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REVIEW ARTICLE

Optimizing Hand Hygiene Protocols in Emergency Settings: A Systematic Review and Meta-Analysis of Agent, Technique, and Procedural Duration

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Abstract: **Introduction:** Hand hygiene is essential for infection prevention, but in busy clinical settings such as emergency departments (EDs), recommended protocols are often performed incompletely. This study aimed to review agent effectiveness, technique complexity, and procedural duration, trying to optimize the hand hygiene protocol in the ED setting. **Methods:** We conducted a systematic review and meta-analysis (PROSPERO CRD420261303149; PRISMA 2020) comparing alcohol-based hand rub (ABHR)-inclusive strategies with washing or antiseptic comparators, the simplified 3-step technique with the World Health Organization (WHO) 6-step technique, and shortened with standard or longer application durations. PubMed, Cochrane CENTRAL, and Scopus were searched for studies published from 2016 to January 2026. Eligible studies enrolled healthcare workers, students, caregivers, or volunteers and reported quantitative microbiological outcomes. Risk of bias was assessed with RoB 2 and Joanna Briggs Institute tools, certainty of evidence with the Grading of Recommendations Assessment, Development and Evaluation (GRADE) approach, and effects were pooled using random-effects models of Hedges' g. **Results:** Twenty-five studies were included. ABHR-inclusive strategies showed a non-significant trend toward lower microbial burden than washing or antiseptic comparators (g = −0.62, 95% confidence interval (CI): −1.32 to 0.09; I^2 = 88.8%); excluding one study comparing alcohol gel with 2% chlorhexidine foam strengthened the effect (g = −0.91, 95% CI: −1.42 to −0.40; I^2 = 67.9%). The simplified 3-step and WHO 6-step techniques showed negligible differences (g = −0.01, 95% CI: −0.46 to 0.44; I^2 = 87.4%). Shortened durations showed a small, non-significant effect favoring longer durations (g = −0.40, 95% CI: −0.87 to 0.08; I^2 = 50.9%). Certainty of evidence was very low to low, and the included studies were conducted in intensive care units, operating rooms, and laboratory simulations rather than emergency settings. **Conclusion:** Selected protocol modifications may achieve comparable microbiological performance and could be attractive where contact time limits compliance, as in resuscitation, triage, and trauma care, but pragmatic non-inferiority trials, including in emergency settings, are needed before guideline modification.

Keywords: Hand Hygiene; Hand Disinfection; Infection Control; Meta-Analysis; Emergency Medicine.

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1. Introduction

Healthcare-associated infections (HAIs) remain a major patient-safety problem, causing avoidable morbidity, mortality, longer hospital stays, antibiotic use, and cost (1). Healthcare workers' hands spread pathogens between patients, staff, and the environment (2, 3). An estimated 7% of hospitalized patients in high-income settings and 15% in low- and middle-income settings acquire at least one HAI, and about one in ten affected patients dies (4). Hand hygiene compliance near 60% has been linked to lower HAI incidence, although causal inference is limited (5).

Reported compliance often overstates real effectiveness, because incorrect technique, insufficient rubbing time, or inadequate product volume reduce microbiological efficacy (6-8). The World Health Organization (WHO) guideline standardizes indications, product choice, technique, and duration, including the "My Five Moments" model and the 6-step hand-rubbing protocol (1), yet workers regularly miss thumbs, fingertips, and interdigital spaces (7-10). Balancing efficacy and feasibility is therefore central. Studies of a simplified 3-step versus the WHO 6-step technique conflict (11-13); the WHO recommends 20-30 seconds for alcohol-based hand rub (ABHR), while some studies find 15 seconds equally effective (4, 14); and comparisons of agents (ABHR versus washing or antiseptics) are hindered by differences in formulation, contamination model, and outcome measures(15-20).

These trade-offs matter particularly in emergency care. In the emergency department (ED), clinicians care for several patients at once, often in crowded or non-traditional areas such as hallways, and much patient contact occurs during time-critical activities (triage, acute resuscitation, and trauma-bay care) in which hand hygiene cannot easily be paused or repeated. ED crowding is a global problem that delays care and lowers its quality (21) , and crowding and care in hallways are barriers to ED hand hygiene compliance (22). Compliance among prehospital emergency providers is also suboptimal, with frequent reliance on gloves (23), and infection-control scores among Iranian emergency medical services personnel were favorable overall but below the optimal level for some items,

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including hand hygiene-related patient care(24). Where contact time and technical complexity decide whether a protocol is completed between successive patient contacts, a technique with fewer steps or a shorter rub-in time may be more likely to be performed, provided microbiological efficacy is preserved. Whether such simplification is microbiologically acceptable has not been examined in a way that informs emergency care.

This systematic review and meta-analysis aimed to examine three modifiable components of hand hygiene protocols: agent type (ABHR-inclusive strategies versus washing or antiseptics), technique complexity (simplified 3-step versus WHO 6-step), and application duration (shortened versus standard or longer). It integrates pooled effects, narrative evidence, heterogeneity, and certainty of evidence to inform practice in high-workload settings, including emergency care.

2. Methods

2.1. *Study design and setting*

The review was registered in PROSPERO (CRD420261303149; registered 15 May 2026) and reported according to the Preferred Reporting Items for Systematic Reviews and Meta-Analyses (PRISMA) 2020 statement (25).

The registered question was retained throughout. Eligibility was structured by PICO (Population, Interventions, Comparators, Outcomes) (Table 1). Studies were included if they evaluated a clearly defined hand hygiene product, technique, or duration against a relevant comparator and reported extractable microbiological outcomes. We excluded studies reporting only self-reported adherence, knowledge, or visual inspection; community or household-only studies; and editorials, protocols, posters, abstracts with insufficient data, and case reports. Studies with spore-forming organisms or viral surrogates were retained for narrative interpretation and pooled only if they met the predefined comparison criteria.

2.2. *Search and study selection*

PubMed, Cochrane CENTRAL, and Scopus were searched for studies published between 2016 and 15 January 2026, with an English-language limit, and reference lists were searched manually. The line-by-line strategies for all three databases, with limits, search date, and record counts, are in Supplementary Appendix S1. Records were managed in Zotero. Two reviewers independently screened titles, abstracts, and full texts, resolving disagreements by discussion or a third reviewer. Studies meeting broader criteria entered the narrative synthesis; only studies with extractable, sufficiently comparable data entered the meta-analyses.

2.3. *Data extraction*

Two reviewers independently extracted study characteristics (design, setting, participants, sample size, agent, technique, duration, contamination model, organism, sampling method, outcome metric, baseline and post-intervention loads in colony-forming units [CFU], reduction factors, dispersion) with a standardized form; all 25 studies are listed in Supplementary Table S1, and pooled studies are summarized in Table 1. Effects were recoded so that negative values favored ABHR-inclusive strategies, positive values favored the 3-step technique, and negative values favored standard or longer duration. When medians with interquartile ranges (IQR) or ranges were reported, means and standard deviations (SD) were approximated using the method of Wan et al. (26); seven effect estimates from five studies required conversion, and CFU counts were treated as approximately log-normal when required (Supplementary Methods).

Suchomel et al. (27) contributed two formulation-specific comparisons (modified ethanol and isopropanol formulations), completed by the same 20 volunteers in a Latin-square crossover design. The two estimates therefore share participants and are not independent, and there was no separate control group to divide. Each comparison was entered with n = 20 per arm, and the dependence was examined in a sensitivity analysis keeping only one comparison. The same volunteers also supplied a third comparison, 80% ethanol rubbed for 15 s versus a different, longer reference rub (60% isopropanol, 2 × 3 mL, 2 × 30 s), which entered the duration analysis. This estimate reuses the 15-second ethanol 6-step arm, so it is not independent of the technique comparisons.

2.4. *Risk of bias*

Randomized and crossover trials were assessed with the Cochrane Risk of Bias 2 (RoB 2) tool (28, 29), and non-randomized and quasi-experimental studies with Joanna Briggs Institute (JBI) checklists (30). Judgments were classified as low, some concerns (RoB 2) or unclear (JBI), or high risk. Overall RoB 2 judgments followed the tool's algorithm, so any domain rated as some concerns produced an overall rating of some concerns. Domain-level judgments are in Supplementary Figures S1 and S2.

2.5. *Statistical analysis*

The effect measure was Hedges' g with random-effects models, specified a priori because heterogeneity was expected. Heterogeneity was summarized with I^2 (>50% considered substantial) (31), τ, τ^2, H^2, and Cochran's Q. Forest plots were produced in Jamovi 2.7.24. Interpretation emphasized magnitude, confidence intervals, heterogeneity, and certainty rather than significance alone. Because fewer than ten studies contributed to each analysis, funnel-plot asymmetry was not tested (29) and publication bias was considered within GRADE.

2.6. *Sensitivity and certainty analyses*

Sensitivity analyses examined clinically distinct comparators, converted distributional statistics, and high-risk-of-bias studies. For the ABHR comparison, the main planned analysis excluded Gil-Peña et al. (29, 32), whose comparator was 2% chlorhexidine foam. For duration, a prespecified ABHR-only analysis excluded Arbogast et al. (19), whose contrast concerned foaming hand-wash lathering rather than ABHR rub-in time. For technique, a post hoc analysis kept only one of the two non-independent Suchomel et al. (27) comparisons. Certainty was rated with the Grading of Recommendations Assessment, Development and Evaluation (GRADE) approach (33): risk of bias, inconsistency, indirectness, imprecision, and publication bias. Because outcomes were microbiological surrogates, indirectness was considered for every comparison, and equivalence or non-inferiority was not claimed without a formal design and margin.

3. Results

3.1. *Study selection and characteristics*

Database and manual searches identified 694 records (Figure 1). After removing 78 duplicates, 616 records were screened,

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582 were excluded, and 34 full texts were assessed; nine were excluded (non-human models, environmental reservoirs, or secondary research). Twenty-five studies were included. Thirteen contributed 16 comparisons to the meta-analyses (Arbogast et al. (19) contributed two and Suchomel et al. (27) three): ABHR-inclusive strategies versus washing or antiseptics (5 studies), simplified 3-step versus WHO 6-step technique (6 estimates from 5 studies), and shortened versus standard or longer duration (5 studies) (Table 2). Designs included randomized, crossover, controlled laboratory, quasi-experimental, and cross-sectional pre-post studies in nurses, physicians, surgeons, caregivers, students, and volunteers, across intensive care units, operating rooms, and laboratory simulations.

The remaining 12 studies (17, 18, 34-43) were not pooled because they did not fit the three comparisons or lacked usable dispersion data: chlorhexidine-containing versus alcohol-only products (Deshpande et al. (17), Hennig et al. (18), Akita et al. (39))viral surrogates (Torko and Gibson (34)); hand-drying method (Mutters and Warnes (35)); different ABHR formulations or pre-post evaluations without a suitable comparator (Setyawati and Wardhani (36), Nawa et al. (37), Matuka et al. (38)); structured versus unstructured handwashing and step-omission variants (Deschênes et al. (40), Gozdzielewska et al. (41)); nail polish type (Arreba et al. (42)); and means without dispersion (Packyanathan and Muralidharan (43)). Endpoints included $\log_{10}$ reduction, CFU counts, percentage reduction, and reduction factors, sampled by glove-juice, fingertip rinsing, swabbing, finger-pad, whole-hand immersion, or handprint methods.

3.2. *Risk of bias*

Seventeen studies were assessed with RoB 2 and eight with JBI checklists. Of the 17 RoB 2 studies, one was at low overall risk (Deshpande et al. (17)), 15 at some concerns, and one at high risk (Akita et al. (39): 16 of 36 randomized nurses were excluded after post-treatment counts exceeded baseline). Lack of participant blinding was the commonest limitation (some concerns in 16 of 17); the randomization domain also raised concerns for Mutters and Warnes (35) and Deschênes et al. (40), whose unstructured technique was always performed first. Of the eight JBI studies, two were at low risk (Gozdzielewska et al. (41), Arreba et al. (42)), three at unclear risk (Vodianyk et al. (44), Packyanathan and Muralidharan (43), López Martín and Erice (16), and three at high risk (Setyawati and Wardhani (36), Nawa et al.(37) , Matuka et al. (38)). Only Vodianyk et al. and López Martín and Erice contributed to meta-analyses (both unclear risk); the other 11 pooled studies were all assessed with RoB 2 and rated some concerns, mainly because participants could not be blinded. Study-level judgments for pooled studies are in Table 2; domain-level judgments are in Supplementary Figures S1 and S2.

3.3. *ABHR-inclusive strategies versus washing or antiseptic comparators*

Five studies(15, 16, 19, 20, 32) compared ABHR-inclusive strategies with washing, foaming hand wash, surgical scrub, soap and water, chlorhexidine products, or heterogeneous presurgical comparators. The pooled estimate favored ABHR but was not significant (Hedges' g = −0.62, 95% confidence interval (CI): −1.32 to 0.09), with very high heterogeneity (I^2 = 88.8%; τ = 0.747; τ^2 = 0.558; H^2 = 8.91; Q = 39.06, degree of freedom (df) = 4, P < 0.001) (Figure 2; Table 3). Arbogast et al. (19), Forer et al. (15), Pourkazemi et al. (20), and López Martín and Erice (16) favored ABHR. In contrast, Gil-Peña et al. (32) found no significant overall difference between 70% alcohol gel and 2% chlorhexidine foam, numerically favoring chlorhexidine.

Excluding Gil-Peña et al. strengthened the pooled effect for ABHR (g = −0.91, 95% CI: −1.42 to −0.40) and reduced heterogeneity (I^2 = 67.9%; Q = 8.63, P = 0.035). The estimate depends strongly on comparator type, formulation, and standardization.

3.4. *Simplified 3-step versus WHO 6-step technique*

Five studies providing six estimates(11-13, 27, 45) showed no significant difference (g = −0.01, 95% CI: −0.46 to 0.44; positive values favor 3-step), with high heterogeneity (I^2 = 87.4%; τ = 0.512; τ^2 = 0.262; H^2 = 7.91; Q = 36.03, df = 5, P < 0.001) (Figure 3; Table 3).

Suchomel et al. (27) contributed two comparisons (ethanol: 3.80 ± 0.71 vs 3.63 ± 0.87 $\log_{10}$ reduction; isopropanol: 4.22 ± 0.92 vs 4.09 ± 0.80; 15 s, 3 mL each) in the same 20 volunteers. Because the pairing was not modeled, standard errors are conservative; keeping only the ethanol comparison gave g = −0.04 (95% CI: −0.58 to 0.51), essentially unchanged. Narratively, Tschudin-Sutter et al., 2019 (45) and Chen et al. (13) reported better compliance, execution accuracy, and preference with 3 steps, and Tschudin-Sutter et al., 2017 (12) favored 3 steps for bacterial reduction, whereas Reilly et al. (11) found greater reduction with 6 steps, at the cost of more time.

3.5. *Shortened versus standard or longer application duration*

Five studies(14, 19, 27, 44, 46) examined shortened versus standard or longer rubbing or lathering. The pooled estimate slightly favored longer times but was not significant (g = −0.40, 95% CI: −0.87 to 0.08), with substantial heterogeneity by the prespecified threshold, although Cochran's Q was not statistically significant (I^2 = 50.9%; τ = 0.385; τ^2 = 0.149; H^2 = 2.04; Q = 6.66, df = 4, P = 0.155) (Figure 4; Table 3). An ABHR-only analysis excluding the lather-duration study of Arbogast et al. (19) was similar (g = −0.44, 95% CI: −1.05 to 0.16; I^2 = 63.1%). Narratively, Pires et al. (14), Harnoss et al. (46) , and Vodianyk et al. (44) supported comparability of 15- and 30-second ABHR application, and Harnoss et al. suggested better compliance with the shorter time.

3.6. *Summary of findings and certainty*

GRADE certainty ranged from very low to low (Table 4), driven by inconsistency (ABHR and technique comparisons), indirectness from surrogate outcomes, and imprecision. Publication bias could not be assessed. The evidence suggests that selected modifications may achieve broadly comparable microbiological performance in specific contexts, but low to very low certainty precludes definitive recommendations.

4. Discussion

This review synthesized comparative evidence on agent, technique complexity, and application duration. No strategy showed conclusive superiority, equivalence, or non-inferiority, which supports context-specific interpretation rather than wholesale guideline revision.

ABHR carried the highest uncertainty. Four of five studies favored ABHR over plain soap, foam wash, or less standardized

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presurgical methods, but the comparison with chlorhexidine foam (32) reversed the direction, and removing it made the pooled effect significant. Comparator type, formulation, and standardization are therefore major sources of variability, and pooling plain washing, foam wash, surgical scrub, and mixed protocols under one label obscures meaningful differences. The findings are consistent with guidance recommending ABHR when hands are not visibly soiled, reserving soap and water for visible soiling and spore-forming organisms (4), but ABHR is not clearly superior to a modern antiseptic such as chlorhexidine foam.

The 3-step versus 6-step comparison was essentially null (g = −0.01, 95% CI: −0.46 to 0.44), with substantial heterogeneity (I^2 = 87.4%) and compatible with a small-to-moderate advantage in either direction, so it supports comparable performance but not formal non-inferiority. A negligible microbiological difference may still have practical value: Tschudin-Sutter et al. (2017, 2019) and Chen et al. (12, 13, 45) suggest that simplification can improve compliance, completion, and preference, and a protocol is of little use if it is not completed correctly. Against this, Reilly et al. (11) found greater reduction with 6 steps, and step-specific studies suggest that fingertips, thumbs, backs of fingers, and large surfaces contribute unequally (41). The question is whether a simplified protocol can ensure thorough coverage while improving consistency. For duration, shorter times may preserve efficacy under selected conditions (g = −0.40, 95% CI: −0.87 to 0.08). Heterogeneity was substantial by the prespecified I^2 threshold (>50%), although borderline, and the confidence interval crossed the null, so the evidence is compatible with little difference or with a potentially meaningful advantage for longer durations. Duration alone is insufficient: efficacy depends on alcohol type, concentration, volume, initial contamination, and organism, with some isopropanol rubs working at shorter times and some ethanol formulations needing longer exposure. Shortened lathering and shortened rubbing are not interchangeable, although the ABHR-only analysis gave a similar estimate.

4.1. *Relevance to emergency care*

Although none of the included studies was conducted in an ED or prehospital setting, the three dimensions examined map directly onto the constraints of emergency care. In acute resuscitation and the trauma bay, clinicians move rapidly between patients and tasks, often with gloves already on, and the window for hand hygiene may be seconds. At triage, one clinician may contact many undifferentiated patients in quick succession, multiplying the hand hygiene indications. In crowded EDs, care in hallways limits access to sinks, making point-of-care ABHR the practical default. Crowding is widespread and degrades the timeliness and quality of care (21), and infection-control practices among emergency medical services personnel were below the optimal level for some items (24). Both contact time and technique complexity plausibly influence whether hand hygiene is completed correctly (22, 23).

The direction favoring ABHR over washing, except against chlorhexidine foam, is consistent with guidance for point-of-care ABHR (4). A simplified 3-step technique or 15-second rub-in may suit settings where contact time limits compliance, but the evidence is of low to very low certainty and comes from laboratory and non-emergency settings. Contamination in trauma care, including blood and body fluids, may differ from the models used, and surrogate outcomes cannot show whether simplified protocols improve ED compliance. Because gloves are used extensively in resuscitation, glove removal and the hand hygiene indications that follow it should be considered alongside technique and duration. Until ED-specific evidence exists, we do not suggest replacing the WHO 6-step technique on this review alone; simplified or shortened protocols could be piloted locally, with audit of technique quality, compliance, and infection indicators.

4.2. *Clinical implications and future research*

The findings support measured, locally evaluated innovation. ABHR may offer advantages over plain soap, foam wash, or traditional presurgical approaches when hands are not visibly soiled and organisms are alcohol-susceptible, but not consistently over chlorhexidine foam. Simplified techniques may help where audits show poor adherence to the 6-step method, provided critical surfaces are covered, and shorter durations may improve practicality if volume, friction, and drying are maintained. Future studies should use adequately powered non-inferiority designs with prespecified margins, combine microbiological endpoints (standardized reporting of baseline and post-intervention loads, organism, sampling, volume, and contact time) with behavioral outcomes (compliance, coverage, time, acceptability, skin tolerance), and, in emergency care, use pragmatic cluster-randomized or stepped-wedge designs in EDs and trauma bays.

4.3. *Limitations*

Strengths include registration in PROSPERO, PRISMA 2020 compliance, multi-database searching with hand-searching, prespecified sensitivity analyses, risk-of-bias evaluation, and GRADE. Limitations include substantial to very high heterogeneity (I^2 = 50.9-88.8%); reliance on surrogate microbiological endpoints; fewer than ten studies per comparison, preventing publication-bias assessment; English-language and date restrictions; limited evaluation of compliance, skin tolerance, and workflow; and no studies in ED or prehospital settings. Several effect sizes were derived from medians and IQRs, adding imprecision. Several pooled studies analyzed paired, clustered, or repeated observations as independent groups (Pourkazemi et al. (20), Tschudin-Sutter et al., 2019 (45), crossover studies, including the two Suchomel et al. (27) estimates), which can distort weights and confidence intervals. The Suchomel et al. duration estimate contrasts different formulations, so duration and formulation effects cannot be separated for that study.

5. Conclusions

Selected modifications to hand hygiene practice may achieve microbiological performance broadly comparable to standard approaches under certain conditions, but certainty was low to very low and no analysis established superiority, equivalence, or non-inferiority. ABHR-inclusive strategies often reduced microbial burden more than some washing and traditional presurgical comparators, with effects depending on comparator and formulation. Simplified 3-step techniques and shorter durations may offer implementation value where adherence or time pressure is a documented problem, but should not replace established standards without stronger evidence. In emergency care, such adaptations should be tested in ED-specific pragmatic trials before adoption.

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6. Declarations

6.1. *Acknowledgments*

Not applicable.

6.2. *Conflict of interests*

The authors declare that they have no conflicts of interest relevant to this manuscript.

6.3. *Funding and supports*

This review did not receive any grant from any funding agency in the public, commercial, or not-for-profit sectors.

6.4. *Authors' contributions*

M.A., F.Z., and Y.R. contributed to the study conception and design. M.A. coordinated the study implementation and screening process. F.Z. and Y.R. performed data extraction, quality appraisal, and evidence synthesis. M.A. drafted the initial manuscript. All authors critically revised the manuscript for important intellectual content and read and approved the final version.

6.5. *Ethical statement*

Ethical approval and informed consent were not applicable because this systematic review used only published data. The review was registered in PROSPERO (CRD420261303149).

6.6. *Informed consent*

Not applicable.

6.7. *Availability of supporting data*

The datasets generated and analyzed during this review consist of study-level data extracted from published literature. The data extraction form and evidence tables are provided as Supplementary Materials, and the authors will provide the data on request.

6.8. *Using artificial intelligence chatbots statement*

An AI assistant was used during revision to support language editing. The authors reviewed and verified all content and take full responsibility for the work.

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Table 1: PICO framework and eligibility criteria

PICO element	Definition used in this review
Population	Healthcare workers, students, surgeons, nurses, physicians, caregivers, or volunteers participating in clinical or controlled laboratory studies that simulated healthcare hand hygiene conditions.
Interventions	ABHR-inclusive strategies; simplified 3-step hand hygiene technique; shortened hand hygiene application duration, including shortened ABHR rub-in protocols and shortened hand-wash lathering protocols.
Comparators	Washing or antiseptic hand hygiene comparators, including soap-and-water washing, foaming hand wash, antiseptic washing or scrubbing, antimicrobial surgical scrub, chlorhexidine-based products, the WHO 6-step technique, or standard/longer application durations such as 30 s or 60 s.
Outcomes	Quantitative microbiological outcomes, including $\log_{10}$ reduction, colony-forming unit counts, percentage reduction, reduction factors, or post-intervention microbial burden.
Study designs	Randomized trials, crossover trials, controlled laboratory studies, quasi-experimental studies, and controlled observational studies with extractable comparative microbiological data.

Note: ABHR: alcohol-based hand rub; PICO: Population, Interventions, Comparators, Outcomes; WHO: World Health Organization.

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Table 2: Characteristics, risk of bias, and summary data of studies contributing to the meta-analyses

Study (ref.)	Country; design	N	Intervention arm (as reported)	Comparator arm (as reported)	Overall risk of bias
ABHR-inclusive strategies versus washing or antiseptic comparators					
Arbogast et al., 2026 (19)	USA; in vivo crossover (ASTM E2784)	12	ABHS (1 dose): mean $\log_{10}$ reduction 4.06 (SD 1.09)	Foaming hand wash, mean $\log_{10}$ reduction: 5 s 2.95 (0.69); 15 s 2.86 (0.57); 20 s 3.00 (0.51)	Some concerns
Pourkazemi et al., 2020 (20)	Iran; randomized, single-blind trial (abstract: cross-sectional)	15 nurses; 50 care episodes per arm	Alcohol rub: CFU/mL after wash, before work 18.33 (SD 33.91)	Soap and water: CFU/mL after wash, before work 54.27 (SD 43.17)	Some concerns
López Martín & Erice, 2017 (16)	Spain; quasi-experimental, within-subject	34	Hydroalcoholic solution: mean post-CFU 0.13 (SD 0.5)	Traditional scrub: mean post-CFU 0.84 (SD 2.5)	Unclear (JBI)
Gil-Peña et al., 2024 (33)	Peru; randomized trial	96 (48/48)	70% alcohol gel: mean post-CFU/mL 1,742 (SD 3,099); baseline 9,108 (SD 9,794)	2% chlorhexidine foam: mean post-CFU/mL 529 (SD 801); baseline 6,050 (SD 7,561)	Some concerns
Forer et al., 2017 (15)	Israel; randomized crossover	20	Alcohol rub: mean $\log_{10}$ CFU 1.59 (SD 1.12)	Routine antimicrobial scrub: mean $\log_{10}$ CFU 3.12 (SD 0.60)	Some concerns
Simplified 3-step versus WHO 6-step technique					
Reilly et al., 2016 (11)	UK; pragmatic parallel-group RCT	120 (60 per arm)	3-step: median post-count 2.88 $\log_{10}$ CFU/mL (IQR 2.11–3.32)	6-step: median post-count 2.58 $\log_{10}$ CFU/mL (IQR 1.78–3.18)	Some concerns
Tschudin-Sutter et al., 2017 (12)	Switzerland; randomized crossover (laboratory)	32	3-step: median log reduction factor 4.45 (IQR 4.04–5.15)	WHO 6-step: median log reduction factor 3.91 (IQR 3.69–4.62)	Some concerns
Chen et al., 2024 (13)	China; randomized crossover (10 departments allocated, 2-week washout)	240 HCWs per technique	3-step: median reduction factor 0.48 $\log_{10}$ (IQR 0.00–0.88)	6-step: median reduction factor 0.43 $\log_{10}$ (IQR 0.00–1.00)	Some concerns
Suchomel et al., 2021 (27), ethanol formulation	Germany; randomized crossover (laboratory), Latin square	20	3-step: mean $\log_{10}$ reduction 3.80 (SD 0.71)	6-step: mean $\log_{10}$ reduction 3.63 (SD 0.87)	Some concerns
Suchomel et al., 2021 (27), isopropanol formulation	As above (same 20 volunteers)	20	3-step: mean $\log_{10}$ reduction 4.22 (SD 0.92)	6-step: mean $\log_{10}$ reduction 4.09 (SD 0.80)	Some concerns
Tschudin-Sutter et al., 2019 (46)	Switzerland; cluster-randomized trial	113 HCWs from 12 wards (56 / 57)	3-step: median reduction factor 0.97 $\log_{10}$ (IQR 0.39–1.59)	6-step: median reduction factor 1.04 $\log_{10}$ (IQR 0.49–1.52)	Some concerns
Shortened versus standard or longer application duration					
Pires et al., 2019 (14)	Switzerland; randomized crossover (laboratory, EN 1500-based), hand-size customized volume	18 (12 sequences each)	15 s rub-in: model-adjusted difference vs 30 s −0.06 $\log_{10}$ (95% CI: −0.34 to 0.22); overall median cRF 2.10 (IQR 1.50–3.10)	30 s rub-in (reference)	Some concerns
Arbogast et al., 2026 (19)	USA; in vivo crossover	12	Foaming hand wash, 15 s lather: mean $\log_{10}$ reduction 2.86 (SD 0.57)	Foaming hand wash, 20 s lather: 3.00 (SD 0.51)	Some concerns
Vodianyk et al., 2025 (45)	Ukraine; experimental laboratory study, 10 volunteers × 10 repetitions (EN 1500-based)	10	15 s, full WHO 6-step (steps 2–7): $\log_{10}$ reduction 2.817; mean CFU 2.039×10^3 (SD 1.435×10^3)	30 s, full WHO 6-step: $\log_{10}$ reduction 2.82; mean CFU 2.024×10^3 (SD 0.879×10^3)	Unclear (JBI)
Harnoss et al., 2020 (47)	Germany; non-blinded randomized crossover (nurses, gynecology ward)	14	15 s: mean $\log_{10}$ reduction factor 0.92 (SD 0.47)	30 s: mean $\log_{10}$ reduction factor 0.89 (SD 0.45)	Some concerns
Suchomel et al., 2021 (27), duration	As above	20	80% ethanol, 3 mL, 15 s: mean $\log_{10}$ reduction 3.63 (SD 0.87)	Reference 60% isopropanol, 2 × 3 mL, 2 × 30 s: 4.27 (SD 0.98)	Some concerns

N: participants (for crossover studies, those completing both arms). Summary statistics are as reported (mean with standard deviation (SD), or median with interquartile range (IQR) or 95% confidence interval (CI)); medians and IQRs were converted following Wan et al. (26). Risk of bias (RoB) 2 for randomized, crossover, and controlled experimental studies; Joanna Briggs Institute (JBI) checklists for non-randomized studies (domain-level judgments in Supplementary Figures S1 and S2). Arbogast et al. (19) contributed two comparisons and Suchomel et al. (27) three (ethanol and isopropanol technique comparisons and one duration comparison), all in the same 20 volunteers. For Pourkazemi et al. (20), the 50 episodes per arm came from 15 nurses. ABHR: alcohol-based hand rub; ABHS: alcohol-based hand sanitizer; ASTM: American Society for Testing and Materials; EN: European Norm; RCT: randomized controlled trial; UK: United Kingdom; USA: United States of America; WHO: World Health Organization; CFU: colony-forming units; HCWs: healthcare workers; cRF: corrected reduction factor.

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Table 3: Heterogeneity statistics for all meta-analyses

Comparison	Studies / estimates	τ	τ^2 (SE)	I^2	H^2	Q	df	p
ABHR-inclusive strategies vs. washing or antiseptic comparators (primary)	5	0.747	0.558 (0.461)	88.8%	8.91	39.06	4	<0.001
Sensitivity: excluding Gil-Peña et al. (33)	4	—	—	67.9%	—	8.63	3	0.035
Simplified 3-step vs. WHO 6-step technique	6 estimates (5 studies)	0.512	0.262 (0.203)	87.4%	7.91	36.03	5	<0.001
Shortened vs. standard or longer application duration (primary)	5	0.385	0.149 (0.216)	50.9%	2.04	6.66	4	0.155
Sensitivity: ABHR-only, excluding Arbogast et al. (19)	4	—	—	63.1%	—	—	—	—

Note. τ: between-study standard deviation; τ^2: between-study variance (standard error); I^2: percentage of variability due to heterogeneity; H^2: relative excess of Q over its degrees of freedom; Q: Cochran's heterogeneity statistic; df: degrees of freedom; —: not reported. Random-effects models of Hedges' g.

Table 4: Summary of findings and GRADE certainty

Comparison	Effect estimate	Heterogeneity	GRADE certainty	Interpretation
ABHR inclusive strategies versus washing or antiseptic hand hygiene comparators (Negative values favor ABHR)	Primary analysis: g = −0.62, 95% CI: −1.32 to 0.09; sensitivity analysis excluding chlorhexidine comparator: g = −0.91, 95% CI: −1.42 to −0.40	I^2 = 88.8% primary; I^2 = 67.9% excluding chlorhexidine	Very low[1]	Pooled effect highly uncertain; comparator differences contributed to heterogeneity. ABHR often performs similarly or better, but universal superiority is not supported.
Simplified 3-step vs. WHO 6-step technique (Positive values favor 3-step)	g = -0.01, 95% CI: -0.46 to 0.44	I^2 = 87.4%	Very low[2]	Negligible point estimate; the confidence interval spans both a small-to-moderate advantage of the 3-step and a small-to-moderate advantage of the 6-step technique. The evidence supports comparable microbiological efficacy but does not establish superiority, formal non-inferiority, or a consistent direction of effect.
Shortened vs. standard or longer hand hygiene application duration (Negative values favor standard or longer duration)	Primary analysis: g = −0.40, 95% CI: −0.87 to 0.08. ABHR-only sensitivity analysis excluding the foaming hand-wash lather-duration study: g = −0.44, 95% CI: −1.05 to 0.16.	I^2 = 50.9% primary; I^2 = 63.1% ABHR-only sensitivity analysis	Low[3]	No clear loss of efficacy; low-certainty evidence precludes definitive guidance.

[1] Downgraded for very serious inconsistency, indirectness due to surrogate microbiological outcomes and clinically diverse comparators, and imprecision because the confidence interval crossed the null. [2] Downgraded for serious inconsistency, indirectness due to surrogate microbiological outcomes, and imprecision because the confidence interval crossed the null and included potentially meaningful effects in both directions. [3] Downgraded for inconsistency and indirectness due to surrogate microbiological outcomes; imprecision was considered because the confidence interval crossed the null. ABHR: alcohol-based hand rub; CI: confidence interval; GRADE: Grading of Recommendations Assessment, Development and Evaluation; WHO: World Health Organization.

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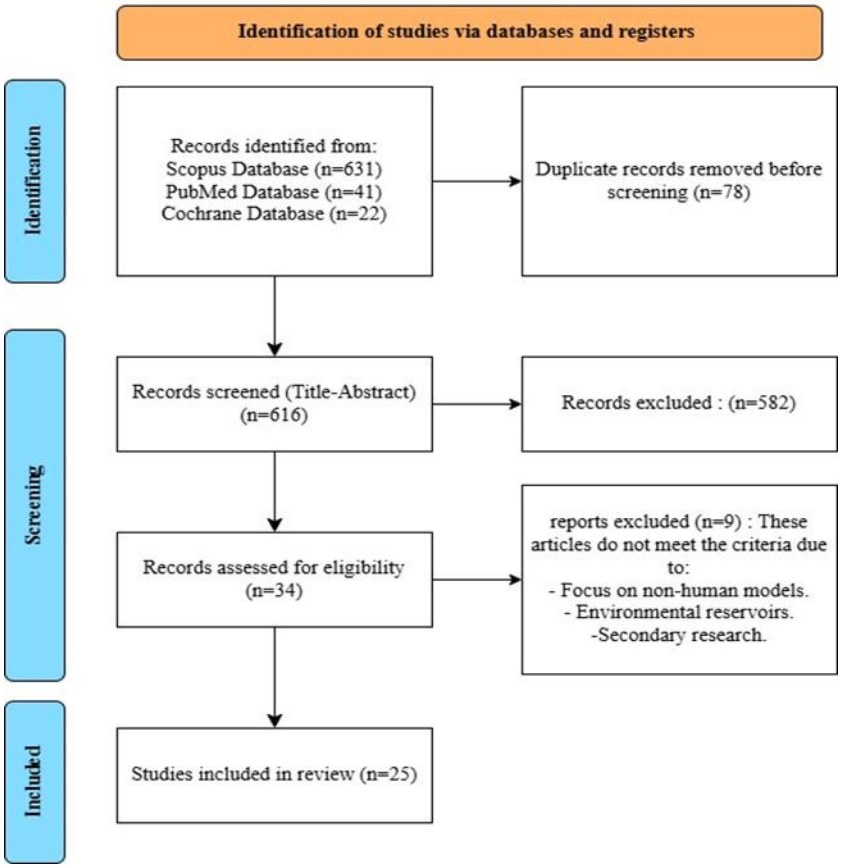


Figure 1: Preferred Reporting Items for Systematic Reviews and Meta-Analyses (PRISMA) 2020 flow diagram of study identification, screening, eligibility assessment, and inclusion.

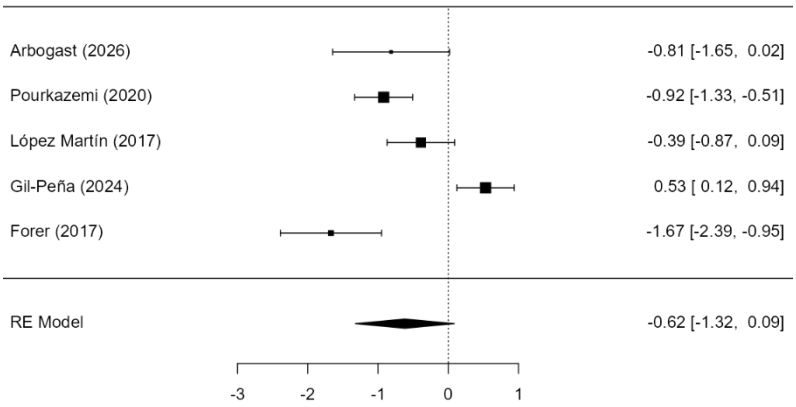


Figure 2: Random-effects (RE) forest plot (Hedges' g) comparing alcohol-based hand rub-inclusive strategies with washing or antiseptic hand hygiene comparators. Negative values favor ABHR. CI: confidence interval; RE: random effects.

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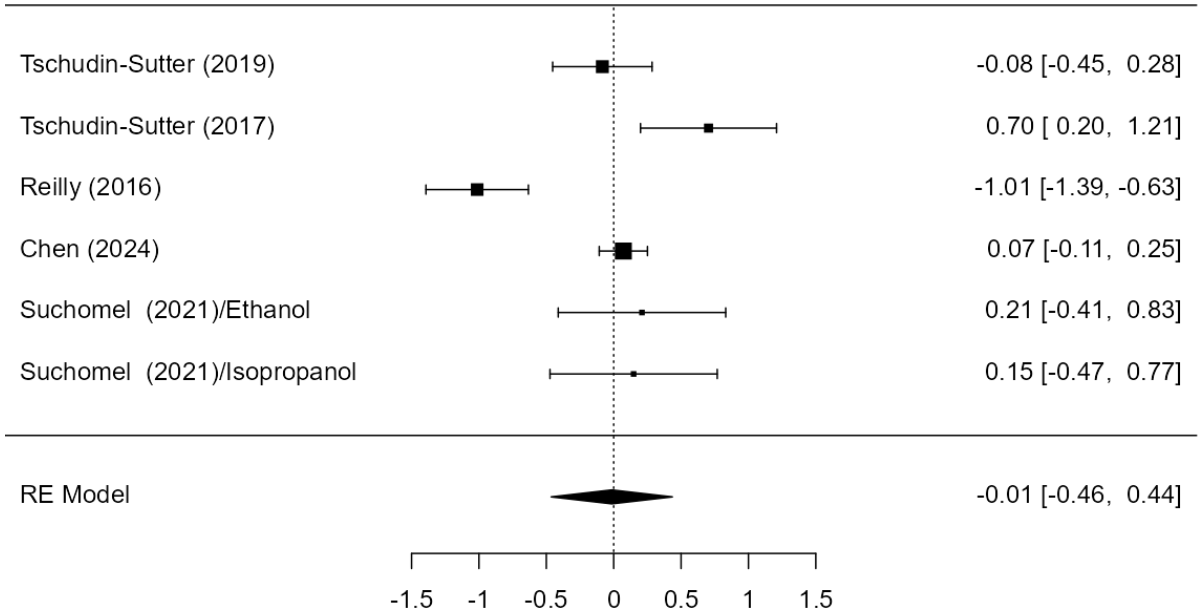


Figure 3: Random-effects (RE) forest plot (Hedges' g) comparing the simplified 3-step with the WHO 6-step technique. Positive values favor the 3-step technique. Suchomel et al. contributed two comparisons (ethanol, isopropanol) from the same 20 volunteers, so the estimates are not independent.

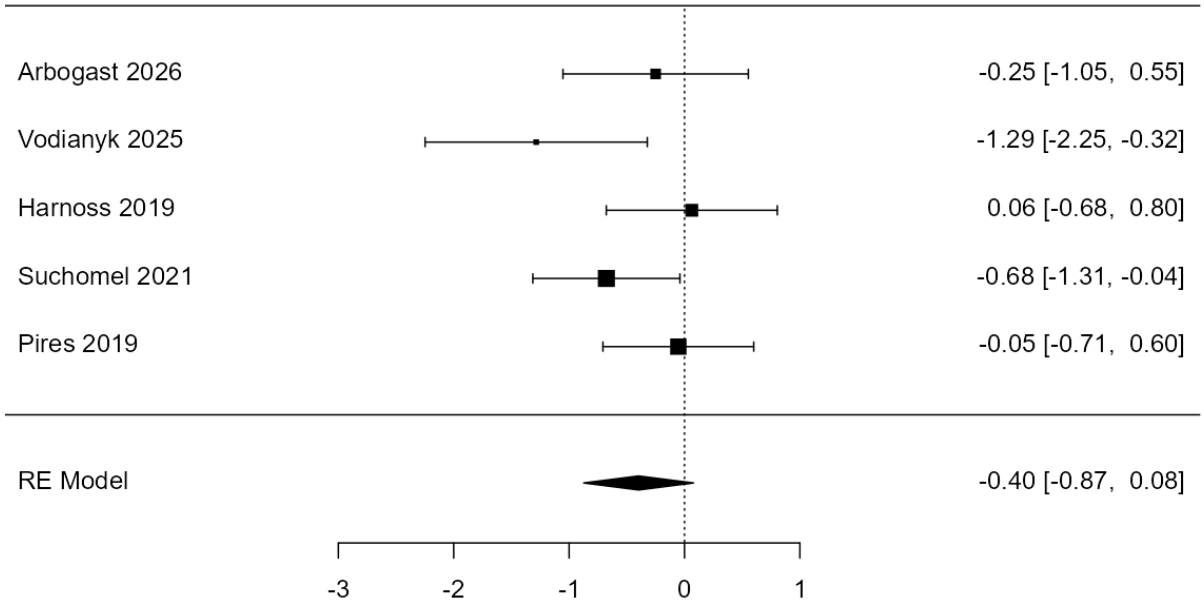


Figure 4: Random-effects (RE) forest plot (Hedges' g) comparing shortened versus standard or longer application duration. Negative values favor standard or longer duration. RE: random effects.

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Appendix

Supplementary Appendix S1: Electronic search strategies

Searches covered from 2016 to 15 January 2026 and yielded 694 records (PubMed 41, Scopus 631, Cochrane CENTRAL 22; see Figure 1). The strings below are the search strategies for each database, adapted to its syntax.
A. PubMed (41 records; search run through 15 January 2026)
(hand hygiene OR handwashing OR hand rub OR alcohol-based hand rub OR ABHR) AND (technique OR 3-step OR 6-step OR WHO technique OR duration OR rub-in time OR compliance OR thoroughness) AND (microbial reduction OR bacterial load OR colony-forming units OR CFU OR log reduction). Filters applied: Clinical Trial, Randomized Controlled Trial, Systematic Review, English, from 2016/6/30 - 2026/1/15.
B. Scopus (631 records; search run through 15 January 2026)
ALL ("hand hygiene" OR "handwashing" OR "hand rub") AND ALL ("technique" OR "quality" OR "compliance" OR "thoroughness") AND ALL ("Pathogen reduction" OR "colony forming units" OR "bacterial load" OR "log reduction") AND PUBYEAR > 2015 AND PUBYEAR < 2027 AND PUBYEAR > 2015 AND PUBYEAR < 2027 AND (EXCLUDE (DOCTYPE , "cp") OR EXCLUDE (DOCTYPE , "le") OR EXCLUDE (DOCTYPE , "no") OR EXCLUDE (DOCTYPE , "ed") OR EXCLUDE (DOCTYPE , "sh") OR EXCLUDE (DOCTYPE , "ch") OR EXCLUDE (DOCTYPE , "bk")) AND (LIMIT-TO (LANGUAGE , "English")) AND (EXCLUDE (SUBJAREA , "MATH") OR EXCLUDE (SUBJAREA , "ENER") OR EXCLUDE (SUBJAREA , "EART") OR EXCLUDE (SUBJAREA , "COMP") OR EXCLUDE (SUBJAREA , "BUSI") OR EXCLUDE (SUBJAREA , "NEUR")) AND PUBYEAR > 2015 AND PUBYEAR < 2027
C. Cochrane CENTRAL (22 records; search run through 15 January 2026)
All text: (hand hygiene OR handwashing OR hand rub OR alcohol-based hand rub OR ABHR) AND (technique OR 3-step OR 6-step OR WHO technique OR duration OR rub-in time OR compliance OR thoroughness) AND (microbial reduction OR bacterial load OR colony-forming units OR CFU OR log reduction).

Supplementary Methods: data extraction and effect-size conventions

Reference numbers refer to the reference list of the main manuscript.
S1. Direction of effects
Effects were recoded so that negative values favored ABHR-inclusive strategies, positive values favored the simplified 3-step technique, and negative values favored standard or longer application duration. For post-intervention bacterial burden, lower values indicate greater efficacy; for log reduction, percentage reduction, or reduction factor, higher values indicate greater efficacy.
S2. Medians, interquartile ranges, and log-normality
When medians with interquartile ranges or ranges were reported, means and standard deviations were approximated using the method of Wan et al. (26). Seven effect estimates from five studies required conversion, and CFU counts were treated as approximately log-normal when required for effect-size estimation. For Pires et al. (14), the effect size was the model-adjusted difference between 15 s and 30 s (−0.06 $\log_{10}$; 95% CI: −0.34 to 0.22) divided by a standard deviation estimated from the overall interquartile range of the corrected reduction factor (1.50–3.10).
S3. Non-independent observations
Suchomel et al. (27) used a Latin-square crossover in which the same 20 volunteers completed all procedures (five groups of four volunteers representing treatment sequences). The ethanol and isopropanol 3-step versus 6-step comparisons therefore share participants and are not independent, and there was no separate control group to divide between them. Each comparison was entered with n = 20 per arm; because the pairing was not modeled, the standard errors are conservative. Keeping only the ethanol comparison gave g = −0.04 (95% CI: −0.58 to 0.51), essentially unchanged from the primary estimate (g = −0.01, 95% CI: −0.46 to 0.44). In the duration comparison, the 15-s arm of Suchomel et al. (80% ethanol) was compared with the reference procedure (60% isopropanol, 2 × 30 s), so formulation and duration are confounded. Pourkazemi et al. (20) analyzed 50 care episodes per arm obtained from 15 nurses as independent observations. Gil-Peña et al. (32) had different baseline counts in the two arms (alcohol gel 9,108 vs chlorhexidine foam 6,050 CFU/mL). Crossover studies (Tschudin-Sutter et al. (12), Chen et al. (13), Pires et al. (14), Harnoss et al. (46)) were analyzed as independent groups, which makes confidence intervals conservative.

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Supplementary Table S1: Extracted data from inclusion articles

Study ID	Study Design	Sample size	Hygiene Agent	Technique Quality (Steps/Duration)	Sampling Method	Pre-Intervention Contamination Status	Post-Intervention (Efficacy)
(Arbogast et al., 2026) USA	Experimental (In vivo crossover)	12	Alcohol-based hand sanitizer (ABHS) gel.	Standardized: 5, 15, 20s lather (wash); 1-2 doses (ABHS)	ASTM E2784 (hand & transfer to food)	7.66 (SD 0.27)	Mean Log_{10} Reduction Hand Wash (5s): 2.95 (SD 0.69) Hand Wash (15s): 2.86 (SD 0.57) Hand Wash (20s): 3.00 (SD 0.51 ABHS (1 dose) :4.06 (1.09)
(Torko & Gibson, 2025) USA	Experimental (In vivo lab-based)	30	Alcohol-based hand rub (ABHR)	Rubbing 10s vs. until dry; 3mL vs. 6mL volume	Modified dish method (ASTM E1838-17 adapted)	MS2 & phi6 (8 log PFU/mL)	Overall mean log reduction (± SD) across all factors: Φ6: 2.83 ± 1.98 MS2: 0.50 ± 0.53 *Difference significant ($P \leq 0.05$)*
(Vodianyk et al., 2025) Ukraine	Experimental laboratory study	10	Alcohol-based hand rub (ABHR)	WHO steps ;15s vs. 30s duration	EN 1500:2013 (modified) for hand contamination and evaluation	Pathogen: *Escherichia coli* K-12 Inoculum. mean CFU: 1.337×10^6 (SD ± 0.637 × 10^6) Log_{10} control: 6.126	6-step technique at 30s: 2.757 log reduction 6-step technique at 15s: 2.281 log reduction
(Gozdzielewska et al., 2026) UK	Stage 1: Laboratory-based design (modified EN 1500). Stage 2: Cross-sectional online survey.	35	ABHR (50–75% propan-2-ol, 1–3% propan-1-ol), 3 mL	WHO six-step technique vs six variations omitting one step each (each step displayed for 4 s); quasi-complete 7 × 7 Latin square	Glove-juice method	$1.6 - 2.7 \times 10^8$ CFU/mL (prepared per EN 1500). Quality control: No significant differences in inoculum concentration between technique variations (p = 0.808) or study groups (p = 0.315).	Mean log_{10} reduction (SD) by variation: Omit step 1: 2.80 (0.96); step 2: 3.22 (0.99); step 3: 3.41 (0.73) Omit step 4: 2.74 (0.99); step 5: 2.85 (0.89); step 6: 3.09 (0.88) Full six-step: 3.36 (0.77) Overall mean 3.07 (SD 0.94)
(Akita et al., 2022) Japan	Randomized, single-blind (evaluators), crossover trial; two hospitals (Japan)	36 enrolled; 20 analysed (16 excluded because CFU immediately after hand hygiene exceeded baseline: 3 used both methods, 10 Waterless only, 3 Two-stage only)	4% CHG, Natural soap, 1% CHG lotion	Two-stage (soap + sanitizer) vs. Waterless (CHG)	Modified EN 12791 method (finger pad/immersion).	samples taken before hand hygiene at the start of surgery.	Reduction factor, mean (SD): Group A: Waterless 0.93 (0.84), Two-stage 1.34 (1.29) Group B: Two-stage 1.43 (0.92), Waterless 0.95 (1.04) Least-squares mean difference (WL – TS) −0.44 (95% CI: −1.25 to 0.39), p = 0.2794

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Study ID	Study Design	Sample size	Hygiene Agent	Technique Quality (Steps/Duration)	Sampling Method	Pre-Intervention Contamination Status	Post-Intervention (Efficacy)
(Harnoss et al., 2020) Germany	Non-blinded, randomized, crossover.	14	Alcohol-based hand rub (ABHR)	Standardized hand rubbing technique. Duration: 15 seconds and 30	EN 1500 fingertip rinsing method	15s 1.78 ± 0.76 log_{10} 30s 1.86 ± 0.71 log_{10}	15 seconds: Mean log: 0.87 ± 0.68 log_{10} Mean log RF: 0.92 ± 0.47 30 seconds Mean post-value: 0.98 ± 0.69 log_{10} Mean log RF: 0.89 ± 0.45
(Tschudin-Sutter et al., 2019) Switzerland	Cluster-randomized controlled trial in a clinical	57	alcohol-based hand rub	WHO 6-step vs. simplified 3-step	Modified EN 1500 method.	Median baseline log_{10} CFU: 3-step group: 2.34 log_{10} (IQR 1.92-2.95) 6-step group: 2.75 log_{10} (IQR 2.13-3.27)	Reduction factor, median (IQR): 3-step 0.97 (0.39–1.59); 6-step 1.04 (0.49–1.52); P = 0.629 Post-value: 3-step 1.60 (0.54–2.44) (n = 56); 6-step 1.67 (0.85–2.53) (n = 57)
(Mutters & Warnes, 2019)Germany	Controlled laboratory study	80	Pure potash soap, pH 10.5 (Urkon Fluessigseife, Algin, Neustadt, Germany).	Washing technique: Standardized as per European Standard Method EN 1499. then Jet air dryer for 1 minute.	Whole-hand immersion method (recommended by German Society for Hygiene and Microbiology - DGHM;	Hands were contaminated by immersion in a bag with the suspension for 5 seconds, then air-dried for 3 minutes. Baseline was not directly measured as pre-wash;	Air samples from washroom showed <100 CFU/m^3 regardless of drying method used.
(López Martín & Erice, 2017)Spain	Quasi-experimental, within-subject design in a clinical (surgical theatre) setting.	34	Hydroalcoholic solution vs. Traditional scrub	WHO surgical protocol	Fingerprint	Traditional wash day: 32.85 ± 25 (range 0-100) Hydroalcoholic solution day: 30.31 ± 24 (range 0-100)	Mean CFU count after hand hygiene (T2): Traditional wash: 0.84 ± 2.5 (range 0-12) Hydroalcoholic solution: 0.13 ± 0.5 (range 0-3)
(Deschênes et al., 2017)Canada/Switzerland	Prospective crossover study; laboratory-based (experimental). Unstructured technique always performed first; order of WHO and WHO-SR randomized	10	Soap and water	WHO-recommended vs. Modified vs. Unstructured	Modified whole-hand test method (ASTM E1174-13). Inoculum: 3×10^6 CFU (in 3 mL of seeding solution). Spore proportion: 90% spores		Unstructured technique: Median RF: 1.30 log_{10} (IQR: 1.27-1.43) WHO technique: Median RF: 1.71 log_{10} (IQR: 1.34-1.91) WHO-SR technique: Median RF: 1.70 log_{10} (IQR: 1.54-2.42)

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Study ID	Study Design	Sample size	Hygiene Agent	Technique Quality (Steps/Duration)	Sampling Method	Pre-Intervention Contamination Status	Post-Intervention (Efficacy)
(Tschudin-Sutter et al., 2017) Switzerland	Randomized, controlled, crossover, laboratory-based trial.	32	Alcohol-based hand rub (ABHR)	3-step vs. WHO 6-step	Modified European Norm (EN 1500) method	Intervention Group: Median baseline load: 6.34 log_{10} CFU (IQR 6.17 - 6.60).	WHO Reference Group: Median 2.34 log_{10} CFU (IQR 1.80 - 2.71). Intervention Group: Median 1.96 log_{10} CFU (IQR 1.25 - 2.52). Log Reduction Factor (RF): WHO Reference Group: Median 3.91 log_{10} CFU (IQR 3.69 - 4.62). Intervention Group: Median 4.45 log_{10} CFU (IQR 4.04 - 5.15).
(Reilly et al., 2016)United Kingdom	Parallel-group, pragmatic randomized controlled trial (RCT) in an acute hospital setting.	120 (60 per arm)	Alcohol-based handrub (ABHR)	WHO 6-step vs. 3-steps	Glove juice technique	6-Step Group: Median baseline: 3.28 log_{10} CFU/mL (95% CI: 3.11-3.38) 3-Step Group: Median baseline: 3.08 log_{10} CFU/mL (95% CI: 2.97-3.27)	6-Step Group: Median post: 2.58 log_{10} CFU/mL (95% CI: 2.08-2.93) 3-Step Group: Median post: 2.88 log_{10} CFU/mL (95% CI: 2.58-3.15)
(Arreba et al., 2025)Spain	Prospective, controlled, within-subject study in a clinical setting.	46	Alcohol-based hand rub (ABHR)	WHO-recommended hand hygiene technique with alcohol-based hand rub.	Fingernail imprint method	Day 21: SP 10.76 (9.30-12.45); UP 9.29 (8.29-10.41); GP 8.07 (6.87-9.48)	ABHR significantly reduced bacterial counts across all groups and all days, with a relative decrease between 81.3% and 89.5% Day 21: SP 1.67 (1.30-2.16); UP 1.15 (0.89-1.49); GP 1.18 (0.89-1.57)
(Chen et al., 2024)China	Randomized controlled cross-over trial; 10 departments randomly allocated (department-level), 2-week washout; Wuhan, China	240 HCWs per technique (each HCW observed with both)	Alcohol-based hand rub (ABHR)	3-step vs. 6-step	Swab method	log_{10} CFU counts before handrub, median (IQR): 3-HT 0.85 (0.24–1.30); 6-HT 0.64 (0.00–1.08)	After hand rub, median (IQR): 3-HT 0.00 (0.00–0.77); 6-HT 0.00 (0.00–0.00) Reduction factor, median (IQR): 3-HT 0.48 (0.00–0.88); 6-HT 0.43 (0.00–1.00); P > .05

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(Gil-Peña et al., 2024) Peru	Randomized controlled trial	96 (48 per arm)	Alcohol gel (70% ethanol) vs. 2% Chlorhexidine foam	WHO-recommended clinical handwashing/hand rub technique (6-step)	Whole-hand immersion method	Mean Baseline CFU/mL (SD): AG Group (Total, n=48): 9,108 (SD 9,794) CF Group (Total, n=48): 6,050 (SD 7,561)	Mean Post-Intervention CFU/mL (SD): AG Group (Total, n=48): 1,742 (SD 3,099) CF Group (Total, n=48): 529 (SD 801)
(Setyawati & Wardhani, 2022)Indonesia	Experimental (Pre/Post design)	60	Three types of Alcohol-based hand rubs	WHO 6-step implied	Swab method	Median Baseline CFU (min-max) (Mean of both observers): Chlorhexidine gluconate group: 445 (200 – 1400) N-propanol group: 470 (180 – 2660) Hydrogen peroxide group: 545 (80 – 2720)	Median Post-Intervention CFU (min-max) Chlorhexidine gluconate group: 20 (0 – 290) N-propanol group: 60 (0 – 280) Hydrogen peroxide group: 25 (0 – 290)
(Nawa et al., 2021)Zambia,	Descriptive cross-sectional study	31	Alcohol-based hand rub (ABHR)	WHO- (6-step implied)	Direct fingerprint method.	Total baseline CFU and Median (IQR) by group: Doctors (n=5): Total CFU = 425; Median = 29.00 (IQR: 25.00-125.00) Caregivers (n=12): Total CFU = 2,224; Median = 40.50 (IQR: 23.75-216.75) Nurses (n=14): Total CFU = 266; Median = 15.50 (IQR: 6.00-22.25)	Total post-intervention CFU and median (IQR) by group: Doctors (n=5): Total CFU = 66; Median = 2.00 (IQR: 1.00-29.00) Caregivers (n=12): Total CFU = 388; Median = 12.00 (IQR: 5.75-18.25) Nurses (n=14): Total CFU = 20; Median = 1.00 (IQR: 0.00-2.50)
(Pourkazemi et al., 2020)Iran	Randomized, single-blind, controlled clinical trial	15	Alcohol-based rub vs. Soap/Water	WHO guidelines (6-step implied).	Swab method.	Alcohol group: 53.11 ± 41.89 CFU/mL Soap group: 42.88 ± 39.84 CFU/mL	Alcohol Group: Mean reduction of 20.58 ± 54.4 . Soap Group: Mean reduction of 2.66 ± 48.17
(Suchomel et al., 2021)Germany,	Randomized, controlled, crossover, laboratory-based study	20	Alcohol-based hand rub (ABHR)	WHO 6 steps and 3 steps (15 s); reference alcohol 2 × 3 mL, 2 × 30 s (6 steps)	EN 1500 fingertip rubbing method.	Bacterial suspension of approximately 10^8 CFU/mL. Hands were artificially contaminated by 5-second immersion.	Mean $\log_{10}$ reduction (SD): 80% ethanol 15 s: 3 steps 3.80 (0.71), 6 steps 3.63 (0.87); 75% isopropanol 15 s: 3 steps 4.22 (0.92), 6 steps 4.09 (0.80); reference (60% isopropanol, 60 s) 4.27 (0.98)

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Study ID	Study Design	Sample size	Hygiene Agent	Technique Quality (Steps/Duration)	Sampling Method	Pre-Intervention Contamination Status	Post-Intervention (Efficacy)
(Packyanathan & Muralidharan, 2019) India	Comparative observational study	50	Alcohol rub, 4% CHG wash, medicated/non-medicated soaps	standard handwashing protocol" for soaps and hand washes, and the "standard hand rub protocol" for the alcohol-based hand rub.	Swab method.	Mean baseline CFU (before disinfection) for each group: Medicated handwash (4% chlorhexidine): 102 CFU Ethanol-based hand sanitizer: 85.6 CFU Medicated soap: 100.6 CFU Non-medicated handwash: 99.8 CFU Non-medicated soap: 101.2 CFU	Mean post-intervention CFU for each group: Medicated handwash (4% chlorhexidine): 10.4 CFU Ethanol-based hand sanitizer: 14.5 CFU Medicated soap: 16.2 CFU Non-medicated handwash: 45.6 CFU Non-medicated soap: 52.8 CFU
(Pires et al., 2019)Switzerland	Randomized, cross-over, experimental laboratory study	18	alcohol-based hand rub (ABHR).	WHO six-step technique in line with their daily routine.	Modified EN 1500 "fingertips method."	Median baseline bacterial counts (log_{10} CFU) - 6.85 (IQR 6.50-7.10)	Overall median corrected reduction factor (cRF): 2.10 log_{10} CFU (IQR 1.50-3.10)
(Matuka et al., 2018)South Africa	Cross-sectional study (pre-post intervention assessment	70	Three types of cleansing formulas used	- Scrub with a brush - Scrub without a brush - Ordinary handwashing	Modified glove juice & fingernail press	Mean S. aureus prewash count (n=29 positive): Arithmetic mean: 1.9 × 10^3 CFU/mL Mean log_{10} CFU/mL: 3.28	Mean S. aureus post-wash count (n=20 positive): Arithmetic mean: 0.5 × 10^3 CFU/mL Mean log_{10} CFU/mL: 2.70
(Deshpande et al., 2018)USA,	Prospective, randomized, double-blind	51	61% Ethanol + 1% CHG vs. 70% Ethanol only	Health care workers(HCWs) applied the assigned product evenly to cover both hands until completely dry.	Handprint method	Baseline sampling was not performed in the traditional sense (pre-contamination).	Mean log_{10} CFU ± SEM (n = 51; randomized, double-blind crossover): Immediately after application: ethanol + CHG 0.27 ± 0.05; ethanol only 0.88 ± 0.08 (p = 0.035) After 4–7 min in ICU common areas: 1.81 ± 0.07 vs 2.17 ± 0.05 (p < 0.0001)

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(Hennig et al., 2017)Switzerland/Germany	Randomized, controlled, cross-over	25	Alcohol-only (A) vs. CHG-Alcohol (B)	90s (Alcohol-only) vs. 180s (CHG-Alcohol)	EN 12791 fingertip rubbing method.	Mean baseline log_{10} counts (pre-values) ± SD: Product A (alcohol-only): 4.68 ± 0.51 log_{10} Product B (CHG-alcohol): 4.79 ± 0.52 log_{10} 6-hour effects testing: Product A (alcohol-only): 4.79 ± 0.51 log_{10} Product B (CHG-alcohol): 4.73 ± 0.50 log_{10}	Log_{10} reduction (mean ± SD), immediately after application: Product A (alcohol-only, 90 s) 1.96 ± 1.06; Product B (CHG-alcohol, 180 s) 1.42 ± 0.79 ($p \leq 0.025$) After 6 h under gloves: A 1.67 ± 0.71; B 1.24 ± 0.90 ($p \leq 0.01$)
(Forer et al., 2017)Israel,	Randomized, crossover, controlled	20	Alcohol rub method	Routine antimicrobial scrub vs. Alcoholic hand rub	Modified glove juice method.	This study did not measure baseline (pre-clean) counts;	Routine scrub Mean log_{10} CFU: 3.12 ± 0.60 Alcohol rub Mean log_{10} CFU: 1.59 ± 1.12

ABHR: alcohol-based hand rub; ABHS: alcohol-based hand sanitizer; AG: alcohol gel; ASTM: American Society for Testing and Materials; CF: chlorhexidine foam; CFU: colony-forming units; CHG: chlorhexidine gluconate; CI: confidence interval; DGHM: Deutsche Gesellschaft für Hygiene und Mikrobiologie (German Society for Hygiene and Microbiology); EN: European Norm; GP: gel polish; HCW(s): healthcare worker(s); HT: hand-rub technique (3-HT, 3-step; 6-HT, 6-step); ICU: intensive care unit; ID: identifier; IQR: interquartile range; MS2 and Φ6 (phi6): bacteriophage virus surrogates; PFU: plaque-forming units; RCT: randomized controlled trial; RF: reduction factor; SD: standard deviation; SEM: standard error of the mean; SP: standard nail polish; T2: time point after hand hygiene; TS: two-stage; UK: United Kingdom; UP: unpolished nails; USA: United States of America; WHO: World Health Organization; WHO-SR: modified WHO technique, as labeled in the source study; WL: waterless.

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Figure S1: Risk of Bias Summary Matrix (RoB 2)

Author (Year)	D1	D2	D3	D4	D5	Overall Risk
(Arbogast et al., 2026)	🟢	🟡	🟢	🟢	🟢	🟡 **Some Concerns**
(Torko & Gibson, 2025)	🟢	🟡	🟢	🟢	🟢	🟡 **Some Concerns**
(Akita et al., 2022)	🟢	🟡	🔴	🟢	🟢	🔴 High
(Harnoss et al., 2020)	🟢	🟡	🟢	🟢	🟢	🟡 **Some Concerns**
(Tschudin-Sutter et al., 2019)	🟢	🟡	🟢	🟢	🟢	🟡 **Some Concerns**
(Mutters & Warnes, 2019)	🟡	🟡	🟢	🟢	🟢	🟡 **Some Concerns**
(Deschênes et al., 2017)	🟡	🟡	🟢	🟢	🟢	🟡 Some Concerns
(Tschudin-Sutter et al., 2017)	🟢	🟡	🟢	🟢	🟢	🟡 **Some Concerns**
(Reilly et al., 2016)	🟢	🟡	🟢	🟢	🟢	🟡 **Some Concerns**
(Chen et al., 2024)	🟢	🟡	🟢	🟢	🟢	🟡 **Some Concerns**
(Gil-Peña et al., 2024)	🟢	🟡	🟢	🟢	🟢	🟡 **Some Concerns**
(Pourkazemi et al., 2020)	🟢	🟡	🟢	🟢	🟢	🟡 **Some Concerns**
(Suchomel et al., 2021)	🟢	🟡	🟢	🟢	🟢	🟡 **Some Concerns**
(Pires et al., 2019)	🟢	🟡	🟢	🟢	🟢	🟡 **Some Concerns**
(Deshpande et al., 2018)	🟢	🟢	🟢	🟢	🟢	🟢 **Low**
(Hennig et al., 2017)	🟢	🟡	🟢	🟢	🟢	🟡 **Some Concerns**
(Forer et al., 2017)	🟢	🟡	🟢	🟢	🟢	🟡 **Some Concerns**

🟢 Low Risk | 🟡 Some Concerns / Unclear | 🔴 High Risk

Risk of bias was evaluated using two validated tools, chosen according to study design. For randomized trials the Cochrane Risk of Bias 2 (RoB 2) instrument was applied; for quasi-experimental, pre-post and analytical cross-sectional studies the Joanna Briggs Institute (JBI) Critical Appraisal Checklists were used. The assessments are summarized in Figure S1 (RoB 2) and Figure S2 (JBI).

1. Randomized trials – Risk of Bias 2 (RoB 2)
The RoB 2 tool addresses five domains
D1 – Bias arising from the randomization process.
D2 – Bias due to deviations from intended interventions (performance bias).
D3 – Bias due to missing outcome data (attrition bias).
D4 – Bias in measurement of the outcome (detection bias).
D5 – Bias in selection of the reported result (reporting bias).
Each domain is rated as low risk, some concerns, or high risk, and an overall risk-of-bias judgement is derived. The domain-level and overall judgements for all included randomized trials are displayed in Figure S1.
2. Quasi-experimental and cross-sectional studies – JBI Critical Appraisal Checklists
For non-randomized designs the JBI checklists assess six key quality criteria:
Q1 – Are the cause-and-effect variables clearly defined?
Q2 – Were participants in any comparisons similar at baseline?
Q3 – Did participants receive similar treatment/care aside from the intervention?
Q4 – Were outcomes measured in a reliable and standardised manner?
Q5 – Were confounding factors identified and controlled for?
Q6 – Was appropriate statistical analysis used?

Figure S2: Risk of Bias Summary Matrix (JBI Checklists)

Author (Year)	Q1	Q2	Q3	Q4	Q5	Q6	Overall Risk
(Vodianyk et al., 2025)	🟢	🔴	🟢	🟢	🟡	🟢	🟡 **Unclear**
(Gozdzielewska et al., 2026)	🟢	🟡	🟢	🟡	🟡	🟢	🟢 **Low**
(Arreba et al., 2025)	🟢	🟢	🟢	🟢	🟢	🟢	🟢 **Low**
(Setyawati & Wardhani, 2022)	🟢	🔴	🟢	🟢	🔴	🟢	🔴 **High**
(Nawa et al., 2021)	🟢	🔴	🟢	🟢	🔴	🟢	🔴 **High**
(Matuka et al., 2018)	🟢	🔴	🟢	🟢	🟡	🟢	🔴 **High**
(Packyanathan & Muralidharan, 2019)	🟢	🟡	🟢	🟢	🟡	🟡	🟡 **Unclear**
(López Martín & Erice, 2017)	🟢	🟢	🟡	🟡	🟡	🟢	🟡 Unclear

🟢 Low Risk | 🟡 Some Concerns / Unclear | 🔴 High Risk

Each question is answered as yes (low concern), no (high concern), unclear, or not applicable. The aggregated judgements for the included quasi-experimental and cross-sectional studies are shown in Figure S2.

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