# Bactericidal Efficacy of Alcohol Solution in Community Hospital and Health Centers

Ratree Tavichakorntrakool PhD*,*****,
Seksit Sungkeeree MSc*,*****, Phitsamai Saisud BSc*,*****,
Pojnicha Chaiyakhot BSc*, Adisak Wongwian BSc*,
Maitree Pakarasang PhD*,*****, Vitoon Prasongwatana MD**,
Narong Asayut BSc***, Narongrit Thipchaksurat BSc****,
Pipat Sribenjalux MSc*,*****, Patcharee Boonsiri PhD**

** Department of Clinical Microbiology, Faculty of Associated Medical Sciences, Khon Kaen University, Khon Kaen, Thailand*
*** Department of Biochemistry, Faculty of Medicine, Khon Kaen University, Khon Kaen, Thailand*
**** National Health Security Office Region 7 Khon Kaen, Khon Kaen, Thailand*
***** Pharmacy Department, Kaengkhro Hospital, Chaiyaphum, Thailand*
****** Centre for Research and Development of Medical Diagnostic Laboratories, Faculty of Associated Medical Sciences, Khon Kaen University, Khon Kaen, Thailand*

***Objective:*** *To evaluate bactericidal efficacy of alcohol solution during actual use and typical storage conditions in community hospital and health centers.*
***Material and Method:*** *The alcohol samples were collected immediately after the first bottle-opening (day 0) and on day 3, 7, 14, 21 and 30 from 10 stations in hospital and community health centers in Pone-na-kaew district, Sakon Nakhon province, Thailand, during May-July 2011. Bactericidal efficacy of these samples against Staphylococcus epidermidis, Staphylococcus aureus, Escherichia coli, Pseudomonas aeruginosa and Klebsiella pneumoniae was evaluated. Ethanol concentration was quantified by a gas chromatography method.*
***Results:*** *Bactericidal efficacy of the alcohol samples still remained on day 30 with ethanol concentration range of 60.91-65.99% v/v.*
***Conclusion:*** *This finding should be considered as a cost-benefit model for using alcohol solution in community hospital and health centers.*

***Keywords:*** *Bactericidal efficacy, 70% v/v alcohol solution, Health centers*

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Alcohol solution is commonly used for hand and wound rubbing in hospitals and health centers as standard precaution to reduce infection from microorganisms including *Staphylococcus aureus*, *Pseudomonas aeruginosa* and others[1-4]. It is significantly more efficient in reducing microbial contamination than antiseptic soap[5]. Different formulations of alcohol solution, composed of different percentages of ethanol and propanol, are commercially available. Ethanol can coagulate protein at cell wall and cytoplasmic membrane of microorganisms, leading to protein denaturation and impairment of cellular functions[6]. Due to alkyl chain length and branching, n-propanol has higher bactericidal activity than isopropanol and ethanol[7,8]. The bactericidal activity of alcohol solution is higher at 30-40°C than at 20-30°C[9].

Bacterial contamination of antiseptic agents was high in provincial hospitals but it was not found in university hospitals[10]. Contamination rate was correlated with the duration of use and most of the contaminated bacteria were from the environment[10]. Storage condition may also affect quality of alcohol solution in bacterial disinfection because ethanol is a volatile substance. It was reported that alcohol solution stored at room temperature and 4°C in sealed polyethylene bottle did not change significantly for at least one year after preparation[11], but 70% ethanol was stable for at least 14 days when kept in a polypropylene syringe at 23-25°C[12]. The 70% v/v alcohol solution produced by Siriraj hospital, Thailand, in package sizes of 60 and 250 ml, preserved their aseptic properties when their lids were opened for 18 and 32

***Correspondence to:***
*Boonsiri P, Department of Biochemistry, Faculty of Medicine, Khon Kaen University, Khon Kaen 40002, Thailand.*
*Phone & Fax: 043-348386*
*E-mail: patcha_b@kku.ac.th*

days, respectively[13]. In Thailand, 70% v/v alcohol solution is aseptically manufactured by Thai Government Pharmaceutical Organization (GPO) in a package size of 450 ml polypropylene bottle for distribution to Thai Government community hospitals and health centers. These alcohol solutions are usually discarded after 1 week after bottle-opening to prevent ethanol evaporation and microbial contamination. This is uneconomical and wasteful. The objective of this study was to evaluate the bactericidal efficacy and ethanol concentration of alcohol solutions manufactured by GPO during actual use and typical storage in community hospital and health centers.

## Material and Method

### *Material*

Alcohol solution was manufactured by GPO of Thailand (Lot number C540216, manufacture date: 2 November 2010, expiration date: 2 November 2013) in a package size of 450 ml polypropylene bottle.

### *Method*

#### *Collection of alcohol samples*

Alcohol samples were collected from 5 medical departments at Pone-na-kaew Hospital (station 1-5) and 5 community health centers (station 6-10) in Pone-na-kaew District, Sakon Nakhon Province, Thailand (Table 1) during May-July 2011. The sample collection was done in 3 rounds. In the first round (May 2011), at the first time of bottle-opening (day 0), 2 ml of each alcohol sample were transferred to an air-seal glass container. During daily used in the hospitals, alcohol samples were also collected on day 3 and 7. After day 7, the alcohol bottles were stored on their original shelves at ambient temperature and bottle-opening was done twice a day (9 am and 5 pm) on the same procedure as actual use, followed by sample collection on day 14, 21 and 30. Temperature of each station on the sample-collection day and cumulative times of bottle-opening were recorded. The samples were kept at 4°C until analysis.

The second and the third rounds of sample collection (June and July 2011) were performed using the same procedure as the first round.

#### *Detection of bacterial contamination in alcohol samples*

Bacterial contamination in alcohol samples was detected by using streak plate method. Briefly, 0.01 ml of each alcohol sample was streak on a blood agar plate using a standard loop. For aerobic bacterial growth, the plates were incubated at 35-37°C for 24-48 hrs. Sterilized distilled water was used as a negative control.

#### *Evaluation of bactericidal efficacy of alcohol solutions*

Five standard bacterial strains including *Staphylococcus epidermidis* ATCC 35984, *Staphylococcus aureus* ATCC 25923, *Escherichia coli* ATCC

**Table 1.** Average temperatures of bottle-opening of all the sample-collection days, cumulative times of bottle-opening on day 0-7 and ethanol concentration on day 30 for 3 rounds of 10 stations at studied sites in Pone-na-kaew District, Sakon Nakhon Province, Thailand. The value are expressed as mean ± SEM

| Stations | Studied sites | Average temperatures (°C) | Cumulative times of bottle-opening on day 0-7 (time) | Ethanol concentrations on day 30 (% v/v) |
|---|---|---|---|---|
| | Pone-na-kaew hospital | | | |
| 1 | Medical technology unit | 26.0±0.0 | 15.67±0.33 | 65.99±1.46 |
| 2 | Pharmaceutical unit | 25.7±0.2 | 19.33±0.33 | 64.65±2.61 |
| 3 | Dentistry unit | 26.1±0.2 | 18.33±0.33 | 61.17±2.54 |
| 4 | Internal patient department | 25.4±0.1 | 15.00±0.58 | 63.84±1.33 |
| 5 | Accidental and emergency unit | 26.7±0.3 | 16.33±0.33 | 63.33±3.05 |
| | Community health center | | | |
| 6 | Primary care unit* | 26.7±0.1 | 20.33±0.33 | 64.07±2.14 |
| 7 | Community health center 1 | 28.8±0.0 | 24.33±0.33 | 62.07±2.58 |
| 8 | Community health center 2 | 28.7±0.2 | 25.67±0.33 | 60.91±3.38 |
| 9 | Community health center 3 | 29.8±0.3 | 27.33±0.67 | 62.97±1.79 |
| 10 | Community health center 4 | 29.3±0.3 | 29.00±0.58 | 62.76±0.63 |

SEM = standard error of mean, * unit of Pone-na-kaew hospital which located 0.5 kilometer far from the hospital

25922, *Pseudomonas aeruginosa* ATCC 27853 and *Klebsiella pneumoniae* ATCC 700603 were tested. The bacterial suspension was adjusted to obtain a turbidity equal to 0.5 McFarland standard using normal saline as a diluent. To confirm the viability of the test organisms, they were streaked on blood agar plates.

To evaluate the bactericidal efficacy, 0.4 ml of each alcohol sample were mixed with 0.1 ml of bacterial suspension for 5, 10, 15 and 30 min. The 0.01 ml of standard loop of this mixture was streaked on blood agar plates and incubated at 35-37°C for 24-48 hrs. The absence of bacterial growth on the medium indicated that the alcohol sample had bactericidal efficacy.

***Determination of ethanol concentration in alcohol samples***

Ethanol concentrations in alcohol samples collected on day 30 were determined by gas chromatography as modified from the method of Thaweethamcharoen & Sasithornrojanachai[13]. A Perkin Elmer gas chromatograph (Massachusetts, USA) was equipped with a flame ionization detector, which was set at 150°C. Capillary column (Agilent, 19091N-133) length was 30 meters. Oven temperature was held at 50°C (isothermal) for 3 min and injector temperature was 200°C. Nitrogen was used as carrier gas, which was held at 9.5 psi. Hydrogen and air flow were set at 40 ml/min and 450 ml/min, respectively. The combined flow was 45 ml/min. The 70% v/v ethanol (analytical grade) was used for preparing standard curve and 2.5% v/v isopropanol (analytical grade) was internal standard. All samples were analyzed in duplicate.

***Statistical analysis***

All data were reported as mean $\pm$ SEM. Statistical analysis was performed using SPSS software (version 11.0). Differences between two sample groups were tested by unpaired t-test. The p-values of $<0.05$ were considered statistically significant.

**Results**

In the present study, 10 stations located in Pone-na-kaew District, Sakon Nakhon Province are shown in Table 1. Station 1-5 were in the hospital in town whereas station 6-10 were in the villages. Average temperature (mean $\pm$ SEM) of the sample-collection day for station 1-5 ($26.0\pm0.2$°C) was significantly lower than station 6-10 ($28.7\pm0.5$°C) ($p = 0.002$). Cumulative times of bottle-opening during the first week of 30-day period of 3 rounds was lower for station 1-5 ($19.74\pm0.71$ times) compared to station 6-10 ($26.46\pm1.14$ times) ($p = 0.001$).

Detection of bacteria in alcohol samples revealed that no bacterial contamination. In addition, no growth of the test organisms was observed on blood agar after 24-48 hrs of incubation.

Quantitative analysis of ethanol by gas chromatography showed that all the samples had ethanol concentration higher than 60% (range of 60.91-65.99% v/v), which was enough to prevent bactericidal growth.

**Discussion**

Alcohol solution is a disinfectant agent widely used in hospitals as a broad spectrum antimicrobial agent to reduce the infection rate[14] due to its effectiveness and low cost. Prolonged use is one of risk factors for the microbial contamination but the duration that bactericidal effect still remains after the first bottle-opening is unclear. The major compounds of alcohol formula include ethanol, n-propanol and isopropanol. The 60-70% v/v ethanol is considered as a standard criterion for its antiseptic property[15]. Formulation and temperature are factors that can influence antimicrobial activity. Thus, stability of alcohol solution is important because it implies to antiseptic quality assurance of hospitals and health centers.

The majority of the flora isolated from the hands were coagulase-negative staphylococci (22%), *Micrococcus* spp. (14%), alpha-hemolytic streptococci (12%), *S. aureus* (3%), *Pseudomonas* spp. (8%), *Corynebacterium* spp. (8%), *Pantoea agglomerans* (8%), *Bacillus* spp. (5%), *Serratia* spp. (3%), *Citrobacter* spp. (3%), *Enterobacter* spp. (3%) and *Klebsiella* spp. (2%)[16]. In the present study, we examined efficacy of the alcohol samples against a variety of bacteria including *S. epidermidis* ATCC 35984, *S. aureus* ATCC 25923, *E. coli* ATCC 25922, *P. aeruginosa* ATCC 27853 and *K. pneumoniae* ATCC 700603. *S. epidermidis* and *S. aureus* are commonly found on skin[17,18], whereas the remaining representatives often cause infection in humans. In the present study, no bacterial growth was found after exposure with alcohol samples for 5, 10, 15 and 30 min. This implied that the bactericidal efficacy of the alcohol solution samples still remained. In all 3 rounds of alcohol sample collection, the test method provided reproducible results. The results reported herein (ethanol concentration more than 60% in a package size 450 ml with bottle-opening times approximately 20 seconds at temperature range 25-30°C) are in accord with the previous study at Siriraj hospital[13] that the

ethanol concentration was not less than 60% in a package size of 250 ml for 49-day period (with bottle-opening times not more than 25 seconds twice a day at room temperature). Not only bottle-opening times, but also the opening period affected the ethanol concentration as reported in a long period study[13], the lid of alcohol container was opened for 24 hrs daily at room temperature in a laboratory and ethanol concentration declined lower than 60% in 32 days for package sizes of 250 ml.

The strength of the present study was that the experiment was conducted in community hospital and health centers in the actual situation. There was, however, a limitation to the present study in that the alcohol samples were not immediately analyzed. Future studies need to explore the bactericidal efficacy of the alcohol solution in a larger number of hospitals and health centers.

## Conclusion

Time taken to achieve antisepsis is important. In the present study, the alcohol samples could preserve their bactericidal efficacy to common pathogenic bacteria within 30 days after the first bottle-opening for daily use. Ethanol concentration was remained in standard range (60.91-65.99% v/v) for at least 30 days under the typical storage condition. This finding may be considered as a cost-benefit model with the economic value of alcohol solutions for community hospital and health centers.

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## Potential conflicts of interest

None.

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## *ประสิทธิภาพการฆ่าเชื้อแบคทีเรียของแอลกอฮอล์ที่ใช้ในโรงพยาบาลชุมชนและโรงพยาบาลส่งเสริมสุขภาพตำบล*

*ราตรี ทวิชากรตระกูล, เสกสิทธิ์ สังคีรี, พิสมัย สายสุด, พจณิชา ไชยโคตร, อดิศักดิ์ วงค์เวียน, ไมตรี ปะการะสังข์, วิทูรย์ ประสงค์วัฒนา, ณรงค์ อาสายุทธ, ณรงค์ฤทธิ์ ทิพย์จักษุรัตน์, พิพัฒน์ ศรีเบญจลักษณ์, พัชรี บุญศิริ*

***วัตถุประสงค์:*** *เพื่อประเมินประสิทธิภาพการฆ่าเชื้อแบคทีเรียของแอลกอฮอล์ในสภาวะการใช้งานและการเก็บรักษาที่โรงพยาบาลชุมชนและโรงพยาบาลส่งเสริมสุขภาพตำบล*

***วัสดุและวิธีการ:*** *เก็บตัวอย่างแอลกอฮอล์ทันทีที่เปิดขวดใช้งานครั้งแรก (วันที่ 0) และเมื่อเปิดใช้งานในวันที่ 3, 7, 14, 21 และ 30 จากโรงพยาบาลโพนนาแก้วและโรงพยาบาลส่งเสริมสุขภาพตำบล จำนวน 10 แห่ง ที่อยู่ใน อ. โพนนาแก้ว จ. สกลนคร ประเทศไทย ในช่วงเดือนพฤษภาคม ถึงกรกฎาคม พ.ศ. 2554 หลังจากนั้นประเมินประสิทธิภาพการฆ่าเชื้อ Staphylococcus epidermidis, Staphylococcus aureus, Escherichia coli, Pseudomonas aeruginosa และ Klebsiella pneumoniae รวมทั้งหาความเข้มข้นของเอทานอล โดยวิธี gas chromatography*

***ผลการศึกษา:*** *หลังจากเปิดขวดใช้แล้วเป็นเวลา 30 วัน แอลกอฮอล์ยังคงมีประสิทธิภาพในการฆ่าเชื้อแบคทีเรีย ที่นำมาทดสอบโดยมีความเข้มข้นของเอทานอลอยู่ในช่วง 60.91-65.99% v/v*

***สรุป:*** *การศึกษานี้เป็นต้นแบบเพื่อประกอบการพิจารณาการใช้แอลกอฮอล์ในโรงพยาบาลชุมชนและโรงพยาบาลส่งเสริมสุขภาพตำบลอย่างคุ้มค่า*