**ABSTRACT**

**Background:** Good implementation of hand hygiene and the availability of cheap, affordable, and effective hand rub can prevent healthcare-associated infection. WHO has issued hand rub formulations that are easily self-produced, effective, and affordable. **Objective:** This study compares the difference between hand rub's effectiveness stored in National Hospital Diponegoro University ER, HCU, and storeroom for 2 and 10 weeks.

**Methods:** This study was an experimental study with pretest post test randomized group design. The effectiveness of hand rub measured by prEN12054 in cfu/ml.

**Results:** There was no significant difference between efficacy of WHO A and B (p=0.458). In the Mann-Whitney test there was no difference between the effectiveness of WHO A and B formulas before storage (p = 0.567), after being stored for two weeks (p=1.000), and ten weeks (p=0.539). In the Kruskal-Wallis test, there was no difference in the effectiveness of the WHO A formula in three sites at weeks 0, 2 and 10 (p = 0.275, 0.538, 0.116), there was no difference in the effectiveness of the WHO B formula in three sites at week 0, the 2nd and 10th (p = 0.289; p = 0.584, 1.000).

**Conclusion:** No significant differences were found on the effectiveness of WHO A or B formulas, before and after being stored for two and ten weeks in three sites.

**Keywords:** alcohol-based hand rub, WHO formula, hand hygiene, prEN12054

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**INTRODUCTION**

Hand hygiene is an important element that is often overlooked in health care facilities. In the Journal of Hospital Infection, hand hygiene compliance from Indonesian health personnel in rural area are still very low that is 20%, (5% before touching a patient and 34% after touching a patient) (Marjadi and McLaws, 2010). This low amount will definitely increase the morbidity due to healthcare-associated infection (Allegranzi and Pittet, 2009, Aiello et al., 2008).

Some approaches can be used in improving hand washing compliance. One of them is a promotive...
program that can be done by hospital management (Huis et al., 2012). From various studies, the hand rub, especially alcohol-based hand rub (ABHR), has been shown to be more effective compared to other materials or hand wash methods in reducing the number of microorganisms on the hands (Guilhermetti et al., 2010, Messina et al., 2008).

To address the problem, the World Health Organization (WHO) has issued an alcohol-based hand rub formula that is easy to make and still proves to be effective (WHQ 2009a, Suchomel et al., 2012). WHO Formula itself consists of 2 types, namely formula A based ethanol, and formula B based isopropyl alcohol. Special handling and proper storage of such alcohol-based disinfectants is required. The risks of improper storage and placement are spores contamination, and safety issues are mainly due to flammable alcohol (WHO, 2009b, an Seifen-und, 2012). Diponegoro National Hospital, Rumah Sakit Nasional Diponegoro (RSND), is a first-rate referral education hospital recently established and managed by Diponegoro University in Semarang, Central Java. This study aims to test the effectiveness of WHO A and B hand rub formulations stored in Emergency Room (ER), High Care Unit (HCU), and RSND store room for 2 weeks and 10 weeks.

METHODS
This research is a quasi experimental research with pretest-post test randomized group design conducted in April-June 2016. The samples were 15 bottles of homemade WHO A formula and 15 bottles of homemade WHO B formula, divided into 5 bottles for each room. The independent variables were Formula A and B, storage duration (0, 2, and 10 weeks), and storage in ER, HCU, and RSND store room. The dependent variable is the effectiveness of the hand rub using a modified method prEN12054 expressed in cfu/ml (Rotter, 2004).

The procedures performed in this study consist of various stages, preparation of ABHR formulas A and B, validation of bacterial suspension, validation of non-toxicity neutralizer, validation of neutralizer effectiveness, determination of handrub A and B effectiveness before storage, handrub storage in ER and ICU, and determination of the effectiveness of handrub A and B after storage.

This study was approved by the Ethics Committees for Health Research, Faculty of Medicine Diponegoro University.

Preparation of ABHR formula A. Ethanol 96% 4167ml, H2O2 3% 208 ml, Glycerol 98% 72 ml, add water up to 5 L then poured into 100 ml plastic bottle.

Preparation of ABHR formula B. Isopropil alcohol 99.8% 3758 ml, H2O2 3% 208 ml, Glycerol 98% 72 ml, add water up to 5 L then poured into 100 ml plastic bottle.

Validation of bacterial suspension. 0.5 McFarland Staphylococcus aureus suspension was diluted 10-6 with diluent (tripton, digestive enzyme pancreas casein, USP 1g, NaCl, EP 8.5g, water 1000 ml) to 1.5 x 102 cfu/ml. 200 µl of homogenized suspension (vortexed) was inoculated with a spread plate method on TSA medium in duplicate. Incubation is carried out at 36° C for 42 to 48 hours. The average cfu was rated as N.

Validation of nontoxicity neutralizer. Neutralizer is made from polysorbate 80 (30 ml), lecithin (30 g / L), and sodium thiosulfate (5 g / L). 0.5 McFarland Staphylococcus aureus suspension was diluted 10-5 diluent to 1.5 x 103 cfu/ml. 1 ml of suspension is added in 9 ml neutralizer and vortexed until homogeneous. The solution is placed in a water bath at 20° C for 1 minute. 200 µl of solution was inoculated with spread plate method on TSA medium in duplicate. Incubation is carried out at 36° C for 42 to 48 hours. The average cfu was rated as N'.

Validation of neutralizer effectiveness. 1 ml diluents homogenized in 9 ml hand rub tested and placed in a water bath at 20° C for 5 min, 1 ml of solution was added in 8 ml neutralizer and put back in water bath at 20° C for 5 min. 1 ml dilution of 103 cfu/ml Staphylococcus aureus was homogenized in the mixture and placed in a water bath at 20° C for 5 minutes. The mixture was homogenized again and taken 200 µl to be inoculated with a spread plate method on TSA medium in duplicate. Incubation is carried out at 36° C for 42 to 48 hours. The average cfu was rated as N'.

Sample storage. Samples were stored in ER, HCU, and RSND store room. Room temperature and humidity are measured daily. The alcohol content was measured at weeks 0, 2, and 10 weeks with alcoholmeter.

Hand rubs bactericidal activity testing. 0.5 McFarland bacterial suspension, neutralizer, and hand rub tested was prepared in a 20 oC water bath. 9 ml hand rub was added to 1 ml of 0.5 McFarland bacterial suspensions. The mixture was vortexed for 5 seconds, and then put back into the water bath, 45 seconds contact time. The mixture was vortexed again for 10 seconds so that the total contact time is 1 minute. 1 ml of mixture was added to 8 ml neutralizer and 1 ml of
sterile aquabidest. 10 ml of the mixture was vortexed for 5 seconds and placed in the water bath for 55 seconds to reach 1 minute contact time. The mixture was taken 200 µl to be inoculated with spread plate method on TSA medium in duplicate. Incubation is carried out at 36º C for 42 to 48 hours. The average cfu was rated as n. Minimal bacterial reduction is log 5.

prEN 12054 requirements. N, and N’ values between 100-300 cfu. N’ equal to or greater than 0.5x N. n’ equal to or greater than 0.5 x N’. The prEN12054 is an in vitro test for the hand rub effectiveness. Due to limitations, the researchers used a modification of the prEN12054 test using only one bacterium, Staphylococcus aureus strain ATCC 29213. It is said to meet prEN12054 if hand rub is capable of killing bacteria from 1x10^7 to 3x10^7 cfu/ ml to no more than 3x10^2 cfu/ ml.

STATISTICAL ANALYSIS
Mann-Whitney test was used to compare formulas A and B after it was made. Kruskal-Wallis test is used to see the difference of each formula in three places. The p value is considered significant if <0.05. These statistical analysis were performed using the SPSS program.

RESULTS
The alcohol content of the WHO A Formula immediately after it was made = 82%. The WHO A formula made has fulfilled prEN12054, with the result of N= 24.5 cfu/ ml, N’= 200 cfu/ ml, n = 24.5 cfu/ ml. The alcohol content of the WHO B Formula immediately after it was made = 77 %. The WHO B formula made has fulfilled prEN12054, with the result of N= 165 cfu/ ml, N’= 142 cfu/ ml, n’ = 185 cfu/ ml. For bacterial reduction results, it is not expressed in log reduction factor due to many sterile plates. Transformation to normalize data cannot be done because there is 0 in the result.

There were no significant differences in Formula A samples before being stored at three locations (p = 0.275), after being stored at three locations for two weeks (p = 0.584), and ten weeks (p = 0.166). There was no significant difference in Formula B samples before being stored at three locations (p = 0.289), after being stored at three locations for two weeks (p = 0.584), and ten weeks (p = 1.000) (Table 1).

There were no significant differences in the effectiveness of formula A and B (p = 0.567) before being stored. The median of the prEN12054 result is 0 cfu/ ml. All samples and replications met the prEN12054 requirements. There were no significant differences in the effectiveness of formula A and B after two weeks (p = 1.000) and ten weeks (p = 0.539) (Table 2).

There were no significant differences between the alcohol levels of WHO A and B formulas measured by alcohol meters at weeks 0, 2, and 10 (Table 3).

There were no significant differences in the temperature and humidity of the three locations measured daily for ten weeks.

DISCUSSION
In this study there were no significant differences in the effectiveness of WHO A and B formulas. Several previous publications have different conclusions regarding effectiveness of basic ingredients in WHO A and B formulas. Isopropyl alcohol, the base ingredient of the WHO B formula is said to be slightly more effective at killing Staphylococcus aureus and E.coli(Rutala and Weber, 2008). Another study stated that there was no significant difference between the effectiveness of hand rub between formula A and formula B (Suchomel et al., 2012).

There were no significant differences from the effectiveness of WHO A hand rub formulations in ER, HCU, and storeroom after being stored for 2 weeks and 10 weeks. The same results were also found in formula B. The WHO's storage recommendation is to store a hand rub in a cool place that is protected from direct sunlight. This is due to the combustible nature of alcohol, and is not directly related to changes in the effectiveness of the hand rub (WHO, 2009a). Alcohol evaporation can be caused by endothermic and exothermic effects, which means external influences or because of the molecular properties of the alcohol itself. The higher the concentration of alcohol, the faster it evaporates because more alcohol molecules are in direct contact with the air. Storage of hand rub in the room that is similar from the temperature (average 28,6ºC, 21,45ºC, 24,7ºC) and in short term makes endothermic and exothermic evaporation not yet occurred (Peeters and Huyskens, 1993).

Storage for 2 weeks did not decrease the alcohol content of WHO A and B. The WHO A formulas in all three places remained 82% and the WHO B formula remained 77%. While at week 10, the alcohol content of formula B fell to 81%, and formula A fell to 75%. The differences are not statistically significant. The previous study states that raising the alcohol content in the WHO formula from vol/ vol to weight/ weight increases effectiveness so it can meet EN12791, and EN1500 procedures for 30 seconds (Suchomel et al., 2013, Kampf and Ostermeyer, 2011).
### Table 1.

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<th>Bactericidal activity hand rub Formula WHO Formula A and B Handrub...</th>
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<td><strong>Week</strong></td>
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* Kruskal-Wallis test
Although almost statistically significant, with significance level of $p = 0.05$ in the WHO A different test formulas were placed in storeroom at weeks 0, 2, and 10 do not meet the conditions for post hoc. The data obtained shows a tendency to have differences compared to other places. This is probably because the storeroom has a relatively higher temperature compared to HCU and ER, although it is not statistically significant. Further research is needed to find out how much storage effect in extreme temperatures in reducing the effectiveness of hand rub.

Storage in this study using plastic lids and additional seals allows only a small amount of alcohol to react with the outside air, thus maintaining its
concentration.

**CONCLUSION**

No significant differences were found on the effectiveness of the WHO A and B formulas. There was no significant difference in the effectiveness of WHO A or B formulas stored in ER, HCU, and storeroom. There were no significant differences in the effectiveness of WHO A or B formulas, before and after being stored for two and ten weeks in three locations.

WHO A and B formulas can be used as an alternative hand rub with a cheaper price. The production cost for five liters hand rub was only 250,000 IDR (±18 USD), same price for one liter commercial hand rub.

Further research are needed on the effectiveness of WHO hand rub formulas that are stored using dispensers, such as their original state and repeated use. Product development is needed and followed by further research on the effectiveness of hand rub if fragrance or softener is added.

**CONFLICT OF INTEREST**

There is no conflict of interest.

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