Quantitative Bactericidal Efficacy of Alcohol-Based Handrub in Thailand Using Modified EN 1276: 2009 Method

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Abstract Alcohol-based handrub (ABHR) products are widely used to reduce hands contamination in Thailand due to their convenience and ease to use in areas where water is not available. In Thailand, ABHR bactericidal efficacy and its test method have not been established. In this study, European Standard EN 1276: 2009 was modified and validated to obtain suitable conditions. The modified method was validated at 34°C with minimizing a test system volume. The bactericidal efficacy was determined at 1 minute contact time in the presence of Bovine Albumin Fraction V using 4 reference bacterial strains; *Staphylococcus aureus* ATCC 6538, *Escherichia coli* ATCC 10536, *Enterococcus hirae* ATCC 10541 and *Pseudomonas aeruginosa* ATCC 15442. The ABHR product was considered as bactericidal effective when log reduction (lg R) of the reference bacterial amount after one minute contact time was ≥ 5. Furthermore, twenty-nine ABHR products marketed in Bangkok Metropolitan Region were evaluated using the proposed method. It was found that 6 ABHR products (20.7%) failed the bactericidal efficacy criterion. The finding emphasized a need for regulatory consideration of bactericidal efficacy of the ABHR product as a supportive scientific data. In addition, regular post-marketing surveillance of ABHR should be undertaken to ensure the bactericidal effectiveness of the products.

Keywords: Alcohol-based handrub, hands disinfection, bactericidal efficacy

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Introduction

Acquired infection through hands of healthcare workers, commuters, including children is one of the critical factors in diseases transmission. Ethyl alcohol (ethanol) and isopropyl alcohol, nonspecific intermediate level germicides, act rapidly by precipitating proteins and solubilising lipids present on cell membranes of vegetative forms of gram-negative and gram-positive microorganisms.[1] World Health Organization[1] and Centers for Disease Control and Prevention[2] hand hygiene guidelines recommend ethanol as a common active agent in handrub formulations as alcohol-based handrub (ABHR) products for hand hygiene when no water is available.[1-4] ABHR products usually found in a ready-to-use form and consisted of 60% to 70% alcohol as a major antibacterial ingredient, however, other additional antimicrobial ingredient such as triclosan may be found in some ABHR formulations.

During the Thailand Great Flood 2011 and several outbreak events of infectious diseases in Thailand, ready-to-use ABHR products in gel form have become popular and frequently used in workplace, hospitals, kindergartens and community as a convenient waterless hand cleansing product for reduction of transient microbial flora from touching soiled surfaces from common objects. In addition, increasing public awareness about personal daily risks of acquired infection has given rise to a large variety of convenient ABHR products. In Thailand, ABHR products in all forms are categorized by the Thai Food and Drug Administration (Thai FDA) as a controlled cosmetic product with alcohol content shall be less than 70% and shown on notification formulation document but may not on the product labelling.[5] To be marketed, ABHR products shall have a Thai language labelling and registration number as regulated but no requirement for quality of ABHR products regard to bactericidal efficacy. Low efficacy ABHR products may not be able to reduce but rather spread out the infectious microorganism.[6]

Chemical methods for determination of alcohol content in the formulation are available but there may be some disadvantages of techniques including interferences from preservatives and other ingredients which may also exert antimicrobial effect.[7,8] The International standard methods for evaluation of bactericidal efficacy on ABHR products are (1) *in vitro* tests in laboratory such as European Standard EN 1276: 2009,[9] a quantitative dilution-neutralization method, under simulated practical conditions appropriate to its intended use, (2) *in vivo* tests under simulated practical conditions in laboratory and (3) *in vivo* field tests.[10] EN 1276: 2009[9] is the recommended *in vitro* method to be used to substantiate claims for hygienic handrub products used in food, industrial, domestic and institutional area which included essential factor simulated a practical use,[6] i.e. organic soil load (dirty condition), and suitable for controlled conditions routinely setting in a laboratory.[7,11] Taylor *et al.* also recommended EN 1276 : 2009 as a useful method to harmonize disinfectant testing across Europe.[12] According to testing process in EN 1276: 2009 method, addition of test organism and interfering substance which consequencely dilute the product concentration to be only 80% of the original concentration. Performing the test on ABHR products

with less than 70% alcohol by this standard method, its active ingredients will be diluted to be only 55.9% or may be less for other formulations which may not demonstrate an actual efficacy of the product.

In another aspect, the obligatory temperature specified in this European standard is 20°C with additional temperatures may be chosen from 4°C, 10°C, 30°C or 40°C. While temperature has been reported to be one of the several factors affecting the antimicrobial products activity,[13-15] WHO Guidelines[1] also stated that "Conditions in suspension and *in vitro* or ex vivo testing do not reflect those on human skin". Therefore, in regard to EN 14885,[10] scientific justification for applying the scheme of testing for specific activity other than the tests specified in this European Standard is recommended. In this study, we modified and validated EN 1276: 2009 method to be suitable for testing the efficacy of a ready-to-use ABHR product at the concentration as high as 98% instead of 80% of original concentration and simulate in-use conditions on human skin temperature by testing at 34°C ± 1°C which is an average human skin temperature.[16-18] The validated modified EN 1276: 2009 method was performed using a range of standard ethanol solutions against each of the 4 reference ATCC bacterial strains in the presence of interfering substance (dirty condition) that was bovine albumin fraction V. For consumer protection, the bactericidal activity of 29 ABHR products marketed in Thailand was also determined using the validated and modified EN 1276: 2009 method. Acceptance criterion for bactericidal efficacy of the ABHR products indicated by a decimal log reduction (lg R) of the reference bacterial amount after 1 minute contact time to be at least 5 lg or 99.999% reduction.[9]

Materials and Methods

Samples: Twenty-nine ABHR products in gel form, collected from retailed and wholesale shops in Bangkok Metropolitan Region during 2013-2015, were tested for bactericidal efficacy.

Standard cultures: Four standard bacterial cultures, *Staphylococcus aureus* ATCC 6538, *Escherichia coli* ATCC 10536, *Enterococcus hirae* ATCC 10541 and *Pseudomonas aeruginosa* ATCC 15442 were used.

Culture media and Reagents: Bovine albumin fraction V (BA; Sigma, USA), Distilled-purified water (Dw; ELGA Purelab option, UK) Dey/Engley neutralizing broth (D/E; DifcoTM, USA), tryptic soy agar (TSA; DifcoTM, USA), tryptone water (Tw; BD, Germany) and Ethanol (Merck, 99.9% purity, Germany)

Equipment: Temperature controlled water bath at 34°C ± 1°C (American Optical, Model 406016 serial, USA), incubator 36°C ± 1°C (Binder, Model BD 720/E2, Germany), Spectrophotometer at wavelength 620 nm (Metertech, Model UV/VIS SP-8001, Taiwan)

Methods: A modified dilution neutralization method specified in European standard EN 1276: 2009 was performed as described.

Preparation of bacterial suspensions: Each of the standard bacterial strains was subcultured. The second and third subcultures were used as working cultures to prepare a test suspension "N" (Figure 1). In parallel to the dilution neutralization tests, the test suspension was determined for bacterial amount "N" which shall be between 1.5×10^9 and 5.0×10^9 cfu/mL. When the test suspension "N" was used in the dilution neutralization method, bacterial amount "N" was diluted 1:100 to be "N0" (=N/100) which shall be between 1.5×10^7 and 5.0×10^7 cfu/mL. Validated bacterial suspension "Nv" was prepared for the amount between 3.0×10^3 and 1.6×10^4 cfu/mL by diluting 1 mL of 10^{-5} "N" with 3 mL of Tw. When the validated bacterial suspension "Nv" was used in the quality control procedure, bacterial amount " Nv" was diluted 1:100 to be "Nv0" (=Nv/100) which shall be ≥ 0.5 x Nv0. The amount of bacterial suspensions used in this study as specified in EN 1276: 2009 was shown in Table 1.

Bactericidal efficacy of ABHR product: The efficacy (Test) was determined including quality control and validation procedures (A, B and C) as followed (Figure 2).

Test: The bacterial suspension "N" (0.1 mL) was added into a tube containing 0.1 mL of 30.0% (w/v) BA. The tube was mixed and placed at 34°C ± 1°C for 2 min ± 10 s, then 9.8 mL of ABHR sample was added, mixed and further placed at 34°C ± 1°C for 1 min ± 5 s (contact time). The test mixture (1.0 mL) was then pipetted to 9.0 mL of D/E. After 5 min ± 10 s (a neutralization period), the test mixture (1.0 mL) was pour plated with TSA in duplicate and incubated at 36°C ± 1°C for 18 h to 24 h prior to enumeration. After the end of contact time, the bacterial amount (cfu/mL) survived from the product test, "Na", was counted.

Experimental conditions control (A): To ensure no biocidal effect from other experimental parameters, the same procedure as Test was performed. Validated bacterial suspension "Nv" (between 3.0×10^3 and 1.6×10^4 cfu/mL) and distilled purified water (Dw) were used in place of "N" and D/E, respectively.

Neutralizer control (B): To verify the absence of toxicity of the neutralizer, the test mixtures of 9.8 mL D/E, 0.1 mL Dw and 0.1 mL "Nv" were prepared and pour plated with TSA at 5 min ± 10 s.

Method validation (C): To validate the dilution neutralization method, the test mixtures of 0.1 mL of BA, 0.1 mL of 0.1% Tw and 9.8 mL of ABHR sample were prepared, mixed and placed at 34°C ± 1°C for 1 min ± 5 s (contact time), then 1.0 mL of the test mixture was transfered to 8.9 mL of D/E. After 5 min ± 10 s, 0.1 mL of "Nv" was added and pour plated with TSA after 30 min ± 1 min.

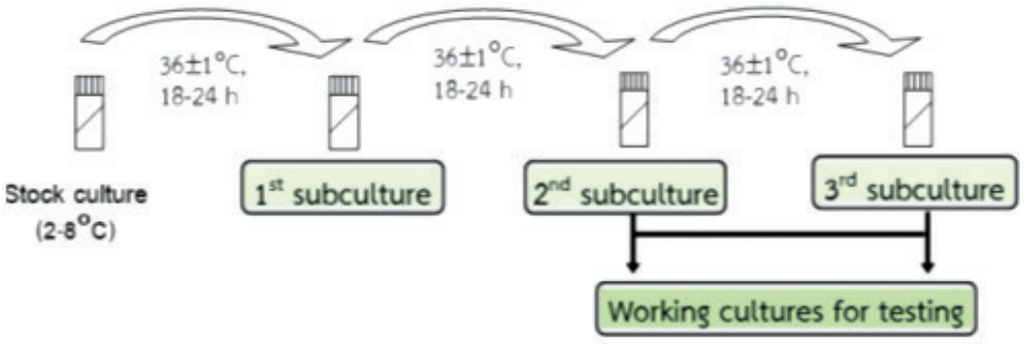

Fig. 1 Preparation of working cultures: each bacterial strain was subcultured on Tryptic Soy Agar and incubated at 36 ± 1°C for 18 h to 24 h. The second and third subcultures were used as the working cultures to prepare a test suspension "N" for testing.

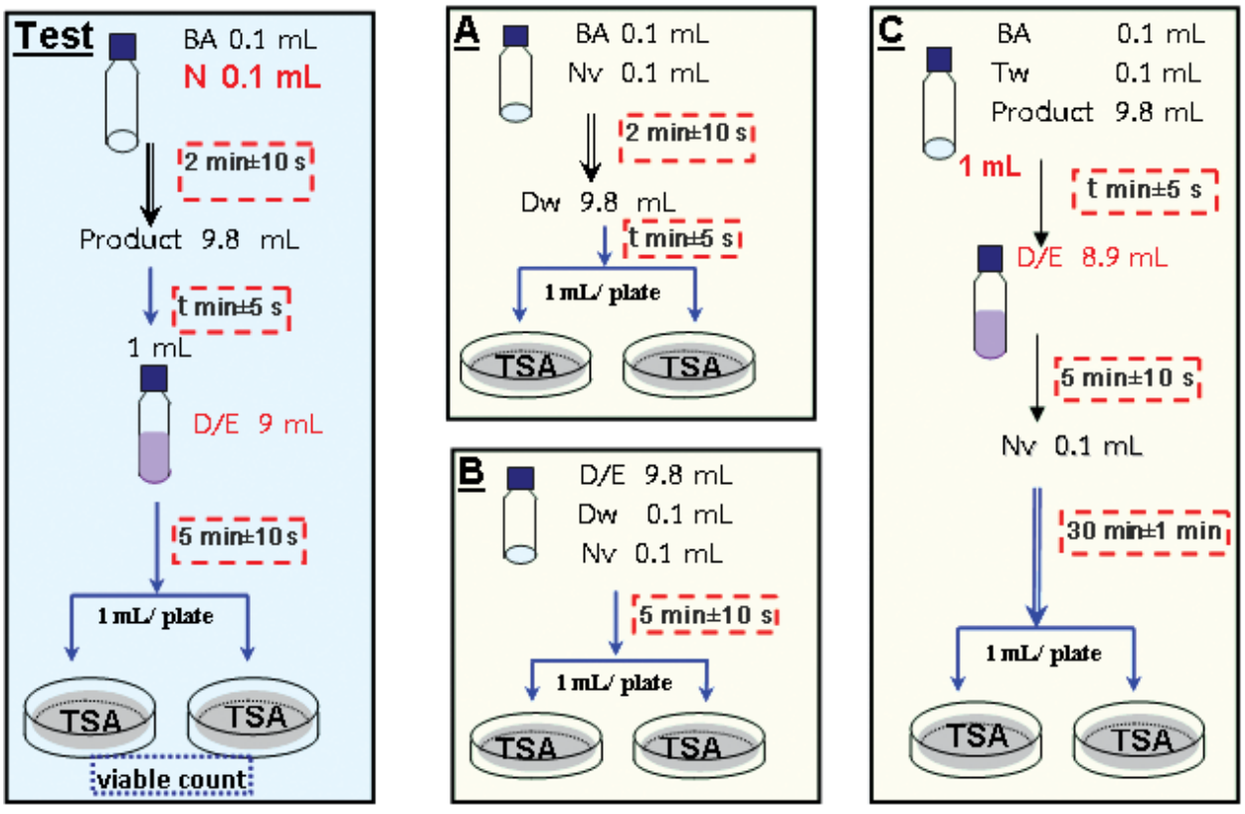

Fig. 2 Diagrams illustrated dilution neutralization method "Test" including quality control "A", "B" and "C". Viable counts of testing bacteria were determined after incubation at 36 ± 1°C for 18 h to 24 h.

BA; Bovine albumin fraction V, N; Culture test suspension, D/E; Dey/Engley neutralizing broth, TSA; Tryptic Soy Agar, Nv; Validation suspension, Dw; Distilled-purified water, Tw; Tryptone water, t; 1 min contact time

The amount of bacterial inoculum, i.e. N, N0, Nv, Nv0 and Na in the test mixtures i.e. "Test" including quality controls "A, B and C" at different contact times must be controlled to achieve the acceptance criterion specified (Table 1).[9] Under the conditions defined by this modified method, lg R for each reference bacterial strain was separately calculated using equation: lg R = lg N0 − lg Na. The lg R ≥ 0.5 indicated the bactericidal efficacy of the product against the test bacteria.[9] The modified EN 1276: 2009 method was validated with the concentrations of standard ethanol suspensions at 20%, 30%, 40%, 45%, 50%, 55% 60% and 70% (by volume) against 4 reference bacterial ATCC strains. Positive control using 60% (v/v) ethanol was included in each experiment. The bactericidal efficacy of the 29 ABHR products was evaluated using this modified method. Test results of products demonstrated lg R less than 5 were repeated.

Table 1 Basic limits of bacterial amount in the test mixtures at the beginning, i.e. *N0* and *Nv0*, and at the end of contact times, i.e. "*A, B* and *C*" as specified in EN 1276: 2009

Experimental Condition	**Bacterial suspension (cfu/mL)**	**Bacterial Amount (cfu/mL) in the test mixtures at different contact times**	
		at the beginning of contact time (time = 0)	**at the end of contact time (survivors)**
Test	*N* $1.5 \times 10^9 - 5 \times 10^9$	*N0* (=N/100) $1.5 \times 10^7 - 5 \times 10^7$ cfu/mL	*Na*[#] (t* min before Neutralization) = viable count x 10
Controls	*Nv* $3.0 \times 10^3 - 1.6 \times 10^4$	*Nv0* (= Nv/100) 30 – 160 cfu/mL	*A* (t* min), B (5 min), C (30 min) ≥ 0.5 x *Nv0* (50% recovery)

[#] Bacterial amount (cfu/mL) survived from the product test after the end of contact time

* 1 min for hands disinfection test

Results

The modified EN 1276: 2009 method was controlled by preparation of 4 bacterial culture suspensions through the quality control conditions. "Method validation C" using 60% (by volume) standard ethanol solution in 4 separate experiments was carried out in 4 different days (Table 2). The concentrations of bacterial suspensions and validation suspensions were prepared to be within the acceptance criterion (Tables 1 and 2). In addition, quality control conditions justified by the recovery ratios were demonstrated to fulfill the acceptance criterion ≥ 0.5 x Nv0.

Table 2 Validation of preparation of bacterial culture suspensions and testing conditions including quality controls "A, B and C" in 4 separate experiments carried out in 4 different days

Acceptance Criteria / Reference Cultures	Validation suspension (cfu/mL)		Recovery Ratio (≥ 0.5 x *Nv0*) Quality Control Conditions		
	N0	*Nv0*	*Experimental control A*	*Neutralizer control B*	*Method validation C*
	$1.5 \times 10^7 - 5 \times 10^7$	30-160			
S. aureus	$1.5 \times 10^7 - 4.8 \times 10^7$	37-117	1.1-1.9	0.9-1.5	1.1-1.7
P. aeruginosa	$3.6 \times 10^7 - 5.1 \times 10^7$	70-127	0.95-1.2	0.8-1.3	0.6-1.2
E. coli	$1.5 \times 10^7 - 2.5 \times 10^7$	35-70	1.0-1.7	1.1-2.0	1.0-1.4
E. hirae	$2.5 \times 10^7 - 4.1 \times 10^7$	65-104	0.9-1.1	0.9-1.2	0.8-1.6

The modified EN 1276: 2009 method was validated also with the concentration ranges of standard ethanol suspensions from 20% to 70% (by volume) against 4 reference bacterial ATCC strains in 2-4 separate experiments carried out in 2 different days (Table 3). Under the defined conditions, lg R for each reference bacterial strain was calculated. The lg R ≥ 0.5 indicated the bactericidal efficacy of the product against the test bacteria.[9]

Table 3 Bactericidal efficacy of standard ethanol suspensions ranged from 20% to 70% (by volume) evaluated by modified EN 1276: 2009 method against 4 reference bacterial strains at 34°C, in dirty condition within 1 min contact time

Ethanol %(v/v)	Mean lg R*			
	S. aureus	***P. aeruginosa***	***E. coli***	***E. hirae***
20	0#	0.3	0.0	0.1
30	0.4	1.6	0.7	0.1
40	6.7	7.7	6.7	3.9
45	6.0	6.0	5.0	6.0
50	6.0	6.0	5.0	6.0
55	6.0	6.0	5.0	6.0
60	7.3	7.6	7.3	7.5
70	6.7	7.7	6.7	7.4

* Mean lg R was an average from 2-4 separate experiments carried out in 4 different days

\# No reduction of the bacterial amount before and after contact time

The modified EN 1276: 2009 method was used to evaluate the bactericidal efficacy of 29 ABHR products. It was found that 23 samples reduced all of the 4 reference bacterial strains by lg R exceeding 5 within 1 min (Table 4). However, 6 samples (20.7%) failed the test criterion: 1 sample demonstrated no bactericidal efficacy against all 4 testing strains, 1 sample failed against 2 strains (i.e. *P. aeruginosa* and *E. hirae*) and 4 samples failed against 1 strain (either *P. aeruginosa*, *E. coli* or *E. hirae*).

Table 4 Bactericidal efficacy of 29 ABHR products evaluated by modified EN 1276: 2009 method against 4 reference bacterial strains at 34°C, in dirty condition within 1 min contact time

Product Code	Ingredients			Product of	lg R			
	Alcohol	Triclosan	Other		*S. aureus*	*P. aeruginosa*	*E. coli*	*E. hirae*
1	Alcohol	–	–	Thailand	7.3	7.7	7.3	7.7
2	Alcohol Denat.	–	–	Thailand	7.3	7.7	7.3	7.7
3	Alcohol Denat.	–	–	UK	7.3	7.7	7.3	7.4
4	Ethyl Alcohol	–	–	Thailand	6.2	6.4	6.4	6.3
5	Ethyl Alcohol	–	–	Thailand	6.6	6.3	6.4	6.3
6	Ethyl Alcohol	–	–	Thailand	6.7	6.9	6.0	7.5
7	Ethyl Alcohol 62%	–	–	USA	7.4	7.6	7.3	7.4
8	Ethyl Alcohol 68%	–	–	Thailand	6.4	6.4	6.5	6.2
9	Alcohol	–	Amino methyl propanol	Thailand	7.4	7.6	7.3	7.4
10	Alcohol	–	Glycerin	Thailand	7.3	7.7	7.3	7.7
11	Alcohol	–	Glycerin and Centella extract	Thailand	7.3	7.7	7.3	5.2
12*	Alcohol 69%	–	Aminomethyl propanol	Thailand	7.4	0#	7.3	7.4
13	Alcohol Denat.	–	Aminomethyl propanol Denatonium benzoate	Thailand	7.4	5.9	7.3	5.3
14*	Alcohol Denat.	–	Potassium hydroxide	Thailand	0#	0#	0#	0#
15	Alcohol Denat.	–	Amnomethyl propanol	Thailand	7.4	7.6	7.3	7.4
16	Alcohol Denat.	–	Triethanolamine, Aloe, Barbadensis	Thailand	7.4	7.6	7.3	7.4
17	Alcohol Denat.	–	Tetrahydroxypropyl Ethylene-diamine, Aloe Barbadensis	Thailand	7.4	7.6	7.3	7.4
18	Ethyl Alcohol	–	Alo Vera Extract	Thailand	7.3	7.7	7.3	7.7
19*	Ethyl Alcohol	–	Vitamin E	Thailand	7.4	7.6	7.3	0#
20	Alcohol	Triclosan	Alo Vera	Thailand	7.4	7.6	7.3	7.4
21*	Alcohol	Triclosan	Aloe Barbadensis with natural Pomelo essential oil	Thailand	7.3	7.7	7.3	4.8
22	Alcohol Denat.	Triclosan	Aloe Barbadensis	Thailand	7.4	7.6	7.3	7.4
23*	Alcohol Denat.	Triclosan	Aloe Barbadensis	Thailand	7.1	5.6	0#	7.7
24	Alcohol Denat.	Triclosan	Cucumber Extract	Thailand	7.3	7.7	7.3	7.7
25	Alcohol Denat.	Triclosan	Triethanolamine	Thailand	7.1	7.7	7.1	7.7
26	Alcohol Denat.	Triclosan	Triethanolamine, Aloe Barbadensis	Thailand	7.3	7.7	7.3	7.7
27*	Alcohol 68.0%	Triclosan	Alo Vera	Thailand	7.1	0#	7.3	2.7
28	Ethyl Alcohol 62%	Triclosan	Alo Vera, Cucumber Extract	Thailand	7.3	7.7	7.3	7.7
29	Ethyl Alcohol 64.13%	Triclosan	Menthol, Moisturizing beads Lemon Soda, Aloe Barbadensis	Thailand	7.1	7.6	7.3	7.4

* Product code 12, 14, 19, 21, 23 and 27 failed to comply with the bactericidal efficacy criterion against at least 1 test bacteria

\# No reduction of the bacterial amount before and after contact time

Discussion

The modified EN 1276: 2009 method utilized 10-time minimizing the test system volume, i.e. volume of bacterial inoculum and interfering substance; BA, from 1 mL to 0.1 mL each, while the same final concentration of inoculum and BA as specified in the method was maintained. Consequently, the volume of tested samples could be increased from 8 mL to 9.8 mL. Alcohol rapidly loses its activity when diluted.[19] The overall 2% additional volume, i.e. 1% inoculum and 1% BA, made the minimum change in the texture of ABHR samples which was in accordance with related international standards[20] that the testing condition shall not cause a substantial change of the test sample integrity.

The temperature is reported to be one of the several factors affecting the antimicrobial products activity.[14-16] WHO Guidelines[1] also stated that "Conditions in suspension and *in vitro* or ex vivo testing do not reflect those on human skin", while the obligatory temperature specified in the European standard is 20°C and additional temperatures could be chosen from 4°C, 10°C, 30°C or 40°C. Therefore, this study demonstrated the modification of testing temperature at 34°C ± 1°C to reflect the temperature of an average human skin temperature[16-18] in practical condition that had been tested using volunteers' hands[21] or fingerpads[22] in *in vivo* testing methods.

The sequence of manipulations in each testing step including the preparation and standardization of reference inoculums is necessary to ensure repeatability of the bactericidal suspension test.[13,15] Calibration data for each bacterial reference strain using spectrophotometer at wavelength of 620 nm[9,20] was used to estimate and achieve a suitable amount of bacterial culture suspension. The quality control especially in the neutralization validation of the method should also be demonstrated.[9] Diluents and/or agar containing neutralizer (s) used to neutralize the antimicrobial properties of each product formulation shall demonstrate that the testing conditions i.e. diluent and testing procedure were suitable and posed no toxic effect on the test bacteria.[23] It was observed that the choice of proper media used throughout the experimental conditions was necessary to achieve the neutralization validation criterion (Tables 2 and 3). Using D/E as a neutralizing diluent and TSA as an enumeration medium, repeatability of bacterial lg R were observed between the 2-4 separate experiments evaluated in our study (Table 3) over the ranges of ethanol standard solution from 20% to 70% (by volume).

Using this validated modified method, 6 ABHR products (Table 4) demonstrated an inadequate bactericidal efficacy even in the presence of triclosan. These results were in concordance with antibacterial efficacy of alcoholic hand rubs in the Kenyan market reported by Ochwoto *et al.*,[24] using a more complicated *in vivo* EN 1500 method,[21] that the combination of alcohol and triclosan in a formulation did not show a synergistic effect but exhibited poor bactericidal efficacy to *E coli*, *S. aureus* and *P. aeruginosa*. Combination of ethanol and aloe was also reported to be the least effective with lg R less than 3.[23-25] Additionally, a study of 12 ABHR products in gel form produced in Brazil containing 70% ethyl alcohol revealed the differences of 17% and 67% of the products that failed in EN 1500 efficacy tests with contact time of 60 s[26] and 30 s,[27] respectively, suggesting that consumer should be aware of application of 60 s to provide adequate effectiveness.

ABHR products are categorized as controlled cosmetic product by the Thai FDA[5] and labelling of ingredients may not show alcohol concentration. To ensure a sufficient effectiveness, regulatory authority should consider setting a criterion on alcohol concentration on ABHR labelling. This concept is confirmed by USFDA statement in "Guidance on studies necessary to support a Generally Recognized as Safe and Effective (GRAS/E)" that the final formulation efficacy testing of antiseptic handrub products marketed as over-the-counter (OTC) drug is necessary in order to confirm effectiveness and label the product appropriately for use.[28,29] USFDA proposed the ABHR's bactericidal efficacy tests to demonstrate only a 3-log reduction (99.9%) or greater in *in vitro* bacterial viability tests with all the 25 representative clinical isolates and 25 reference strains.[29] However, the susceptibility of 4 European test bacterial strains used in an obsolete prEN 12054 method which was identical to EN 1276: 2009, were reported to be comparable to the clinical isolates and reference strains specified by USFDA and sufficient to determine a comprehensive bactericidal activity of ABHR products.[30] In addition to the bactericidal efficacy evaluation of ABHR products, consumer shall also realize that ABHR may be used as an alternative to hand washing with soap and warm water which is the most effective washing.[31]

Conclusion

The modified EN 1276: 2009 method demonstrated minimizing the test system volume up to 98% concentration of ABHR products could be tested under the simulated used conditions. The modified method was not only suitable for *in vitro* testing but also practical for routine test of bactericidal effctiveness for any abcohol-based sanitizers with intention to use as undiluted. Using this modified EN 1276: 2009 method, 20.7% of ABHR products failed the bactericidal efficacy, suggesting that not all marketed products had adequate efficacy. Therefore, the bactericidal efficacy of the products is important and appropriate regulation and monitoring of the post-marketing surveillance for consumer protection are needed.

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ประสิทธิภาพเชิงปริมาณในการฆ่าเชื้อแบคทีเรียของเจลทามือในประเทศไทย ชนิดที่มีแอลกอฮอล์เป็นส่วนประกอบ เมื่อทดสอบด้วย Modified EN 1276: 2009 method

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บทคัดย่อ ผลิตภัณฑ์สำหรับรักษาสุขภาพอนามัยมือแบบพร้อมใช้ ที่มีแอลกอฮอล์เป็นส่วนประกอบหลัก (alcohol-based handrub-ABHR) เป็นที่นิยมอย่างแพร่หลายในประเทศไทย เนื่องจากความสะดวกและรวดเร็วในการใช้งานแม้ในบริเวณที่ไม่มีน้ำเพื่อลดปริมาณเชื้อก่อโรคซึ่งอาจส่งผ่านจากมือที่ปนเปื้อน อย่างไรก็ตาม ผลิตภัณฑ์ ABHR ที่วางจำหน่ายในประเทศไทยยังไม่มีการกำหนดเกณฑ์ประสิทธิภาพการฆ่าเชื้อรวมถึงวิธีทดสอบ จึงได้นำวิธีทดสอบสากล EN 1276: 2009 มาปรับให้เหมาะสม โดยทำการทดสอบที่อุณหภูมิ 34 องศาเซลเซียส และปรับลดการเจือจางในขั้นตอนทดสอบ ในสภาวะที่มี Bovine Albumin Fraction V ในเวลาทดสอบ 1 นาที โดยทดสอบกับเชื้อมาตรฐาน 4 ชนิด ได้แก่ *Staphylococcus aureus* ATCC 6538, *Escherichia coli* ATCC 10536, *Enterococcus hirae* ATCC 10541 และ *Pseudomonas aeruginosa* ATCC 15442 ผลิตภัณฑ์ที่สามารถลดปริมาณเชื้อได้ในเวลาสัมผัสเชื้อ 1 นาที ต้องมีค่า log reduction (lg R) ≥ 5 จากการประเมินคุณภาพผลิตภัณฑ์ ABHR ที่จำหน่ายในท้องตลาด จำนวน 29 ตัวอย่าง ด้วยวิธีที่ปรับใหม่นี้พบว่ามีตัวอย่างไม่ผ่านเกณฑ์ 6 ตัวอย่าง (ร้อยละ 20.7) แสดงให้เห็นว่าข้อมูลด้านประสิทธิภาพ ด้านการฆ่าเชื้อแบคทีเรียของผลิตภัณฑ์ ABHR เป็นข้อมูลที่จำเป็นเพื่อสนับสนุนการควบคุมคุณภาพและพิจารณากำหนดมาตรฐานผลิตภัณฑ์ ABHR ของประเทศ นอกจากนี้ควรมีการเฝ้าระวังคุณภาพหลังจำหน่ายเพื่อคุ้มครองผู้บริโภคและพัฒนาคุณภาพผลิตภัณฑ์ต่อไป