HP)

Table 4. Significance of the influence of hand-gloves and swab techniques on the count of microorganism

Count colonies Hand-gloves vs swab	Z score	*Sig.*
Observer 1		
Beforehand rub	-6.449	0.000
After hand rub	-6.449	0.000
Observer 2		
Beforehand rub	-6.736	0.000
After hand rub	-6.736	0.000

DISCUSSION

The results of this study confirm that hand washing using an alcohol-based hand rub is an effective effort to eliminate the number of microorganism colonies on the surface of the hands. Alcohol has an antimicrobial effect through the mechanism of protein denaturation. Hand rub with an alcohol concentration of 60-80 % is most effective in eliminating microorganisms from the surface of the hands. Alcohol has been shown to have bactericidal effects against vegetative Gram-positive and Gram-negative (including multidrug-resistant pathogens such as MRSA and VRE) as well as several types of fungi (Pittet et al., 2009; WHO, 2006; Sandoval Vergara et al., 2019).

The combination of the active ingredients contained in the alcohol-based hand rub did not affect the number of microorganisms that were eliminated after the application of the hand rub. This can be observed in Table 3, alcohol-based hand rub, both with a combination of chlorhexidine gluconate, n-propanol, and hydrogen peroxide, has the same effectiveness in eliminating the number of microorganisms on the surface of the hands (Sig > 0.005). The results of this study are different from several previous studies. Research by Hennig and colleagues, Kampf and Ostermeyer, as well as research by Zandiyeh and Roshanaei, found that alcohol-based hand rubs in combination with n-propanol were significantly better than chlorhexidine gluconate and hydrogen peroxide. The three studies used the same culture sampling technique, which was taken from the tips of the fingers that were dipped/rubbed in the culture medium without covering the surface of the palms or between the fingers (Kampf & Ostermeyer, 2005; Zandiyeh & Roshanaei, 2015).

Assessment of the effectiveness of a hand hygiene product is recommended by calculating the total bacterial colony count on the surface. The difference in results with previous studies can be caused by differences in the surface area of culture samples so that a too narrow surface area does not reflect the distribution of microorganisms on the surface of the hands after washing hands.

Researchers compared the total colony count results from hand cultures taken using hand-gloves and swab techniques. The two sampling techniques gave the same results in assessing the effectiveness of hand rub products (Table 4) but resulted in a total colony count that was significantly different between the two techniques. Culture sampling using the hand-gloves technique resulted in a total colony

count greater than the swab technique. The volume of media exposure to the culture surface can affect these differences in the colony count results. The swab technique uses less volume of media (2 mL) than the hand-gloves technique (100 mL). The use of a cotton swab in the swab technique further reduces the volume of media exposure to the surface of the hand, while the hand-gloves technique allows exposure to a larger volume of media in a standard time.

The researcher also analysed the colony count results using the two culture sampling techniques and found a constant difference between the application of the hand-gloves and swab techniques to the assessment of hand culture results. If Y is the result of the colony count using the hand-gloves technique, and X is the result of the colony count using the swab technique, then the equation Y = 6123.7 + 6.8 X. This indicates that the swab technique can be used as an alternative to the hand-gloves technique in health facilities with limited resources. The use of these equations is limited to hand surface cultures in the research laboratory. Further research is needed with a larger sample size to validate the equation.

CONCLUSION

The use of alcohol-based hand rub with a combination of chlorhexidine gluconate, n-propanol, and hydrogen peroxide, has the same effectiveness in eliminating microorganisms on the surface of the hands. The hands-glove technique is better at describing the elimination of hand microorganisms after hand washing. Although it cannot replace the hand-gloves technique, the swab technique may be used as an alternative technique in peripheral health facilities.

REFERENCES

DEMŠAR J. Statistical comparisons of classifiers over multiple data sets. *Journal of Machine Learning Research*. 2006; 7.

EHRENKRANZ N.J., ALFONSO B.C. Failure of Bland Soap Handwash to Prevent Hand Transfer of Patient Bacteria to Urethral Catheters. *Infection Control & Hospital Epidemiology*. 1991; 12 (11), 654-662.

FAVERO M.S., MCDADE J.J., ROBERTSEN J.A. et al. Microbiological Sampling of Surfaces. *Journal of Applied Bacteriology.* 1968; 31(3): https://doi.org/10.1111/j.1365-2672.1968.tb00375.x

FOO A., SHENTHILKUMAR N., KIN-SZE CHONG A. The “hand-in-gloves” technique: vacuum-assisted closure dressing for multiple finger wounds. *Journal of Plastic, Reconstructive and Aesthetic Surgery.* 2009; 62 (5): https://doi.org/10.1016/j.bjps.2008.05.030

GANIME A.C., LEITE J.P.G., DE ABREU CORRÊA A. et al. Evaluation of the swab sampling method to recover viruses from fomites. *Journal of Virological Methods.* 2015; 217: https://doi.org/ 10.1016/j.jviromet.2015.02.009

GIROU E., LOYEAU, S., LEGRAND P. et al. Efficacy of handrubbing with alcohol based solution versus standard handwashing with antiseptic soap: Randomised clinical trial. *British Medical Journal.* 2002; 325 (7360): https://doi.org/ 10.1136/bmj.325.7360.362

HANSEN T.B., KNØCHEL S. Image analysis method for evaluation of specific and non-specific hand contamination. *Journal of Applied Microbiology.* 2003; 94 (3): 483–494. https://doi.org/10.1046/j.1365-2672.2003.01855.x

HENNIG T.J., WERNER S., NAUJOX K. et al. Chlorhexidine is not an essential component in alcohol-based surgical hand preparation: A comparative study of two handrubs based on a modified EN 12791 test protocol. *Antimicrobial Resistance and Infection Control*. 2017; 6 (1): 1-6. https://doi.org/10.1186/s13756-017-0258-0

KAMPF G., KRAMER A. Epidemiologic background of hand hygiene and evaluation of the most important agents for scrubs and rubs. *Clinical Microbiology Reviews*. 2004; 17 (4): https://doi.org/10.1128/CMR.17.4.863-893. 2004

KAMPF G., OSTERMEYER C. Efficacy of two distinct ethanol-based hand rubs for surgical hand disinfection - A controlled trial according to prEN 12791. *BMC Infectious Diseases*. 2005; 5: 1-4. https://doi.org/10.1186/1471-2334-5-17

KINGSTON L.M., O’CONNELL N.H., DUNNE C.P. Survey of attitudes and practices of Irish nursing students towards hand hygiene, including handrubbing with alcohol-based hand rub. *Nurse Education Today*. 2017; 52: https://doi.org/10.1016/j.nedt.2017.02.015

KOÇAK TUFAN Z., IRMAK H., BULUT C. et al. The effectiveness of hand hygiene products on MRSA colonization of health care workers by

using CHROMagar MRSA. *Mikrobiyoloji Bulteni.* 2012; 46 (2).

LARSON E.L., STROM M.S., EVANS C.A. Analysis of three variables in sampling solutions used to assay bacteria of hands: Type of solution, use of antiseptic neutralizers, and solution temperature. *Journal of Clinical Microbiology*. 1980; 12 (3): 355-360. https://doi.org/10.1128/jcm.12.3.355-360.1980

LUCERO S., DRYDEN M. Antisepsis, asepsis and skin preparation. *Surgery (United Kingdom).* 2019; 37 (1): https://doi.org/10.1016/j.mpsur.2018.11.008

MÜLLER S.A., DIALLO A.O.K., WOOD R. et al. Implementation of the WHO hand hygiene strategy in Faranah regional hospital, Guinea. *Antimicrobial Resistance and Infection Control*. 2020; 9 (1): https://doi.org/10.1186/s13756-020-00723-8

PINCOCK T., BERNSTEIN P., WARTHMAN S. et al. Bundling hand hygiene interventions and measurement to decrease health care-associated infections. *American Journal of Infection Control.* 2012; 40 (4 SUPPL.): https://doi.org/10.1016/j.ajic.2012.02.008

PITTET D., ALLEGRANZI B., BOYCE J. The World Health Organization Guidelines on Hand Hygiene in Health Care and Their Consensus Recommendations. *Infection Control & Hospital Epidemiology.* 2009; 30 (7): 611-622. https://doi.org/10.1086/600379

PRADHAN D., BISWASROY P., KUMAR NAIK P. et al. A Review of Current Interventions for COVID-19 Prevention. *Archives of Medical Research.* 2020; 51 (5): https://doi.org/10.1016/j.arcmed.2020.04.020

RIDLEY N. Effective hand hygiene-wash your hands and reduce the risk. *British Journal of Nursing.* 2020; 29 (1), 10. https://doi.org/10.12968/bjon.2020.29.1.10

ROSENTHAL M. The Healthcare Workers' Hand Microbiome: Community Structure and Dynamics. *Journal of Chemical Information and Modeling.* 2013; 53.

SANDERS E.R. Aseptic laboratory techniques: Plating methods. *Journal of Visualized Experiments.* 2012; 63: 1-18.

SANDOVAL VERGARA A., FARFÁN CÓRDOVA M., SALAZAR CASTILLO M.L. et al. Antibacterial effect of the hydroalcoholic extract of Mauritia flexuosa leaves on gram-negative and gram-positive bacteria. *F1000Research.* 2019; 8: :1487.

STAPOR K. *Linear Regression and Correlation. In: Introduction to Probabilistic and Statistical Methods with Examples in R.* Intelligent Systems Reference Library, 2020; vol 176. Springer, Cham. https://doi.org/10.1007/978-3-030-45799-0_3

TURGEON M.L. *Clinical hematology: Theory and procedures*, Sixth edition. In Clinical hematology: Theory and procedures, 2017; Third edition.

VISALACHY S., KUMAR K., KOPULA S.S. et al. Carriage of multidrug resistant bacteria on frequently contacted surfaces and hands of health care workers. *Journal of Clinical and Diagnostic Research.* 2016; 10 (5): DC18-DC20.

WEISSGERBER T.L., GARCIA-VALENCIA O., GAROVIC V.D. et al. Why we need to report more than 'data were analyzed by t-tests or ANOVA'. *eLife.* 2018; 7: e36163.

WHO. 2006; *World Alliance For Patient Safety: WHO Guidelines on Hand Hygiene in Health Care. WOrld Health Organization.*

WIDMER A.F., CONZELMANN M., TOMIC M. et al. Introducing Alcohol-Based Hand Rub for Hand Hygiene The Critical Need for Training. *Infection Control & Hospital Epidemiology.* 2007; 28 (1): 50-54.

WILKINSON I.B., RAINE T., WILES K. et al. *Oxford Handbook of Clinical Medicine. In Oxford Handbook of Clinical Medicine.* 2017; https://doi.org/10.1093/med/9780199689903.001.0001

ZANDIYEH M., ROSHANAEI G. Effectiveness of three surgical alcohol-based hand rubs on skin flora. *Iranian Journal of Nursing and Midwifery Research*. 2015; 20 (2): 221-225.

ZAPKA C., LEFF J., HENLEY J. et al. Comparison of standard culture-based method to culture-independent method for evaluation of hygiene effects on the hand microbiome. *MBio*. 2017; 8 (2): 1-17.