

**Comparing the effectiveness of hand hygiene techniques in reducing the microbial load and covering hand surfaces in healthcare workers: updated systematic review**

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*Published in:*  
American Journal of Infection Control

*DOI:*  
[10.1016/j.ajic.2022.02.003](https://doi.org/10.1016/j.ajic.2022.02.003)

*Publication date:*  
2022

*Document Version*  
Author accepted manuscript

[Link to publication in ResearchOnline](#)

*Citation for published version (Harvard):*

Price, L, Gozdzielewska, L, Matuluko, A, Pittet, D, Allegranzi, B & Reilly, J 2022, 'Comparing the effectiveness of hand hygiene techniques in reducing the microbial load and covering hand surfaces in healthcare workers: updated systematic review', *American Journal of Infection Control*. <https://doi.org/10.1016/j.ajic.2022.02.003>

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**Title: Comparing the effectiveness of hand hygiene techniques in reducing the microbial load and covering hand surfaces in healthcare workers: updated systematic review**

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**Declaration of interest:** LP, LG, AM and JR report a grant from WHO to do the study. AM also reports being a remote volunteer in the International Pharmaceutical Federation (FIP) Commission on Antimicrobial Resistance (Jan 2021 to present) and previous Chairperson of Projects FIP Young Pharmacists Group (Sept 2019 to Dec 2020). The remaining authors (DP and BA) do not report any declarations of interest

**Title: Comparing the effectiveness of hand hygiene techniques in reducing the microbial load and covering hand surfaces in healthcare workers: updated systematic review**

## **Abstract**

**Background:** This review, commissioned by the World Health Organization (WHO), examined the effectiveness of the WHO 6-step hand hygiene (HH) technique in reducing microbial load on hands and covering hand surfaces, and compared its effectiveness to other techniques.

**Methods:** Medline, CINAHL, ProQuest, Web of Science, Mednar, and Google Scholar were searched for primary studies, published in English (1978 - February 2021), evaluating the microbiological effectiveness or hand surface coverage of HH techniques in healthcare workers. Reviewers independently performed quality assessment using Cochrane tools. The protocol for the narrative review was registered (PROSPERO 2021: CRD42021236138).

**Results:** Nine studies were included. Evidence demonstrated that the WHO technique reduced microbial load on hands. One study found the WHO technique more effective than the 3-step technique ( $P=0.02$ ), while another found no difference between these two techniques ( $P=0.08$ ). An adapted 3-step technique was more effective than the WHO technique in laboratory settings ( $P=0.021$ ), but not in clinical practice ( $P=0.629$ ). One study demonstrated that an adapted 6-step technique was more effective than the WHO technique ( $P=0.001$ ). Evidence was heterogeneous in application time, product, and volume. All studies were high risk of bias.

**Conclusions:** Eight studies found that the WHO 6-step technique reduced microbial load on healthcare workers' hands; but the studies were heterogeneous and further research is required to identify the most effective, yet feasible technique.

**Keywords:** hand hygiene; technique; systematic review; effectiveness; microbial load

## **Highlights**

- WHO 6-step technique effectively reduces bacterial load on hands
- Effectiveness of hand hygiene techniques in covering all hand surfaces is limited
- Evidence for the most effective and feasible hand hygiene technique is inconclusive
- Substantial heterogeneity exists in the body of evidence
- Hand hygiene technique studies require standardisation

## Background

It is widely acknowledged that effective hand hygiene (HH) among healthcare workers (HCWs) is one of the most important infection prevention strategies available<sup>1,2</sup>; and therefore, is a key element of infection prevention and control guidelines.<sup>3</sup> However, uncertainty remains concerning a range of issues related to HH.<sup>2,4</sup>

One major issue relates to which technique to use when performing HH.<sup>4-11</sup> The World Health Organization (WHO) recommends the adoption of a 6-step technique,<sup>2</sup> which was originally developed in 1978 by Professor Graham Ayliffe to standardize testing of HH products.<sup>12</sup> Elements 2-7 on Figure 1 shows the areas on hands that should be covered with alcohol-based handrub (ABHR) when performing handrubbing, or with soap when performing hand washing using the 6-step technique.

The 6-step technique has now been adopted globally as the gold standard for hand washing with soap and water and for hand rubbing ABHR in clinical practice;<sup>13</sup> compliance, however, is usually low.<sup>14-16</sup> One possible way to increase compliance with the technique is to provide HCWs with evidence of how effective the recommended 6-step technique is in decontaminating their hands and in covering all hand surfaces.<sup>17</sup> A previous review identified the body of evidence in relation to the most effective HH technique offered conflicting findings.<sup>18</sup> Given the current increased interest in improving HH practice in response to the COVID-19 pandemic<sup>19,20</sup> and the continuously growing body of evidence, we updated our previous review.

Figure 1. World Health Organization's 6-step technique for hand rubbing with alcohol-based handrub.<sup>21</sup>

# How to Handrub?

RUB HANDS FOR HAND HYGIENE! WASH HANDS WHEN VISIBLY SOILED

**⌚** Duration of the entire procedure: 20-30 seconds



 **World Health Organization**

**Patient Safety**  
A World Alliance for Safer Health Care

**SAVE LIVES**  
Clean Your Hands

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May 2009

The aims of our updated review, commissioned by WHO, were to examine the effectiveness of the WHO 6-step technique in reducing microbial load on hands and covering all hand surfaces, and to compare its effectiveness to that of other techniques. The other techniques involved adaptations to the order of performance of the WHO's 6-step technique or to techniques involving three steps. The 3-step techniques were based on the Centers for

Disease Control and Prevention's (CDC) 3-step HH technique which involves putting product on hands and rubbing hands together until dry while covering all hand surfaces.<sup>22</sup>

## **Methods**

This systematic review was an update of the original review conducted in 2018.<sup>18</sup> The findings of the studies included in the original and in the updated review are presented in this paper. The updated review used the same search strategy as the original 2018 review<sup>18</sup> but upgraded the quality assessment methods to use, where appropriate, Cochrane Collaboration's Effective Practice and Organization of Care (EPOC) tools.<sup>23</sup> The updated review protocol was prospectively registered with the international prospective register of systematic reviews (PROSPERO 2021: CRD42021236138) (Available from: [https://www.crd.york.ac.uk/prospero/display\\_record.php?ID=CRD42021236138](https://www.crd.york.ac.uk/prospero/display_record.php?ID=CRD42021236138)) and is reported in accordance with the Preferred Reporting Items for Systematic Reviews and Meta-Analyses (PRISMA) statement.<sup>24</sup>

### ***Inclusion criteria***

Studies were included in the review if they referred to the WHO 6-step technique, or the technique described was consistent with the WHO technique. Studies focusing on HCWs, (including, but not limiting to physicians, nurses, nursing assistants, allied health professionals, or healthcare students), performing either a hand rubbing or handwashing within any healthcare context, in any country were included.

### ***Exclusion criteria***

Studies based in operating theatres using surgical hand preparation protocols were excluded, since the HH techniques and duration differ within this setting.<sup>2</sup> Studies were also excluded if



they were not specifically about HH technique but were, instead, investigating the efficacy of HH products or evaluating HH compliance. Studies not conducted with HCWs were excluded as well, as were those that did not measure microbial load.

### ***Outcomes***

The primary outcomes required in the reviewed studies were reduction in the microbial load or surface coverage of HCWs' hands following application of the tested HH techniques. Studies were included if their aims did not include testing the effectiveness of the technique, but quantification of the effect of using the technique was one of the outcomes measured and reported separately. Secondary outcome was a measure of time of hand decontamination.

### ***Types of study***

To enable the identification of the current available evidence, the review considered empirical research designs, including randomized controlled trials (RCTs), non-randomized controlled trials (NRTs), before and after studies, case control studies, cohort studies and observational descriptive studies. Reviews, conference proceedings and non-primary research records, such as editorials, opinion-based papers and commentaries were excluded.

### ***Search strategy***

The search included sources published between 1978 (the first year we were aware of the technique being used<sup>12</sup>) and February 2021. A three-stage search strategy was employed. Keywords and index terms were searched in CINAHL, Medline, ProQuest and Web of Science databases with the search restricted to sources published in English language. The full search strategy applied for Medline (Supplementary file 1) was individualised for the other databases according to their functionality. Secondly, as keyword terms cannot be combined in Mednar and Google Scholar, only the broadest keywords were searched in these

databases. Finally, the reference lists of included papers were searched manually to identify any additional relevant articles.

### ***Study selection***

The titles and abstracts of all articles retrieved from the search were independently screened for relevance by two reviewers against the eligibility criteria relating to study design, population, intervention, and outcomes, as described above. The full-texts of articles that met the inclusion criteria after the title and abstract search, and those in which there was insufficient evidence in the title and abstract to make a decision, were reviewed independently by the two reviewers. Discrepancies were resolved through discussion.

### ***Quality assessment and data extraction***

Two reviewers independently extracted data from the eligible studies, using a standardised data collection tool (Supplementary file 2), which was used previously and described elsewhere.<sup>18</sup> Full-text copies of all articles included in the review were independently reviewed by two reviewers to assess their quality. Studies meeting the EPOC design criteria,<sup>25</sup> i.e. RCTs, NRTs, controlled before-and-after studies, interrupted time series studies and repeated measures studies,<sup>25</sup> were assessed for quality using the recommended EPOC tool<sup>23</sup>; while design-specific, Joanna Briggs Institute critical appraisal checklists<sup>26</sup> were used for the remaining study designs. Disagreements were resolved through discussion or by the involvement of another reviewer.

### ***Analysis***

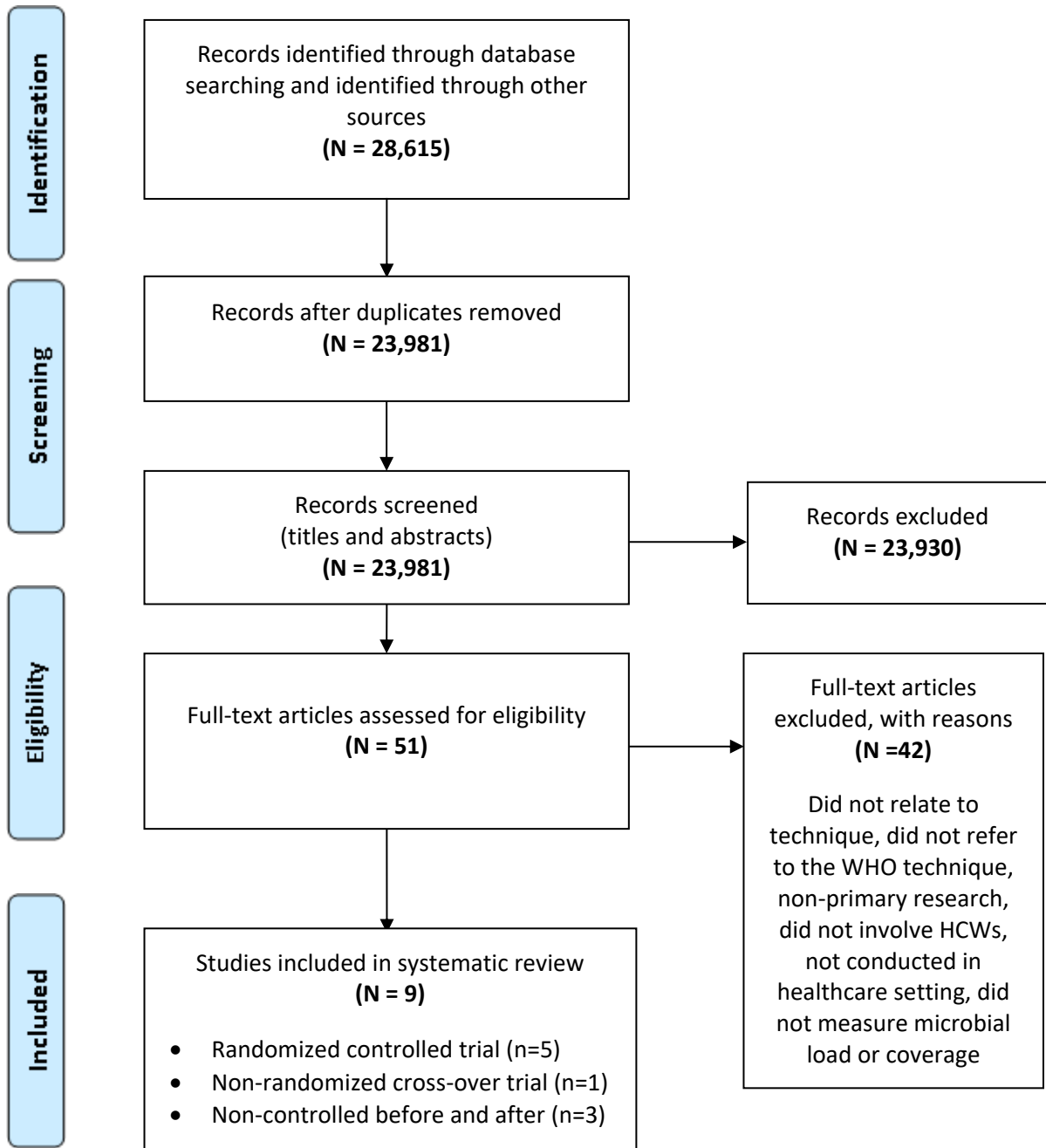
The results are presented in a narrative summary because it was not appropriate to conduct a meta-analysis nor to use the Grading of Recommendations Assessment, Development and Evaluation (GRADE) approach,<sup>27</sup> due to the substantial heterogeneity of the studies.

# Results

## *Search results*

As shown in Figure 2, the search resulted in a total of 28,615 records. Of these, 4634 were duplicates, resulting in 23,981 records being eligible for stage two of the selection process. After screening of titles and abstracts for eligibility, 51 records were selected for full-text review, of which nine<sup>6, 28-35</sup> were included in the review. The characteristics of these studies are shown in Table 1.

Figure 2. PRISMA 2009 Flow Diagram<sup>23</sup>



**Table 1. Characteristics of studies**

Author Date Country	Aim	Design	Setting	Participants	Intervention(s)	Outcome measure(s)	Results
Widmer et al. (2007) <sup>35</sup> Switzerland	To evaluate the impact of the WHO 6-step technique	NCBA	University affiliated geriatric hospital	All physicians & 10 nurses per ward were selected by an infection control professional (n = 180)	WHO 6-step technique  Product used: Fluorescent ABHR (Sterillium, Bode; (isopropanol + mecetronium acid + fluorescent dye); volume used: 3ml; application time: 30 seconds; technique adherence verified & accounted for	<u>Primary outcome:</u> CFUs from finger imprint technique	<u>Before training with WHO handrub technique:</u> 31% of HCWs used proper technique, and achieved a mean RF of 1.4 log <sub>10</sub> CFU <u>After training:</u> 74% of HCWs used proper technique with mean RF of 2.2 log <sub>10</sub> CFU. Increased antimicrobial effect of the technique (P<0.001) with improvement in application of the technique.
Laustsen et al. (2008) <sup>6</sup> Denmark	To investigate the use of the correct application of WHO 6-step technique before and after performance of a clinical procedure	NCBA	University hospital	HCWs from 10 departments working during a randomly chosen weekday (n = 117). HCWs with hand dermatitis were excluded (n = 2)	WHO 6-step technique before and after a clinical procedure  Product used: ABHR Gel 85% (v/v) ethanol + 0.5% Glycerine; volume used: 2-3ml; application time: 30 seconds; technique adherence verified & accounted for	<u>Primary outcome:</u> CFUs from finger imprint technique of the dominant hand	<u>Before clinical procedure:</u> 56% (n = 66/117) performed correct WHO technique <b>Correctly performed technique</b> - significant reduction in the number of CFUs by 90% (from 18.1 [95% CI, 13.5-24.2] to 1.8 CFU [95% CI, 1.1-2.7] per plate; P<0.001) <b>Incorrectly performed technique</b> - significant reduction in the number of CFUs by 60% (from 25.5 [95% CI, 18.4-35.1] to 10.2 [95% CI, 7.2-14.3] CFU per plate; P<0.001) <u>After clinical procedure:</u> 58% (n = 68/117) performed correct WHO handrub technique <b>Correctly performed technique</b> - significant reduction in the number of CFUs by 82% (from 10.0 [95% CI,

Author Date Country	Aim	Design	Setting	Participants	Intervention(s)	Outcome measure(s)	Results
							7.4-13.5] to 1.8 [95% CI, 1.1-2.7] CFU per plate; P<0.001) <b>Incorrectly performed technique</b> - significant reduction in the number of CFUs by 54% (from 16.3 [95% CI,11.6-22.7] to 7.5 [95% CI, 5.2-10.7] CFU per plate; P<0.001)
Tschudin-Sutter et al. (2010) <sup>32</sup> Switzerland	To evaluate the level of bacterial killing on hands of medical students using the WHO technique	NCBA	University hospital training facilities	Medical students (n=563)	WHO 6-step technique  Product used: ABHR 2-propanol 45g + 1-propanol 30g + mecetronium ethylsulfate 0.2 g + fluorescent dye; volume in cupped hand; application time: 20-30 seconds; technique monitored & supervised	<u>Primary outcomes:</u> (1) CFUs from finger imprint technique before and after use of handrub; (2) Hand coverage	<u>Before WHO handrub technique:</u> Bacterial density was 26-100 CFU per plate (n = 259, 46%); >100 CFU per plate (n = 207, 36.8%); <26 CFU per plate (n = 97, 17.2%) <u>After WHO handrub technique:</u> No detectable bacteria (n = 244, 43.3%) <25 CFUs per plate (n = 262, 46.5%) 25-100 CFUs per plate (n = 45, 8%) <100CFUs per plate (n = 12, 2.1%) Highly significant (P<0.001) difference in the density of CFUs before and after WHO handrub technique.
Chow et al. (2012) <sup>28</sup> Singapore	1. To compare the efficacy of HH protocols during routine inpatient clinical care 2. To evaluate the time effectiveness of each protocol	RCT	Adult, tertiary care general hospital	Medical and nursing staff (n = 120); 20 medical & 20 nursing staff to each of the 3 intervention groups	<u>Three intervention groups:</u> 1. CDC 3-step technique (hand rubbing with ABHR covering all hand surfaces in no particular order) 2. WHO 6-step technique (hand rubbing with ABHR using the WHO technique)  Product used: ABHR 70% ethanol + 2.5% chlorhexidine gluconate; handwashing- chlorhexidine gluconate 4% hand wash; volume of product used: no details	<u>Primary outcomes:</u> CFUs using the modified glove juice technique of the dominant hand of each participant (a) after patient contact but before HH and (b) after HH <u>Secondary outcome:</u>	Overall, HH resulted in a substantial reduction in bacterial load of 77.65 x10 <sup>2</sup> CFU/ml (P<0.01). After adjusting for staff category compared with protocol 1, protocol 2 (-5.17 x 10 <sup>2</sup> CFU/ml, P=0.07) resulted in slightly greater, but non-significant bacterial load reduction. Both protocols were effective in reducing hand bacterial load. Protocol 1 required less time (Mdn: 26.0 seconds) than protocol 2 (Mdn: 38.5 seconds; P=0.04)

Author Date Country	Aim	Design	Setting	Participants	Intervention(s)	Outcome measure(s)	Results
					provided; technique adherence verified & accounted for	Mean time of each HH protocol	
Reilly et al. (2016) <sup>30</sup> Scotland	To evaluate the microbiologic effectiveness of the WHO 6-step and the CDC 3-step HH techniques using ABHR	RCT	Acute care inner city teaching hospital	Medical and nursing staff (n = 120); doctors (n = 42) & nurses (n = 78)	<p><u>Two intervention groups:</u></p> <p>1. WHO 6-step technique 2. CDC 3-step technique</p> <p>Product used: Softcare Med ABHR, Diversey (isopropyl alcohol and n-propanol; details on concentration not provided), and ABHR with fluorescent dye- Spirigel, Ecolab (coverage); volume used- 3ml (via a pump dispenser); technique adherence verified &amp; accounted for</p>	<p><u>Primary outcomes:</u></p> <p>(1) CFUs (residual bacterial load) using the modified glove juice technique of each participant (a) after patient contact but before HH and (b) after HH; (2) hand coverage</p> <p><u>Secondary outcomes:</u></p> <p>(1) Compliance with the technique; (2) duration (seconds)</p>	<p>Protocol 1 reduced the count from 3.28 (95% CI, 3.11- 3.38) to 2.58 (2.08-2.93) CFU/mL, whereas Protocol 2 from 3.08 (2.977-3.27) to 2.88 (-2.58 to 3.15) CFU/mL, (P=0.02).</p> <p>Only 65% (n = 39/60) were fully compliant with Protocol 1. Among those fully compliant, the median bacterial load went from 3.18 (before) to 2.08 (after HH) log<sub>10</sub> CFU/mL, compared with 3.36 (before) to 2.55 (after HH) log<sub>10</sub> CFU/mL among those not fully compliant (P=0.01)</p> <p>No significant difference in total hand coverage between Protocol 1 (98.8%) vs Protocol 2 (99.0%, P=0.15)</p> <p>Median percentage of hand area not covered was 1.20 for Protocol 1 and 1.02 for Protocol 2 (P=0.97)</p> <p>Protocol 1 required 15% (95% CI, 6-24%) more time than the Protocol 2 (42.5 vs 35 seconds, P=0.002)</p>
Pires et al. (2017) <sup>29</sup> Switzerland	To evaluate whether modifying the sequence of the WHO technique by performing step 6 first would result in greater bacterial	NRT (crossover)	Laboratory, university hospital	HCWs (n = 16) Nurses (n = 7) & medical doctors/ pharmacists/ biologists (n = 9)	<p><u>Two intervention groups:</u></p> <p>1. WHO 6-step technique 2. Modified WHO 6-step technique, in which last step of the standard WHO technique (rubbing of the fingertips) was performed first</p> <p>Product used:</p>	<p><u>Primary outcome:</u></p> <p>CFUs using the finger imprint technique at baseline and after each of the</p>	<p>Overall, the log<sub>10</sub> reduction in bacterial concentration was significantly higher when performing Protocol 2 (3.44 [±1.33, 3.20]) compared with Protocol 1 (2.68 [SD: 1.48, 2.85])</p> <p>After adjustment for hand size</p>

Author Date Country	Aim	Design	Setting	Participants	Intervention(s)	Outcome measure(s)	Results
	reduction on HCWs' hands				ABHR 60% (v/v) isopropanol; volume used: 3ml; application time: 30 seconds	two different techniques	and gender, the mean reduction of bacterial concentration was 0.77 log <sub>10</sub> greater (95% CI, 0.27-1.26; P=0.002) for Protocol 2 compared to Protocol 1.
Tschudin-Sutter et al. (2017) <sup>33</sup> Switzerland	To assess a modified 3-step technique and compare it to the conventional WHO 6-step technique in terms of bacterial counts reduction on HCWs' hands	RCT (crossover)	Laboratory, university hospital	Medical students (n=32)	<u>Intervention group:</u> Modified 3-step technique consisting of: a. covering all surfaces of the hands b. rotational rubbing of fingertips in the palm of the alternate hand c. rotational rubbing of both thumbs <u>Control group:</u> WHO 6-step technique  Product used: ABHR 60% (v/v) 2-propanol; volume used: 3ml; application time: 30 seconds; artificial contamination ( <i>E. coli</i> ); technique adherence verified (100% for both protocols)	<u>Primary outcome:</u> CFUs using the modified glove juice technique	<u>Before HH:</u> Mdn bacterial counts did not differ between the control group (6.37 log <sub>10</sub> CFU, IQR: 6.19-6.54) and the intervention group (6.34 log <sub>10</sub> CFU, IQR: 6.17-6.60, one-sided P=0.513) <u>After HH:</u> Reductions in CFU were evident for both groups. Median bacterial counts were lower in the intervention group (1.96 log <sub>10</sub> CFU, IQR 1.25-2.52) compared to control group (2.34 log <sub>10</sub> CFU, IQR: 1.80-2.71, one-sided P=0.055) The Mdn log RF was higher in the intervention group (4.45 log <sub>10</sub> CFU, IQR: 4.04-5.15 versus 3.91 log <sub>10</sub> CFU, IQR: 3.69-4.62, one-sided P=0.010, two-sided P= 0.021)
Sakmen et al. (2019) <sup>31</sup> Germany	To compare the effectiveness of two techniques of hand disinfection in undergraduate surgical education	Cluster RCT	University hospital training facilities	Medical students (n=198)  6-step (n=103); self-responsible disinfection: (n=95)	<u>Intervention groups:</u> 1. WHO 6-step technique 2. Self-responsible application (no instructions)  Product used: Fluorescent ABHR (no details provided); volume used: 3-5ml (sufficient to fill cupped hand); application time: 30 seconds	<u>Primary outcome:</u> Hand coverage at three points of time: (1) directly after HH teaching was delivered (2) at the end of the one-week training of practical clinical skills	The mean (SD) percentage hand coverage deteriorated at each data collection point in group 1 [Point 1: 97.40% (2.94); Point 2: 96.60% (2.78); Point 3: 78.93% (22.04)]. For group 2, it did not deteriorate between data collection point 1 [97.26% (3.04)] & 2 [97.88% (2.90)] but did for the third [82.52% (19.48)]. No significant difference between hand coverage at data point 1 (P=0.584) and 3 (P=0.123)



Author Date Country	Aim	Design	Setting	Participants	Intervention(s)	Outcome measure(s)	Results
						(3) at students OSCE examination at the end of the semester (5–12 weeks after the initial training)	but there was for data point 2 (P=<0.001).
Tschudin-Sutter et al. (2019) <sup>34</sup> Switzerland	To compare compliance between 3-step and 6-step HH techniques for hand rubbing, as well as the relative reductions of bacterial loads on the hands of HCWs in clinical practice.	Cluster RCT	A tertiary, academic care centre in a university hospital	HCWs (n=113)	<u>Intervention group:</u> Modified 3-step technique consisting of: a. covering all surfaces of the hands b. rotational rubbing of fingertips in the palm of the alternate hand c. rotational rubbing of both thumbs  <u>Control group:</u> WHO 6-step technique  Product used: ABHR 75% (propan-2-ol 45/100g + propan-1-ol 30/100g + mecetroniumetilsulfate 0.2/100g); volume used: 3ml; application time: 30 seconds	<u>Primary outcome:</u> CFUs using the broth bag technique before & after application of ABHR <u>Secondary outcome:</u> Compliance with the technique	RF did not differ between techniques (P=0.629) <u>3-step technique</u> - Mdn: 0.97 log <sub>10</sub> CFU; IQR: 0.39–1.59 <u>6-step technique</u> - Mdn: 1.04 log <sub>10</sub> CFU; IQR: 0.49–1.52  Compliance with the 3-step technique was significantly greater in comparison with the 6-step technique (Odds Ratio: 6.27; 95% CI, 3.52-11.17; P=<0.001). Significant difference in compliance with two steps of 3-step technique corresponding to two of the 6-steps between the two groups: 75.8% and 56.7% on 3-step wards and 53.7% and 30.5% on 6-step wards.

ABHR – alcohol-based handrub; CDC – Centers for Disease Control and Prevention; CFU – colony-forming unit; CI – confidence intervals; HCWs – healthcare workers; HH – hand hygiene; Mdn – median; NCBA – non-controlled before and after study; NRT – non-randomized controlled trial; RCT – randomized controlled trial; RF – reduction factor; SD – standard deviation; WHO – World Health Organization

Using the EPOC study design criteria,<sup>25</sup> of the studies included, four were RCTs,<sup>28, 30, 31, 34</sup> one was a crossover RCT,<sup>33</sup> three were non-controlled before and after studies (NCBAs)<sup>6, 32, 35</sup> and one was a crossover NRT.<sup>29</sup> All studies used ABHR to investigate some aspects of the WHO 6-step HH technique. The primary outcome of all studies was the bacterial load on the hands of HCWs measured in colony-forming units (CFUs), apart from Sakmen et al.,<sup>31</sup> in which the primary outcome measure was percentage hand surface coverage. Time and hand coverage were assessed in addition to bacterial load outcome in two<sup>28, 30</sup> and three<sup>30, 32, 35</sup> studies, respectively. The participants were doctors and nurses,<sup>28, 30, 35</sup> medical students,<sup>31-33</sup> or HCWs,<sup>6, 29, 34</sup> while the settings included hospitals,<sup>6, 28, 30, 34, 35</sup> hospital laboratories<sup>29, 33</sup> or university hospital training facilities.<sup>31, 32</sup> The studies that compared the 6-step technique to other approaches<sup>28-31, 33, 34</sup> and the studies that investigated the effectiveness of the 6-step technique were analysed and discussed separately.<sup>6, 32, 35</sup> The studies were further categorised per type of settings, while the studies comparing 6-step technique to other techniques were further grouped according to the comparator.

### ***Studies comparing two hand hygiene techniques***

#### *Clinically based studies comparing the 6-step and the 3-step technique*

Two clinically based RCTs<sup>28, 30</sup> compared the WHO 6-step technique with the CDC 3-step technique amongst 120 doctors and nurses. Reilly et al.<sup>30</sup> and Chow et al.<sup>28</sup> both observed a reduction in bacterial load after application of the WHO 6-step technique. However, findings were inconsistent. Chow et al.<sup>28</sup> found that the WHO 6-step technique was no more effective than the CDC 3-step technique (P=0.07). In contrast, Reilly et al.<sup>30</sup> reported that the WHO 6-step technique was more effective than the CDC 3-step technique (P=0.02).

Chow et al.<sup>28</sup> and Reilly et al.<sup>30</sup> both monitored, as a secondary outcome measure, the median time for conducting HH and both observed the CDC 3-step technique required significantly

less time to complete than the WHO 6-step technique ( $P=0.04^{28}$  and  $P=0.002^{30}$ ). In addition, Reilly et al.<sup>30</sup> also evaluated hand coverage and found that the WHO 6-step technique did not increase the total hand coverage area ( $P=0.15$ ) and that a reduction in bacterial count was not related to hand coverage ( $P=0.97$ ).

#### *Laboratory-based studies adapting the 3-step or 6-step technique*

Two laboratory-based studies, including a crossover RCT<sup>33</sup> and a crossover NRT<sup>29</sup> compared the WHO 6-step technique with an adapted 3-step<sup>33</sup> or adapted 6-step technique<sup>29</sup>. In Tschudin-Sutter et al.<sup>33</sup>, this adapted 3-step technique was different from that used by Chow et al.<sup>28</sup> and Reilly et al.<sup>30</sup> in that it consisted of covering all surfaces of the hands and, in addition, rotationally rubbing fingertips in the palm of the alternate hand and rotationally rubbing both thumbs. Tschudin-Sutter et al.<sup>33</sup> observed that the adapted 3-step technique was significantly more effective at reducing bacterial load than the WHO 6-step technique ( $P=0.021$ ) when tested amongst 32 medical students.

Pires et al.<sup>29</sup> recruited 16 HCWs to compare the WHO 6-step technique with its variation, in which step six (rotational rubbing of fingertips against the opposite palm and vice versa) was performed as first in the sequence of steps. Pires et al.<sup>29</sup> reported that this modified “Fingertips First” 6-step technique resulted in a significantly greater reduction in bacterial load than the standard WHO 6-step technique ( $P=0.002$ ).

#### *Clinically based study adapting the 3-step or 6-step technique*

Tschudin-Sutter et al.<sup>34</sup> also performed a clinically based study comparing the effectiveness of the WHO 6-step technique with an adapted 3-step technique in reducing microbial load on hands amongst 113 HCWs. As in their previous laboratory-based study<sup>33</sup>, the modified 3-step technique involved covering all surfaces of the hands followed by rubbing of fingertips against the opposing palm and rubbing of thumbs. In addition, HCWs’ compliance with the

assigned handrubbing technique was also measured. Although no significant difference in reducing microbial load was found between the two techniques ( $P=0.629$ ), HCWs' compliance with the HH technique was significantly greater on wards assigned to the adapted 3-step technique in comparison with wards assigned to the WHO 6-step technique ( $P<0.001$ ).<sup>34</sup>

*University hospitals training facilities-based study comparing the 6-step technique and “self-responsible application”*

A recent RCT by Sakmen et al.<sup>31</sup> compared hand surface coverage with ABHR between two groups of medical students ( $n=198$ ), of which one was taught the WHO 6-step technique while the other the “self-responsible application” approach, in which no particular instructions with regards to the application technique were provided. Hand coverage was assessed at three time points: (1) directly after training, (2) at the end of the week in which training took place and (3) 5–12 weeks later. Sakmen et al.<sup>31</sup> reported that while there were no significant differences in the total hand coverage between the two groups directly after training ( $P=0.584$ ) and after 5-12 weeks ( $P=0.123$ ); at the end of the week coverage was significantly greater in the “self-responsible application” group ( $P<0.001$ ).

***Studies demonstrating effectiveness of the 6-step hand hygiene technique***

*Clinically based studies demonstrating effect of the 6-step technique*

Two clinically based NCBA studies investigated the effectiveness of the WHO 6-step technique in reducing microbial load on hands.<sup>6, 35</sup>

Widmer et al.<sup>35</sup> studied microbial load reduction on hands of 180 doctors and nurses after the use of ABHR using the 6-step technique prior to, and following HH training, while Laustsen et al.<sup>6</sup> observed 117 clinical procedures and investigated microbial load reduction on HCWs' hands after the use of ABHR prior to and following the completion of the clinical procedure.

Both studies<sup>6, 35</sup> reported that when participants performed the WHO 6-step technique either correctly or incorrectly, bacterial load was reduced. But, the correct application of the technique, as opposed to the incorrect application, resulted in a greater reduction (Widmer et al.<sup>35</sup>:  $P < 0.001$ ; Laustsen et al.<sup>6</sup>: P value not reported). This finding was also supported by Reilly et al.,<sup>30</sup> who found a significant difference between those who performed the WHO 6-step technique with 100% accuracy and compared to those who did not ( $P = 0.01$ ).

In addition, Widmer et al.<sup>35</sup> also reported examining the hand surface coverage using an ultraviolet light box to detect areas missed on the hands after HH, but did not report specific results on this.

#### *University hospitals training facilities-based study demonstrating effect of the 6-step technique*

One NCBA study,<sup>32</sup> based in the university hospitals training facilities investigated microbial load on hands of 563 medical students before and after they applied ABHR using the WHO 6-step technique following HH training. Tschudin-Sutter et al.<sup>32</sup> found that the bacterial load on the hands of medical students was reduced after the use of the WHO 6-step technique ( $P < 0.001$ ). Tschudin-Sutter et al.<sup>32</sup> also report investigating hand surface coverage following HH using an ultraviolet light box, but findings of this investigation were not provided.

#### ***Methodological quality of included studies***

##### *Studies meeting the EPOC study design criteria*

Six of the included studies met the EPOC study design criteria.<sup>25</sup> As shown in Table 2, the overall risk of bias of these studies was assessed as high. High risk of bias was most commonly associated with the lack of, or insufficient protection against contamination resulting from the allocation to study groups occurring at the participant's or ward level within a single hospital<sup>28, 30, 34</sup> or from crossover design.<sup>29, 33</sup> High risk of bias was also

related to the lack of randomisation,<sup>29</sup> risk of selection bias resulting from the crossover approach<sup>29, 33</sup> and potential bias resulting from imbalanced missing outcome data between the groups.<sup>31</sup> In addition, all studies had at least one item assessed as unclear risk. This was related to the lack of sufficient information regarding blinding,<sup>29, 31, 33, 34</sup> baseline outcome measures<sup>31</sup> or baseline participants' characteristics,<sup>28, 30, 31</sup> the process of random sequence generation,<sup>31, 33</sup> allocation sequence concealment<sup>31</sup> or protection against contamination.<sup>31</sup> More details on the reasons for risk of bias assessment decisions of the EPOC design studies can be found in Supplementary file 3.

**Table 2. Risk of bias of studies meeting the EPOC criteria**

	Chow et al. (2012) <sup>28</sup>	Reilly et al. (2016) <sup>30</sup>	Pires et al. (2017) <sup>29</sup>	Tschudin-Sutter et al. (2017) <sup>33</sup>	Sakmen et al. (2019) <sup>31</sup>	Tschudin-Sutter et al. (2019) <sup>34</sup>
Adequate sequence generation?	L	L	H	U	U	L
Allocation concealment?	L	L	H	H	U	L
Baseline outcome measurements similar?	L	L	L	L	U	L
Baseline characteristics similar?	U	U	L	L	U	L
Incomplete outcome data addressed?	L	L	L	L	H	L
Blinding?	L	L	U	U	U	U
Protected against contamination?	H	H	H	H	U	H
Selective outcome reporting?	L	L	L	L	L	L
Free of other bias?	U	U	U	U	U	U
<b>Overall risk of bias</b>	<b>H</b>	<b>H</b>	<b>H</b>	<b>H</b>	<b>H</b>	<b>H</b>

H=high risk; L=low risk; U=unclear risk

Furthermore, a number of other potential biases were identified in relation to the analysis and data collection methods. While four studies<sup>28, 30, 33, 34</sup> were powered, and recruitment targets

were achieved, two studies<sup>29, 31</sup> did not mention this, so it is unclear whether their sample size was adequate.

Four studies used the modified glove juice sampling method,<sup>28, 30, 33</sup> or its variation in which sterile bag was used instead of the glove,<sup>34</sup> while fingertip imprint method was used in one study.<sup>29</sup> Chow et al.<sup>28</sup> stated that glove juice method provides a more accurate measurement of the actual bacterial load that could be transferred via hand contact. However, it could be argued that the glove juice method measures the reduction in resident skin flora as well as transient skin flora. Thus, although the RCTs using this method demonstrated reductions in CFUs, they were not necessarily measuring reductions relevant to the transmission of infection in a clinical setting. Furthermore, in clinically based studies<sup>28, 30, 34</sup> the CFUs detected after patient contact but before HH would be affected by the number of transient organisms acquired during the clinical procedure(s). In addition, the glove juice sampling technique might have also removed some bacteria from participants' hands before ABHR was applied, leading to an overestimation of the bacterial reduction. This number will vary considerably; and if the comparison of reduction outcomes is valid, evidence is required to show that there is a true random distribution of contamination density across the two groups. It is unknown whether this can be guaranteed in a relatively small sample of clinicians delivering different aspects of care.

In two laboratory-based studies<sup>29, 33</sup> participants' hands were artificially contaminated which standardised baseline bacterial load. However, using a single type of microorganism does not reflect the natural conditions and the actual bacterial flora present on HCWs' hands,<sup>36, 37</sup> reducing the external validity of their findings.

Furthermore, apart from Reilly et al.<sup>30</sup> and Sakmen et al.,<sup>31</sup> studies were unable to determine whether specific areas of the hand had been missed by the HH techniques, because they did not evaluate or did not report hand coverage and sites missed. Previous studies<sup>8, 35</sup> showed

that the thumb and fingertips are the most frequently missed areas on the hands. In the study by Reilly et al.,<sup>30</sup> correlation between bacterial reduction and hand surface coverage was also a limitation, because these data were collected at two different time points. Therefore, Reilly et al.<sup>30</sup> could not be certain that the technique was performed by participants in exactly the same way each time, although standardization by showing each participant an instruction card with a diagram of the allocated technique should have helped minimise the risk.

*Methodological quality of other study designs*

Three of the studies included in the review were NCBA design; thus, did not meet the EPOC study design criteria.<sup>25</sup> They are by the nature of their design consider to be high risk of bias. Nevertheless, quality assessment was performed using the relevant Joanna Briggs critical appraisal tool.<sup>26</sup> As shown in Table 3, two of these studies<sup>32, 35</sup> had at least one item assessed as high risk.

**Table 3. Risk of bias of studies not meeting the EPOC criteria**

	Widmer et al. (2007) <sup>35</sup>	Laustsen et al. (2008) <sup>6</sup>	Tschudin-Sutter et al. (2010) <sup>32</sup>
Inclusion criteria in the sample clearly defined?	N	Y	N
Were the study subjects and the setting described in detail?	Y	U	U
Was the exposure measured in a valid and reliable way?	Y	Y	N
Were confounding factors identified?	Y	U	N
Were strategies to deal with confounding factors stated?	Y	U	N
Were the outcomes measured in a valid and reliable way?	Y	Y	Y
Was appropriate statistical analysis used?	Y	Y	Y

Y=yes; N=no; U=unclear



In two studies, high risk of bias was related to a lack of clearly defined eligibility criteria.<sup>32, 35</sup> In addition, for Tschudin-Sutter et al.,<sup>32</sup> high risk of bias also resulted from the lack of details on potential confounding factors and on how participants' compliance with the 6-step technique was monitored. Two studies<sup>6, 32</sup> also had at least one item assessed as unclear risk resulting from a lack of clear description of the study subjects<sup>6, 32</sup> and insufficient detail to assess whether confounding factors were identified and appropriately accounted for in the analysis.<sup>6</sup> Further details on the risk of bias assessment decisions of the non-EPOC design studies can be found in Supplementary file 4.

It should also be noted that none of the non-EPOC design studies<sup>6, 32, 35</sup> had control groups, making it difficult to differentiate between the observed effect being due to the HH technique or to other confounding variables, such as application time or ABHR volume, thereby affecting the validity of the outcomes. Although all three NCBAAs used appropriate statistical analysis, none of these studies mentioned if they were powered, so it is unclear whether their sample sizes were adequate for the analyses they performed.

In addition, despite all three studies microbial load reduction outcomes being measured using objective, valid and reliable methods, involving collecting fingertip imprint samples and using standard microbiological procedures to assess bacterial growth, a limitation of the finger-imprint technique is it only allows bacterial measurement from the fingertips. As the study by Reilly et al.<sup>30</sup> showed, the back of the hands, the back of the thumbs, and the back of the index fingers were the most frequently missed sites regardless of the technique used. However, it could be argued that the finger imprint technique is perhaps a more appropriate method of bacterial measurement in terms of transmission of infections because it does not involve any massage of the hands that could remove resident organisms.

## Discussion

HH is the single most important intervention to reduce the risk of cross transmission of infection.<sup>2</sup> Despite this, to our knowledge, our previous systematic review<sup>18</sup> was the first to evaluate the evidence for the WHO technique in reducing the microbial load on the hands of HCWs and has now been updated. The findings of our updated review showed that of the studies evaluating microbial load as an outcome, all found that the WHO 6-step technique reduced bacterial load on the hands of HCWs; however, the strongest evidence came from five studies<sup>28-30, 33, 34</sup> that met the EPOC design criteria<sup>25</sup> and which compared different HH techniques with the WHO 6-step technique. Yet, the studies are heterogeneous in terms of techniques being compared, and where similar techniques have been compared, the findings are inconsistent.

Chow et al.<sup>28</sup> found no difference in the effectiveness of the WHO 6-step technique compared to the CDC 3-step technique, whereas Reilly et al.<sup>30</sup> found the WHO 6-step technique to be more effective. Tschudin-Sutter et al.<sup>33</sup> reported that an adapted 3-step technique that focused on the fingertips and thumbs was more effective than the WHO 6-step technique when tested in laboratory settings; however, when the authors compared the two techniques in clinical settings, no difference was found.<sup>34</sup> Finally, Pires et al.<sup>29</sup> provided evidence on the superiority of the modified 6-step technique (“Fingertips First” approach) over the standard WHO 6-step technique in reducing bacterial load on hands. However, this was a laboratory-based study limited by the lack of randomization. The remaining evidence comes from studies with poor-quality research designs due to their lack of control or comparator groups. As a result, only limited conclusions can be drawn from these studies.

Of particular note is the study by Chow et al.,<sup>28</sup> which found that covering of all aspects of the hands with no instructions was as effective as the WHO 6-step technique and quicker.

Reilly et al.<sup>30</sup> also reported the CDC 3-step technique required significantly less time to perform than the WHO 6-step technique. This supports similar findings from an earlier study by Kampf et al.,<sup>8</sup> which was not included in our review because it involved non-healthcare participants. It is possible that having a simple and quick technique, may effectively reduce key reported behavioural barriers,<sup>2</sup> and could be important to achieve better compliance in clinical practice.

Furthermore, some of the reviewed evidence<sup>30,34</sup> suggests that simpler HH technique may also increase compliance and potentially improve HH practice within the clinical setting, given that suboptimal rates of HCWs' compliance with the WHO 6-step technique have been previously reported in studies worldwide.<sup>14-16</sup> However, according to current understanding, when using ABHR the hands should be allowed to dry after performing “the technique” and before proceeding to clinical duties.<sup>2</sup> Evidence shows that depending on ABHR products, 2ml or 3mL volume of ABHR requires between 27- 50 and 35-67 seconds, respectively, to dry when rubbed into the hands.<sup>38-42</sup> Thus, there are limits to the amount of time that can be saved with different techniques.

Interestingly, Reilly et al.<sup>30</sup> found that the efficacy of the WHO 6-step technique was enhanced when it was performed with 100% accuracy (correct steps, correct order), whereas Pires et al.<sup>29</sup> showed that the efficacy of the WHO 6-step technique was enhanced when the order of steps was changed—when the finger tips, normally the last step, was performed first. This not only raises questions about what technique is most effective but also suggests that techniques may be modifiable to enhance their effectiveness.

From the whole body of evidence, there is consistency in that all techniques reduce the microbial load on the hands; however, it is difficult to differentiate between the efficacy of different HH techniques. Furthermore, with the exception of the “self-responsible application” technique, for which no specific instructions were provided,<sup>31</sup> all techniques

identified in our review involved covering all surfaces of the hands, which may have confounded the comparisons. In addition, relevant confounding factors, such as product used, time taken to perform HH, volume of HH product and accuracy in performance of the technique,<sup>4</sup> have not always been controlled for and may have influenced the results. Therefore, inconsistencies in evidence could result from the influence of these potential confounders.

Furthermore, different hand sampling methods for the collection of microbiological samples were used across the studies, including glove juice,<sup>28, 30, 33</sup> broth bag<sup>34</sup> and fingertips<sup>6, 29, 32, 35</sup> methods. While the need for standardising hand sampling methods has been stressed previously,<sup>43</sup> the best method remains a subject of debate. When conducting experiments on HH, the European Standard EN 1500 guidelines<sup>44</sup> recommend the finger-imprint method, whereas the U.S. Food and Drug Administration<sup>45</sup> recommends the glove juice method. It was argued that glove juice technique provides a more accurate measurement as it reflects the bacterial count present on the entire surface of the hand,<sup>28, 30</sup> including the dorsal and interdigital areas, whereas fingertip methods allows recovering bacterial load present on the fingertips only.<sup>46</sup> Fingertips are thought to be most likely to come into contact with patients or patient surroundings and therefore, to play an important role in infection transmission,<sup>2</sup> which could justify the choice of the fingertip sampling method. However, while the fingertips method may be appropriate and practical for testing the effectiveness of HH products, given that reviewed hand rubbing techniques aim to cover all hand surfaces with ABHR, glove juice technique appears to be a more suitable choice. Furthermore, provision of patient care might lead to contamination of other parts of the hands, as activities, such as, taking blood pressure or performing physical examination are not limited to using fingertips only, while the risk of transmission resulting from a direct contact with parts of the hand other than fingertips is unknown, and thus, cannot be excluded. On the contrary, glove juice

technique has been criticised for reflecting the reduction of both, the transient and resident flora. Yet, if transmission is thought to occur either from direct contact or from skin cells shedding, glove juice method would in fact provide a more comprehensive and accurate representation of the transmission risk.<sup>47</sup>

Historically, in infection prevention and control studies, the default research design has been observational studies; however, RCT studies of HH techniques are feasible.<sup>28, 30, 34</sup> The studies included in this review provide some relevant and interesting findings that demonstrate that the techniques reduce bacterial load on HCWs' hands; however, overall the level of evidence is low and the generalizability of the findings is limited. Furthermore, all EPOC design studies included in this review were assessed as being high risk of bias. It should be however acknowledged that the EPOC risk of bias criteria<sup>23</sup> consider as high risk if a study was not randomized or if the allocation of the intervention is at the participant level. Another limitation is that in all of the included studies, participants' were observed performing hand hygiene. Their performance may differ in clinical practice due to competing workload pressures. These studies can, however, form the basis of more robust future studies. Therefore, we recommend that RCTs directly comparing the effectiveness of the different techniques be performed in clinical practice.

## **Conclusion**

### ***Implications for Research***

Further robust research, using well-designed multi-arm parallel RCTs that specifically focus on the different HH techniques, are required to determine which technique is the most effective and in what context. All of the following measures are required: determining the bacterial load on the hands of HCWs before and after application of techniques, ideally, using

the glove juice method during clinical practice in acute-care hospitals; controlling the time of application, the products used and the product volume; including inter-rater reliability testing of data collectors; blinding microbiologists to study protocols; and having adequate sample sizes to power the studies. Studies should also report missing data, baseline outcomes, baseline participant characteristics and accuracy of performing the HH technique and, if necessary, appropriately adjust for these factors in the analysis. Randomisation of the population should help control for differences in participant experiences, previous training, and expectations of the HH technique; but the reporting of these data will demonstrate if this has been achieved. Randomization should occur at the healthcare facility level to avoid contamination of the intervention by contact between groups performing the different techniques.

Thus, the development of a protocol for standardising HH research studies, taking account of HH product and volume, and application time, and using the glove juice method for hand sampling in clinically based HH technique studies could improve future research and should be part of HH research agenda.<sup>48</sup>

### ***Implications for Practice***

This review provides evidence on the effectiveness of the WHO 6-step HH technique, supporting the use of this approach in clinical practice. There is also some evidence demonstrating the microbiological efficacy of the WHO 6-step, adapted 6-step, CDC 3-step and adapted 3-step approaches, but it is insufficient as a body of evidence to be definitive. Compliance with correct application of HH technique is usually suboptimal and developing new techniques demonstrating optimal efficacy but being simpler and faster will likely help increase compliance. However, HH is an essential part of infection prevention, and control

measures and current practices should be maintained and reinforced while additional evidence is gathered.

Regarding the performance of HH systematic reviews, our search retrieved a large number of articles that were excluded because they were not empirical studies. We recommend that others performing similar searches include study design as one of the search domains.

Finally, when reporting the findings of HH research, this review identified the need to include a thorough description of the HH techniques, sampling strategy, and population/sample in each study. A checklist for reporting HH studies is warranted to help improve the evidence base, similar to reporting templates such as CONSORT,<sup>49</sup> STROBE<sup>50</sup> or ORION statement.<sup>51</sup>

This review highlighted current evidence regarding the effectiveness of the WHO HH technique in reducing microbial load on the hands of HCWs. The findings provide direction for current practice and future research. HH research must continue to evolve to inform global action to prevent and control healthcare-associated infections and contain antimicrobial resistance.

## **Acknowledgements**

We would like to thank the WHO Technical Expert Group for their constructive comments when this work was presented at the WHO Technical Expert Meeting on Hand Hygiene Technique. We would also like to acknowledge the Scottish Healthcare Associated Infection Prevention Institute (SHAIPi) research consortium who financially supported a post of the staff member (L.G.) who has contributed to this review.

## **Funding**

This review was supported by the funding from the World Health Organization (2021/1102079-0). Glasgow Caledonian University was responsible for conducting and sponsoring this review.



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