ORIGINAL ARTICLE

Shortening the Application Time of Alcohol-Based Hand Rubs to 15 Seconds May Improve the Frequency of Hand Antisepsis Actions in a Neonatal Intensive Care Unit

Axel Kramer, MD;[1] Didier Pittet, MD, MS;[2] Romana Klasinc, MD;[3,8] Stefan Krebs, MD;[1] Torsten Koburger, PhD;[4] Christoph Fusch, MD, PhD;[5,6] Ojan Assadian, MD, DTMH[3,7]

BACKGROUND. For alcohol-based hand rubs, the currently recommended application time of 30 seconds is longer than the actual time spent in clinical practice. We investigated whether a shorter application time of 15 seconds is microbiologically safe in neonatal intensive care and may positively influence compliance with the frequency of hand antisepsis actions.

METHODS. We conducted in vitro experiments to determine the antimicrobial efficacy of hand rubs within 15 seconds, followed by clinical observations to assess the effect of a shortened hand antisepsis procedure under clinical conditions in a neonatal intensive care unit (NICU). An independent observer monitored the frequency of hand antisepsis actions during shifts.

RESULTS. All tested hand rubs fulfilled the requirement of equal or even significantly higher efficacy within 15 seconds when compared to a reference alcohol propan-2-ol 60% (v/v) within 30 seconds. Microbiologically, reducing the application time to 15 seconds had a similar effect when compared to 30-second hand rubbing, but it resulted in significantly increased frequency of hand antisepsis actions (7.9 ± 4.3 per hour vs 5.8 ± 2.9 per hour; $P = .05$).

CONCLUSION. Time pressure and workload are recognized barriers to compliance. Therefore, reducing the recommended time for hand antisepsis actions, using tested and well-evaluated hand rub formulations, may improve hand hygiene compliance in clinical practice.

Infect Control Hosp Epidemiol 2017;38:1430–1434

Hand hygiene is the single most important measure to prevent healthcare-associated infections. Since the late 1960s and their first commercial availability, alcohol-based hand rubs (ABHRs) have been increasingly used for hand hygiene.[1] Many in vitro and in vivo studies have shown their preventive potential, and both the US Centers for Disease Control and Prevention (CDC)[2] and the World Health Organization (WHO) Guidelines for Hand Hygiene in Healthcare[3] favor ABHR over hand washing with antimicrobial soaps.[4–8] Alcohol-based hand rubs have additional benefits over antimicrobial soaps, such as no necessity for using sinks with water supply for washing and rinsing,[9] better dermal tolerance, and higher acceptability by healthcare professionals,[10–12] as well as a significantly shorter time to carry out the procedure.[13,14] Although there is consensus on the importance of hand hygiene and general agreement on the use of ABHR, the major challenge today remains healthcare worker compliance with this practice, which is generally reported to be low.[3,15] One frequently given reason for noncompliance is lack of time for this action.[16–24] Although recommendations for the application time of ABHRs have decreased over the past years from several minutes to a minimum of 30 seconds,[25] even this short time still seems to be too long in clinical practice. The actual time spent on a hand antisepsis action was reported to range between 5 and 24 seconds,[2,3] and the WHO Guideline on Hand Hygiene in Health Care recommends a duration of 20–30 seconds.[26] However, concerns have been raised that application times that are too short may decrease ABHR antimicrobial efficacy, and it remains unproven that further shortening the application time will encourage

Affiliations: 1. Institute of Hygiene and Environmental Medicine, University Hospital of Greifswald, Greifswald, Germany; 2. Infection Control Program and WHO Collaborating Centre on Patient Safety, University of Geneva Hospitals and Faculty of Medicine, Geneva, Switzerland; 3. Department of Infection Control and Hospital Epidemiology, Medical University of Vienna, Vienna General Hospital, Vienna, Austria; 4. Hygiene North GmbH, Greifswald, Greifswald, Germany; 5. Department of Pediatrics, University Hospital of Greifswald, Greifswald, Germany; 6. Center for the Newborn, Children, and Adolescents, General Hospital, Paracelsus Medical School, Nuremberg, Germany; 7. Institute for Skin Integrity & Infection Prevention, School of Human & Health Sciences, University of Huddersfield, Queensgate, Huddersfield, United Kingdom; 8. Institute for Hygiene and Applied Immunology, Center for Pathophysiology, Infectiology and Immunology, Medical University of Vienna, Vienna, Austria.

Received May 23, 2017; accepted September 21, 2017; electronically published October 30, 2017

 0899-823X/2017/3812-0005. DOI: 10.1017/ice.2017.217

healthcare workers to perform hand antisepsis actions more frequently.[27]

We investigated whether shortening the required application time from 30 seconds to 15 seconds may alter the antimicrobial efficacy of ABHRs and simultaneously influence healthcare worker compliance with the recommended frequency of hand antisepsis actions under real working conditions in a neonatal intensive care unit (NICU).

MATERIALS AND METHODS

In total, 10 commercially available ABHRs were included in the in vitro and in vivo tests. The evaluated ABHRs were based on the following compositions: (1) 70% (weight/weight [w/w]) ethanol, (2) 76.7% (w/w) ethanol, (3) 79.9% (w/w) ethanol, (4) 15% (w/w) ethanol + 55% (w/w) propan-1-ol, (5) 45% (w/w) ethanol + 18% (w/w) propan-1-ol, (6) 53.9% (w/w) ethanol + 21.6% (w/w) propan-1-ol, (7) 54% (w/w) ethanol + 10% (w/w) propan-1-ol, (8) 73.4% (w/w) ethanol + 10% (w/w) propan-2-ol, (9) 14.3% (w/w) propan-1-ol + 63.1% (w/w) propan-2-ol, 70% (w/w) propan-2-ol, and (10) 30% (w/w) propan-1-ol + 45% (w/w) propan-2-ol + 0.2% mecetroniumetile sulfate. Additionally, the 2 ABHRs based on the WHO formulation were tested: (1) 80% (w/w) ethanol + 1.45% glycerol + 0.125% H_2O_2 and (2) 75% (w/w) propan-2-ol + 1.45 glycerol + 0.125% H_2O_2.

The study was approved by the Ethic Committee of the University Hospital of Greifswald (Reg. No. BB 109/10).

In Vitro Antimicrobial Efficacy of Hand Rubs

The bactericidal and yeasticidal efficacy of a representative sample of ethanol- and propanol- based commercially available hand rubs was tested in quantitative suspension essays following the European norms (EN) 13727[28] and EN 13624.[29] All tested products were liquid ABHRs; gel formulations were not included because their drying times were longer than those of liquid rubs, thus potentially confounding comparisons.[25]

Efficacy of Artificially Contaminated Hands of Volunteers

The antimicrobial efficacy of ABHRs was evaluated following the EN 1500.[30] Alcohol-based hand rubs were tested against the reference alcohol propan-2-ol 60% (volume/volume [v/v]) in a blinded, controlled, crossover design with 15 volunteers for the in vivo test. Briefly, fingertips, including thumbs, were sampled in soy broth with the addition of Tween 80 3% (weight/weight [w/w]), saponine 3% (w/w), histidine 1% (w/w), and cysteine 1% (w/w) to eliminate the residual effect of a potential bacteriostatic detergent or compound. The fingertips of each hand were tested separately in a petri dish filled with sample fluid. The initial number of colonies (ie, pretreatment value) was determined, followed by a standardized hand-rubbing motion[30] at the respective observation time (15 seconds or 30 seconds). The result (ie, posttreatment value) was then recorded.

Sampling Culture and Calculation of Efficacy in Reducing Microorganisms

A volume of 0.1 mL sample fluid from hands was plated within 30 minutes after sampling in serial dilutions on Columbia blood agar plates. Culture plates were incubated for 48 hours at 36°C ± 1°C under aerobic conditions, and the number of colony-forming units (CFUs) was determined thereafter. The log reduction factor (log RF) was calculated by subtracting the log posttreatment values from the log pretreatment values. Log RFs were calculated separately for each participant, and the arithmetic mean of all samples was calculated.

Efficacy Under Practical Working Conditions

In a second step, a clinical observation trial was performed including 14 registered nurses working at the NICU of the University Hospital of Greifswald, Greifswald, Germany. Inclusion criteria were healthy skin condition, and short and clean fingernails without artificial or gelled fingernails and without nail polish. Exclusion criteria were open wounds and skin irritations on hands and lower forearms. All 14 participants underwent training on correct hand-rubbing technique using a pictogram according to EN 1500[30] and practical training using fluorescent dye together with the use of an ultraviolet light to control correct application. In addition, on-site training was repeated individually with each participant before the trial. Pictograms demonstrating the correct hand-rubbing technique were placed next to each hand rub dispenser.

For the clinical observation trial, an ABHR containing the active ingredients 45% (w/w) propan-2-ol + 30% (w/w) propan-1-ol + 0.2% mecetroniumetile sulfate was used. This product showed noninferiority when tested at 15 seconds compared to the reference alcohol 60% (v/v) propan-2-ol tested at 30 seconds. Furthermore, it was available as routine ABHR at the NICU under observation. Before the beginning of each shift and before the first hand rubbing, the initial bacterial colony count on fingertips of both hands of participating nurses was determined. This procedure was repeated hourly during the entire shift.

Frequency of ABHR Use

Participant compliance with hand antisepsis recommendations in terms of frequency was monitored during a complete working shift of 8 hours, starting at the beginning of a shift. Registered nurses were randomly allocated to a 15-second (group A) or 30-second (group B) hand antisepsis cohort. During the shift, a trained, independent observer monitored the frequency of hand antisepsis actions and recorded whether the assigned application time (15 seconds or 30 seconds) was followed. Parts of the WHO hand hygiene observation method[31,32] were used to measure compliance in terms of frequency of hand antisepsis moments. The overall consumption of ABHR and the count of hand antisepsis actions in each group were used to determine the average use of ABHR for each hand antisepsis action.

https://doi.org/10.1017/ice.2017.217 Published online by Cambridge University Press

Statistical Analysis

Bacterial counts were logarithmically transformed and expressed as means ± standard deviation (SD) before statistical analysis. Viable CFU $\log_{10}$ differences were calculated as $\log_{10}$ CFU of the tested ABHR product minus $\log_{10}$ CFU of a reference alcohol for each corresponding pair. Continuous variables such as frequency of hand antisepsis action were calculated as the mean of all separate counts ± SD. For the in vivo experiments on artificially contaminated hands, means ± SD of test and the reference group were compared using a matched Wilcoxon's matched-pairs signed-ranks test. To assess differences of the antimicrobial efficacy or the frequency of use under practical working conditions between group A (15 seconds) and group B (30 seconds), an unpaired 2-tailed t test was applied. All tests for significance were run as 2-sided tests, with α set at the 5% level.

RESULTS

In Vitro Antimicrobial Efficacy of ABHRs and Efficacy on Artificially Contaminated Hands

The in vitro suspension test demonstrated log RF of >5 for *Staphylococcus aureus*, *Enterococcus hirae*, *Escherichia coli*, *Pseudomonas aeruginosa*, and *Proteus mirabilis* and >4 for *Candida albicans* within 15 seconds of application time for all tested commercially available ABHRs (Table 1). All tested ABHRs fulfilled within 15 seconds the requirement of the EN 1500 as being equal or even more effective than the reference alcohol 60% (v/v) propan-2-ol within 30 seconds (Table 2).

Efficacy Under Practical Working Conditions

No significant differences between CFUs retrieved from hands before hand rubbing was observed in the groups rubbing hands for 15 seconds and 30 seconds ($P = .73$, Wilcoxon test, asymptotic significance; Table 3) or in terms of antimicrobial log RF of ABHRs applied for 15 seconds or 30 seconds ($P = .59$, Wilcoxon test). These results indicate that the application of ABHRs for 15 seconds has antimicrobial efficacy similar to that at 30 seconds. On average, 3.4 mL ABHR was used for each hand antisepsis action, with no difference in ABHR consumption between the 2 different application times (3.4 mL, group A vs 3.3 mL, group B; $P > .05$). Furthermore, there was no difference between the 2 groups in terms of retrievable CFU counts from hands before, during, and at the end of shifts. During the entire shifts, the mean retrievable CFU counts ranged from 0.2 to 4.4 log (15-second group: 2.13 ± 1.1 log CFU; 30-second group: 2.26 ± 1.3 log CFU; $P = .87$, Wilcoxon test). These results indicate that reducing the ABHR application time to 15 seconds had no influence on a possible accumulation of bacteria on hands.

When hands were rubbed with an application time of 15 seconds, the frequency of hand antisepsis action was significantly higher (7.9 ± 4.3 times per hour) than with the application time of 30 seconds (5.8 ±2.9 times per hour; $P < .05$). These results indicate that shortening the application time for hand antisepsis significantly increased the frequency of hand antisepsis actions.

DISCUSSION

The results of this investigation are based on clinical practice and provide evidence for the feasibility and safety of shortening the time required for hand antisepsis from 30 seconds to 15 seconds during real patient care. The prerequisite for such an approach is the proof of the efficacy of ABHRs fulfilling the requirements of the EN 1500 within an application time of 15 seconds. Our results also show increased frequency of hand antisepsis action in the group of healthcare workers at a 15-second application time compared to a 30-second hand antisepsis application time. This finding may suggest that the recommendation of the application time of ABHR alone seems to influence clinicians' willingness to use ABHRs more frequently. Notably, there was no difference in duration for hand antisepsis action between both groups in our observation. Although time recommendation did not practically shorten the hand antisepsis action, it encouraged the healthcare workers to follow hand antisepsis recommendations more frequently.

TABLE 1. Antimicrobial Efficacy of Commercially Available Alcohol-Based Hand Rubs at 15-Second Application Time

		Log Reduction Factor	
Formulation (% w/w)	Tested Dilution, %	*S. aureus,E. hirae, E. coli, P. aeruginosa, P. mirabilis*	*C. albicans*
Ethanol (79.9)	75	>5	>4
Ethanol (76.0)	97		
Ethanol (15.0), propan-1-ol (55.0)	75	>5	>4
Ethanol (73.4), propan-2-ol (10.0)	80	>5	>4
Ethanol (53.9), propan-1-ol (21.6)	90	>5	>4
Propan-1-ol (70.0)	75	>5	>4
Propan-1-ol (30.0), propan 2-ol (45.0), mecetroniumetile sulfate (0.2)	50	>5	>4

https://doi.org/10.1017/ice.2017.217 Published online by Cambridge University Press

TABLE 2. Log Reduction Factors of Commercially Available and WHO-Recommended ABHRs Within 15 Seconds Compared to the EN 1500 Reference Alcohol at 30 Seconds After Artificial Contamination With *Eschericha coli K12*

Formulation (% w/w)	Log Reduction Factor ± SD	
	Test Product	Reference[a]
Ethanol (70.0)	4.4 ± 1.04	4.0 ± 0.53
Ethanol (80.0), H_2O_2 (0.1)[b]	4.1 ± 0.60	4.4 ± 0.57
Ethanol (45.0), propan-1-ol (18.0)	4.5 ± 0.77	4.7 ± 0.94
Ethanol (54.0), propan-1-ol (10.0)	4.8 ± 0.69[c]	4.5 ± 0.77
Ethanol (15.0), propan-1-ol (55.0)	4.4 ± 0.80	4.4 ± 0.75
Ethanol (73.4), propan-2-ol (10.0)	4.7 ± 0.66[c]	3.8 ± 0.77
Propan-2-ol (70.0), H_2O_2 (0.1) [b]	4.9 ± 0.80[c]	4.5 ± 0.70
Propan-1-ol (30.0), propan-2-ol (45.0), mecetroniumetile sulfate (0.2)	5.2 ± 0.62[c]	5.1 ± 0.63
Propan-1-ol (14.3), propan-2-ol (63.14)	4.8 ± 0.55	4.5 ± 0.72

NOTE. WHO, World Health Organization; ABHR, alcohol-based hand rub; SD, standard deviation.
[a]EN 1500 reference alcohol: propan-2-ol 60% (v/v).
[b]WHO-recommended ABHR formulations.
[c]$P < .05$ (Wilcoxon test).

TABLE 3. Comparison of the Efficacy of an ABHR[a] at 15-Second and 30-Second Application Times Before and After Hand Rubbing During Clinical Practice in a NICU

Duration	Pretreatment Value, log		Posttreatment Value, log		Log Reduction Factor	
	Mean	SD	Mean	SD	Mean	SD
15 s	1.79	0.64	0.55	0.75	1.24	0.68
30 s	1.78	0.51	0.47	0.68	1.31	0.61

NOTE. ABHR, alcohol-based hand rub; NICU, neonatal intensive care unit; SD, standard deviation.
[a]Content (% v/v) of tested formulation: propan-1-ol (30.0), propan-2-ol (45.0), mecetroniumetile sulfate (0.2).

The precondition for investigating a shortened application time of 15 seconds for ABHRs in comparison to the currently recommended 30 seconds in a clinical setting was the assessment of their antimicrobial efficacy in vitro and under practical experimental conditions on artificially contaminated hands. Our in vitro experiments confirmed that the shorter application time was not associated with a statistically significant reduction in antimicrobial efficacy of ABHRs, as recently demonstrated by Pires et al[33] in a laboratory based study in conditions closely mimicking clinical practice. In our study, all tested ABHRs fulfill this precondition according to one of the most stringent[3] standard testing norms (ie, EN 1500).[30]

For the study under practical clinical conditions, the shortened application time was the only different factor applied during the study period. All participants were registered nurses and performed all procedures without any dropout. To achieve standardization under clinical conditions, all participants were carefully supervised by a trained infection-control nurse. Indeed, direct observation of staff during their clinical work is the preferred method to assess compliance with hand antisepsis recommendations.[3,31,32,34] Because there were no differences in antimicrobial efficacy of the ABHRs among both groups and because the reduction of the recommended application time was associated with a significantly increased frequency of hand antisepsis actions, a reduction of the time for hand rubbing can be recommended to encourage healthcare workers to perform hand antisepsis, provided that the ABHR in use has proven efficacy after 15 seconds. This efficacy does not extend to all available ABHRs, and particularly not gel formulations of ABHRs. Many available commercial products may not fulfill the requirements of the EN 1500 within 15 seconds.[25,35]

Although they are encouraging, our results must be regarded with some caution. This study lacked monitoring compliance with hand hygiene practices in full accordance with the definition proposed by the WHO in 2009.[3,26,32] Although the strength of our study is that the observer was physically present in the NICU and directly observed hand antisepsis actions, the quality of performance was not assessed. However, all 14 participants were trained to perform the correct hand-rubbing technique using appropriate approach and repeated practical training before the trial to control correct ABHR application. Nevertheless, we report only the frequency of hand antisepsis actions, which technically does not equal compliance as defined by the WHO.[36] This gap should be closed in future studies.

In conclusion, shortening the application time of ABHRs from 30 seconds to 15 seconds showed no statistically significant difference in the antimicrobial efficacy of applied ABHR products on the hands of healthcare workers, but it significantly increased the frequency of hand antisepsis actions under clinical conditions. Because time pressure and workload are recognized barriers to compliance,[17,20–24] reducing the currently recommended 20–30 seconds duration of ABHR application,[26] using tested and well evaluated hand rub formulations, may constitute a further step toward improving compliance with hand hygiene recommendations in clinical practice.[36]

ACKNOWLEDGMENTS

Financial support: This study was conducted with funds from the routine departmental research budgets of the Institute of Hygiene and Environmental Medicine, University Hospital of Greifswald, Greifswald, Germany. No external funds from sources outside the institution were received.

Potential conflicts of interest: Prof. Ojan Assadian was member of the medical advisory boards of Hutchinson santé, Beckon Dickenson, and Mölnlycke Health Care until end of 2016, and he declares having received consulting and lecture fees, travel compensation, and speaker's honoraria from Altrazeal Europe, Ltd., Antiseptica Chemical GmbH, Bode GmbH Germany, B. Braun Melsungen AG, Ethicon, Ltd., Kinetic Concepts, Inc., Lohmann & Rauscher, Smith & Nephew, Ltd., Quantum Management & Service GmbH, and Schulke & Mayr GmbH in the past. All other authors report no potential conflicts of interest relevant to this article.

Address correspondence to Romana Klasinc, MD, Department for Infection Control and Hospital Epidemiology, Medical University of Vienna, General Hospital of Vienna Waehringer Guertel 18-20, 1090 Vienna, Austria (romana.klasinc@meduniwien.ac.at).

https://doi.org/10.1017/ice.2017.217 Published online by Cambridge University Press

REFERENCES

1. Kampf G. State-of-the-art hand hygiene in community medicine. *Int J Hyg Environ Health* 2003;206:465–472.
2. Boyce JM, Pittet D. 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. 2002;51(No. RR-16):1–45.
3. Pittet D, Allegranzi B, Boyce J, for the World Health Organization World Alliance for Patient Safety First Global Patient Safety Challenge Core Group of Experts. The World Health Organization Guidelines on Hand Hygiene in Health Care and their consensus recommendations. *Infect Control Hosp Epidemiol* 2009;30:611–622.
4. Rotter ML, Kampf G, Suchomel M, Kundi M. Population kinetics of the skin flora on gloved hands following surgical hand disinfection with 3 propanol-based hand rubs: a prospective, randomized, double-blind trial. *Infect Control Hosp Epidemiol* 2007;28:346–350.
5. Tavolacci MP, Pitrou I, Merle V, Haghighat S, Thillard D, Czernichow P. Surgical hand rubbing compared with surgical hand scrubbing: comparison of efficacy and costs. *J Hosp Infect* 2006;63:55–59.
6. Kramer A, Bernig T, Kampf G. Clinical double-blind trial on the dermal tolerance and user acceptability of six alcohol-based hand disinfectants for hygienic hand disinfection. *J Hosp Infect* 2002;51:114–120.
7. Larson EL, Aiello AE, Bastyr J, et al. Assessment of two hand hygiene regimens for intensive care unit personnel. *Crit Care Med* 2001;29:944–951.
8. Pittet D, Boyce JM. Revolutionising hand hygiene in health-care settings: guidelines revisited. *Lancet Infect Dis* 2003;3:269–270.
9. Boyce JM. Using alcohol for hand antisepsis: dispelling old myths. *Infect Control Hosp Epidemiol* 2000;21:438–441.
10. Pittet D, Allegranzi B, Sax H, Chraiti M-N, Griffiths W, Richet H. Double-blind, randomized, crossover trial of 3 hand rub formulations: fast-track evaluation of tolerability and acceptability. *Infect Control Hosp Epidemiol* 2007;28:1344–1351.
11. Kampf G, Löffler G. Dermatological aspects of a successful introduction and continuation of alcohol-based hand rubs for hygienic hand disinfection. *J Hosp Infect* 2003;55:1–7.
12. Souweine B, Lautrette A, Aumeran C, et al. Comparison of acceptability, skin tolerance, and compliance between handwashing and alcohol-based handrub in ICUs: results of a multicentric study. *Intensive Care Med* 2009;35:1216–1224.
13. Voss A, Widmer AF. No time for handwashing!? Handwashing versus alcoholic rub can we afford 100% compliance? *Infect Control Hosp Epidemiol* 1997;18:205–208.
14. Rotter ML. Arguments for alcoholic hand disinfection. *J Hosp Infect* 2001;48:4–8.
15. Pittet D. Improving compliance with hand hygiene in hospitals. *Infect Control Hosp Epidemiol* 2000;21:381–386.
16. Boyce JM. Hand hygiene compliance monitoring: current perspectives from the USA. *J Hosp Infect* 2008;70(Suppl 1):2–7.
17. De Wandel D, Maes L, Labeau S, Vereecken C, Blot S. Behavioral determinants of hand hygiene compliance in intensive care units. *Am J Crit Care* 2010;19:230–239.
18. Barrett R, Randle J. Hand hygiene practices: nursing students' perceptions. *J Clin Nurs* 2008;17:1851–1857.
19. Longtin Y, Sax H, Leape LL, Sheridan SE, Donaldson L, Pittet D. Patient participation: current knowledge and applicability to patient safety. *Mayo Clin Proc* 2010;85:53–62.
20. Dunn-Navarra A-M, Cohen B, Stone PW, Pogorzelska M, Jordan S, Larson E. Relationship between systems-level factors and hand hygiene adherence. *J Nurs Care Qual* 2011;26:30–38.
21. Takahashi I, Turale S. Evaluation of individual and facility factors that promote hand washing in aged-care facilities in Japan. *Nurs Health Sci* 2010;12:127–134.
22. Buffet-Bataillon S, Leray E, Poisson M, Michelet C, Bonnaure-Mallet M, Cormier M. Influence of job seniority, hand hygiene education, and patient-to-nurse ratio on hand disinfection compliance. *J Hosp Infect* 2010;76:32–35.
23. Korniewicz DM, El-Masri M. Exploring the factors associated with hand hygiene compliance of nurses during routine clinical practice. *Appl Nurs Res* 2010;23:86–90.
24. Allegranzi B, Pittet D. Role of hand hygiene in healthcare-associated infection prevention. *J Hosp Infect* 2009;73:305–315.
25. Kramer A, Rudolph P, Kampf G, Pittet D. Limited efficacy of alcohol-based hand gels. *Lancet* 2002;359:1489–1490.
26. World Health Organization. *WHO Guidelines on Hand Hygiene in Health Care. First global patient safety challenge clean care is safer care. World Alliance for Patient Safety*. Geneva: World Health Organization Press; 2009.
27. Pittet D. Hand hygiene: it's all about when and how. *Infect Control Hosp Epidemiol* 2008;29:957–959.
28. EN 13727. Chemical disinfectants and antiseptics. Quantitative suspension test for the evaluation of bactericidal activity in the medical area. Test method and requirements (phase 2, step 1). Brussels: European Committee for Standardization; 2003.
29. EN 13624. Chemical disinfectants and antiseptics. Quantitative suspension test for the evaluation of fungicidal activity of chemical disinfectants for instruments used in the medical area. Test method and requirements (phase 2, step 1). Brussels: European Committee for Standardization; 2003.
30. EN 1500. Chemical disinfectants and antiseptics—hygienic handrub—test method and requirements (Phase 2/Step 2). Brussels: European Committee for Standardization; 1997.
31. Sax H, Allegranzi B, Chraïti M-N, Boyce J, Larson E, Pittet D. The World Health Organization hand hygiene observation method. *Am J Infect Control* 2009;37:827–834.
32. Pittet D, Mourouga P, Perneger TV. Compliance with handwashing in a teaching hospital. Infection control program. *Ann Intern Med* 1999;130:126–130.
33. Pires D, Soule H, Bellissimo-Rodrigues F, Gayet-Ageron A, Pittet D. Hand hygiene with alcohol-based handrub: how long is long enough? *Infect Control Hosp Epidemiol* 2017;38:547–552.
34. Pittet D, Hugonnet S, Harbarth S, et al. Effectiveness of a hospital-wide programme to improve compliance with hand hygiene. *Lancet* 2000;356:1307–1312.
35. Dharan S, Hugonnet S, Sax H, Pittet D. Comparison of waterless hand antisepsis agents at short application times: raising the flag of concern. *Infect Control Hosp Epidemiol* 2003;24:160–164.
36. Pittet D, Donaldson L. Clean care is safer care: the first global challenge of the WHO World Alliance for Patient Safety. *Infect Control Hosp Epidemiol* 2005;26:891–894.