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Evaluation of 15-second alcohol-based hand rub efficacy: a multi-laboratory study using a modified EN 1500 protocol

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SUMMARY

Introduction: Hands are a key vector for pathogen transmission in healthcare, making effective hand antisepsis crucial for infection prevention. According to the European standard EN 1500, the reference method for evaluating hand antiseptics, a minimum rub-in time of 30 s is required. However, observations show healthcare workers typically spend less time on hand antisepsis.

Method: To assess the feasibility of a reduced rub-in time under standardized conditions, the German Association for Applied Hygiene conducted a multi-centre ring trial in 14 laboratories using a modified EN 1500 protocol (15 s, 3 mL of 60% v/v propan-2-ol). In a randomized crossover design, volunteers' hands were contaminated with *Escherichia coli* K12 and treated either with the reference (2 × 3 mL/2 × 30 s) or the test protocol (1 x 3 mL/15 s). Microbial reduction was measured and non-inferiority statistically analysed.

Results: The 15-second protocol yielded significantly lower $\log_{10}$ reductions than the reference in 13 out of 14 laboratories but demonstrated consistent reproducibility and satisfactory interlaboratory performance. Challenges in completing the full rub-in technique within 15 s were reported, indicating the need for targeted training.

Conclusion: These findings support the methodological feasibility of a shortened protocol and are consistent with evolving clinical guidelines advocating reduced rub-in times, as well as with real-world practice, where healthcare workers typically spend less than 30 s on hand antisepsis. Nonetheless, any revision of EN 1500 should proceed cautiously to ensure antimicrobial efficacy, emphasizing complete hand coverage and strict adherence to technique.

Introduction

Hands are one of the most important vectors for the transmission of pathogens in the healthcare setting. Hygienic hand antisepsis is therefore regarded as an essential tool for preventing infections [1,2]. Hand antiseptics in the European Union (EU) are biocides that must be approved in accordance with the requirements of Regulation (EU) No. 528/2012 (Biocidal Product Regulation [BPR]) [3]. In Germany, hand antiseptics were previously classified as pharmaceutical products and those still in the market are protected under existing law. In the future, however, only biocides will be placed in the market.

The efficacy of hand antiseptics must be thoroughly tested before they can be introduced in the clinical practice. This evaluation is carried out in accordance with the requirements of EN 1500, which is the most stringent standard for testing the efficacy of hygienic hand antiseptics in Europe [4]. In this standard, the efficacy of a new product is compared with the efficacy of a reference procedure (60% v/v propan-2-ol, 2 x 3 mL/2 x 30 s) on hands of volunteers artificially contaminated with *Escherichia coli* K12 in a crossover design [4]. The current standard EN 1500 requires a minimum application time of 30 s for the product under test and adherence to a standardized six-step rub-in technique [4] which is also found in the World Health Organization (WHO) recommendation for clinical use [1]. However, observational studies have demonstrated that the actual duration of alcohol-based hand antisepsis in clinical settings is often considerably shorter than 30 s. Median hand hygiene durations of 17.4 s have been reported with values as low as 7.3 s depending on the facility [5], and similarly short application times of 7.6 s have been observed in intensive care units [6].

In several studies, it has been demonstrated that the antibacterial efficacy of alcohol-based hand rubs (ABHRs) can be achieved in 15 rather than 30 s [7–11]. Based on the data available from the literature, rub-in times for at least 15 s were recommended by Action 'clean hands' in 2020 [12], in the USA recommendations for hand hygiene in 2022 [13], in the Arbeitsgemeinschaft der Wissenschaftlichen Medizinischen Fachgesellschaften S2k guideline on hand antisepsis and hand hygiene in 2023 [14], and by the WHO task force in 2024 [15].

In accordance with the BPR guidelines of the European Chemicals Agency (ECHA), a minimum rub-in time of 30 s is permissible for hygienic hand disinfection [16], which complies with the requirements of EN 1500 [4]. Also, the German Association for Applied Hygiene (VAH) currently certifies only those hand antiseptics that demonstrate efficacy with a minimum rub-in time of 30 s, and shorter application times are not accepted [17]. However, this requirement which is based on EN 1500 conflicts with typical clinical workflows and raises legitimate concerns about the extent to which the existing test protocol still adequately reflects actual conditions in healthcare. Given that the antimicrobial efficacy of a 15-second ABHR has been consistently demonstrated in previous studies and is reflected in current guidelines, the primary aim of this study was to evaluate the feasibility, repeatability, and reproducibility of a 15-second hand rub procedure under controlled laboratory conditions. A secondary aim was to evaluate whether the reduced rub-in time maintains sufficient antimicrobial efficacy according to the EN 1500 standard.

To address this issue, the VAH initiated an international ring trial in 2024, designed to evaluate the methodological and statistical feasibility of a modified EN 1500 protocol featuring a 15-second rub-in time while preserving the structural integrity of the original standard. The present study reports on the

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design, execution, and statistical analysis of this ring trial and aims to ascertain whether such a shortened protocol can serve as a valid basis for efficacy testing.

Material and method

Selection of participating laboratories

At the end of 2024, laboratories that carry out efficacy tests according to EN 1500 were invited by the VAH as the organizer to participate in a cluster-randomized trial. Participation in this interlaboratory study was voluntary and offered free of charge by the VAH. A total of 14 laboratories, eight of which were located in Germany, three in Austria, two in Italy, and one in Malaysia, agreed to participate. The participating laboratories contributed substantial time, effort, and financial resources to conduct the tests under standardized conditions. Their commitment and collaboration were indispensable for the successful execution of the trial. In addition, participation in this ring trial can be used by laboratories as part of their accreditation processes, supporting ongoing quality assurance and demonstrating methodological competence in accordance with international standards.

Test product and reference alcohol

The test product used in this ring trial consisted of the same active ingredient as the EN 1500 reference alcohol (60% v/v propan-2-ol) but was applied according to the modified test protocol (1 × 3 mL for 15 s) rather than the standard reference procedure (2 x 3 mL for 2 x 30 s). The alcohol was locally produced in a hospital pharmacy in Bonn, Germany. The laboratories each received 500 mL of the test product and 500 mL of the reference alcohol in plastic bottles.

Neutralizer

The following neutralizer was added to the sampling fluids to stop the action of the alcohol immediately after the specified rub-in time and to ensure accurate assessment of microbial survival: polysorbate 80 (30 g/L), saponin (30 g/L), L-histidine (1 g/L), and cysteine (1 g/L). It was validated during the ring trial by each laboratory.

Test procedure

The study followed the EN 1500 methodology. A randomized crossover design was used to reduce intersubject variability. Each participant tested both the reference alcohol and the test product. The order in which participants applied the test product and the reference alcohol was randomly assigned. Healthy adult volunteers at least 18 years of age with intact, non-irritated skin on both hands were included. Exclusion criteria were visible skin conditions, known allergies to alcohol, recent use of systemic antibiotics (within 48 h), or acute infections. The volunteers in each laboratory gave written informed consent prior to inclusion. Hand size was recorded based on surgical glove sizes (XS, S, M, L, and XL).

Before contamination, hands were washed for 1 min with soft soap (sapo kalinus, 20 g/L) and dried with paper towels. For contamination, both hands of the volunteers were immersed for 5 s up to the mid-metacarpal level in a contamination fluid of *E. coli* K12 (NCTC 10538) grown in tryptic soy broth (TSB) for 24 h at 36 ± 1 °C, targeting a final concentration of 1.5–5.0 × 10^8 colony-forming units (cfu)/mL. Hands were air-dried for 3 min after immersion. To determine pretreatment values, fingertips were rubbed for 1 min on the bottom of a Petri dish containing TSB, one for each hand. The hands were then treated with either the reference procedure (60% v/v propan-2-ol, 2 x 3 mL for 2 x 30 s) or the test protocol (60% v/v propan-2-ol, 1 x 3 mL for 15 s). For the reference hand rub procedure, 3 mL of the reference alcohol was applied to dry hands and rubbed for 30 s up to the wrists using the standard hand rub procedure according to EN 1500 [4]. The following sequence ensured complete coverage: (1) palm to palm including wrists (1x), (2) palm over the dorsum of the opposite hand (5×), (3) palm to palm with fingers interlaced (5x), (4) backs of fingers to opposing palms with fingers interlocked (5x), (5) rotational rubbing of thumbs (5x), and (6) rotational rubbing of clasped fingers in the opposite palm (5x). Steps 2–6 were repeated as needed to reach 30 s. The procedure was repeated with an additional 3 mL for a total rub-in time of 60 s. For the test product, 3 mL of alcohol was applied to dry hands and rubbed for a total of 15 s up to the wrists using the same technique and step sequence as for the reference alcohol. If the six-step sequence was completed before 15 s had elapsed, steps 2–6 were repeated as needed to ensure that the full rub-in time consisted of continuous hand-rubbing movements. Immediately after the rub-in, post-treatment values were collected as described above in TSB containing validated neutralizing agent, irrespective of whether the hands were still moist or already dry.

All samples were serially diluted and spread onto tryptic soy agar containing sodium deoxycholate, which suppresses the growth of resident skin flora. Culture plates were incubated at 36 ± 1 °C for 24–48 h, and cfus were counted.

Evaluation of results and statistics

Log_{10} values from the left and right hands of each volunteer were averaged separately for both pretreatment and post-treatment values. These values were used to calculate the log_{10} reductions, defined as the difference between the mean log_{10} pretreatment and post-treatment bacterial counts, reflecting the magnitude of microbial reduction achieved by the hand rub procedure. Subsequently, the differences between the log_{10} reduction factors from the reference and the test product were tested for significance by a non-parametric non-inferiority test according to Hodges and Lehmann.

Inferiority of the test product was rejected and non-inferiority assumed if the Hodges–Lehmann upper 97.5% confidence limits for the differences in log_{10} reductions between test product and reference product were smaller than the agreed inferiority margin of <0.6 log_{10}. The level of significance was set at $P = 0.025$ (one sided). In addition, each laboratory evaluated the acceptance criteria of EN 1500 [4] and documented the hand sizes of the individual study volunteers according to glove sizes as XS, S, M, L, and XL.

To evaluate the laboratory performance in terms of mean log_{10} reductions and Hodges and Lehmann values, a z-score was calculated in accordance with DIN EN ISO 13528 [18]. The z-scores were calculated by subtracting the mean value of all values from each value and dividing by the standard deviation of all values. A satisfactory performance was indicated by z-

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Table I
Results from 14 laboratories on the efficacy of the test procedure applied with 3 mL for 15 s according to EN 1500

Laboratory	Volunteers (*N*)	Reference procedure (mean $\log_{10}$ reduction ± SD)			Test procedure (mean $\log_{10}$ reduction ± SD)			Upper 95% confidence interval (Hodges and Lehman)	*P* value mean	*P* value SD
		Pretreatment values	Post-treatment values	$\log_{10}$ reduction	Pretreatment values	Post-treatment values	$\log_{10}$ reduction			
1	20	6.83 ± 0.61	2.07 ± 0.80	4.76 ± 1.23	6.99 ± 0.32	3.30 ± 0.84	3.70 ± 1.04	1.75[a]	0.003	0.707
2	20	5.72 ± 0.36	1.55 ± 0.40	4.17 ± 0.48	5.54 ± 0.32	2.30 ± 0.59	3.24 ± 0.64	1.30[a]	0.000	0.527
3	20	5.88 ± 0.36	2.48 ± 0.50	3.40 ± 0.46	5.91± 0.33	3.45 ± 0.38	2.46 ± 0.48	1.23[a]	0.000	0.641
4	20	5.99 ± 0.24	1.18 ± 0.83	4.80 ± 0.90	6.02 ± 0.21	2.19 ± 0.65	3.83 ± 0.63	1.40[a]	0.001	0.069
5	20	5.78 ± 0.41	0.59 ± 0.61	5.19 ± 0.78	5.58 ± 0.55	1.21 ± 0.86	4.37 ± 0.93	1.10[a]	0.006	0.750
6	18	6.00 ± 0.29	0.84 ± 0.63	5.17 ± 0.67	6.00 ± 0.40	2.10 ± 0.71	3.90 ± 0.77	1.57[a]	0.000	0.577
7	20	5.83 ± 0.29	0.92 ± 0.83	4.92 ± 0.91	5.79 ± 0.53	1.81 ± 0.73	3.98 ± 1.02	1.22[a]	0.004	0.962
8	20	5.93 ± 0.38	0.71 ± 0.74	5.22 ± 0.68	6.03 ± 0.31	1.97 ± 0.81	4.06 ± 0.74	1.47[a]	0.000	0.510
9	19	5.87 ± 0.56	1.27 ± 0.68	4.60 ± 0.92	5.96 ± 0.49	2.15 ± 0.67	3.81 ± 0.71	1.16[a]	0.005	0.507
10	20	6.45 ± 0.45	1.84 ± 0.71	4.60 ± 0.68	6.38 ± 0.41	2.29 ± 0.66	4.09 ± 0.76	0.76[a]	0.030	0.745
11	18	6.23 ± 0.47	3.12 ± 0.64	3.11 ± 0.55	6.24 ± 0.41	3.06 ± 0.96	3.18 ± 0.87	0.29[b]	0.591	0.294
12	20	6.35 ± 0.33	0.89 ± 0.82	5.45 ± 0.89	6.48 ± 0.31	1.74 ± 0.83	4.75 ± 0.72	1.20[a]	0.005	0.857
13	20	6.25 ± 0.32	2.75 ± 0.70	3.50 ± 0.72	6.43 ± 0.24	3.63 ± 0.60	2.80 ± 0.64	1.00[a]	0.002	0.765
14	20	5.17 ± 0.39	0.67 ± 0.58	4.50 ± 0.66	5.13 ± 0.39	1.71 ± 0.86	3.42 ± 0.84	1.43[a]	0.000	0.320
All	275	6.02 ± 0.39	1.49 ± 0.68	4.53 ± 0.75	6.03 ± 0.37	2.35 ± 0.72	3.68 ± 0.77	n. a.		

SD, standard deviation; n. a., not applicable.
[a] test product is not non-inferior to reference procedure.
[b] test product is non-inferior to reference procedure.

scores ≤2.0, a questionable result that represents a warning signal was indicated by z-scores between 2.0 and <3.0, and an unsatisfactory result that triggers an action signal was indicated by z-scores ≥3.0. Reproducibility and repeatability were assessed using R Core Team 2024, version 4.3.3. Difference between procedures (60 s vs. 15 s rub in time) was tested using the Mann–Whitney test and Kruskal–Wallis test; the latter was used to assess the influence of volunteers, hand size, and chronological sequence. Homogeneity of variance was checked using the Levene test.

Results

As shown in Table I, the mean pretreatment values between the participating laboratories showed only minor variations. In experiments with the reference alcohol, the pretreatment values ranged between 5.17 (laboratory 14) and 6.83 (laboratory 1). When using the test product, the corresponding pretreatment values ranged from 5.13 (laboratory 14) to 6.99 (laboratory 1). In contrast, the mean post-treatment values showed greater variations between laboratories (Table I). With the reference alcohol, the post-treatment values after treatment ranged from 0.59 (laboratory 5) to 3.12 (laboratory 11). When using the test product, the post-treatment values ranged from 1.21 (laboratory 5) to 3.63 (laboratory 13). The mean $\log_{10}$ reductions observed with the reference alcohol ranged from 3.11 (laboratory 11) to 5.45 (laboratory 12), with an overall mean across all datasets of 4.53 (Table I). The corresponding z-scores indicated acceptable performance in all 14 laboratories, as shown in Figure 1. For the test product, the mean $\log_{10}$

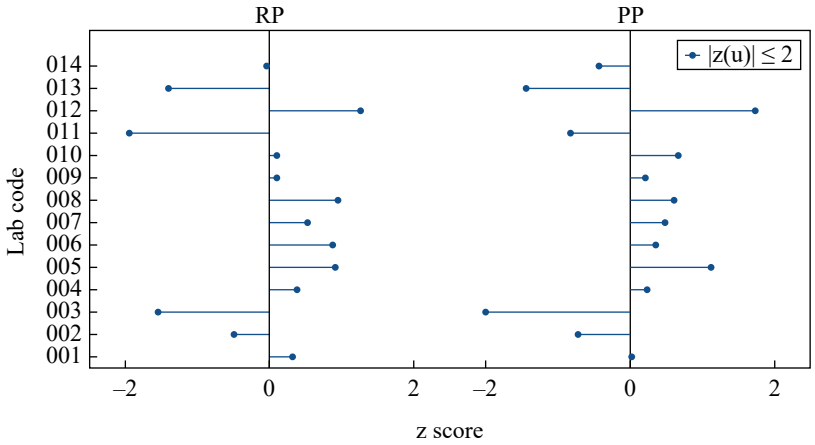

Figure 1. Z-scores of the mean $\log_{10}$ reductions for the reference procedure (2 x 3 mL/2 x 30 s) and the test procedure (1 x 3 mL/1 x 15 s) calculated for each laboratory. RP, reference procedure; PP, test procedure.

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Table II
Statistical parameters for the mean $\log_{10}$ reduction of *Escherichia coli* with the reference procedure and test procedure according to EN 1500

	Reference procedure	Test procedure
Applied volume – application time	2 x 3 mL – 60 s	1 x 3 mL – 15 s
Number of laboratories (n) that submitted results	14	14
Mean $\log_{10}$ reduction ± 95% CI[a]	4.53 ± 0.75	3.68 ± 0.77
Repeatability SD[b] S_r	0.757	0.776
Reproducibility SD S_R	0.730	0.612

[a] CI, confidence interval
[b] SD, standard deviation.

reductions varied between 2.46 (laboratory 3) and 4.45 (laboratory 12), resulting in an overall mean for all data sets of 3.68 (Table I). Again, the z-score analysis demonstrated satisfactory performance in all participating laboratories (Figure 1).

The repeatability within the individual laboratories was comparable for the test product (0.757) and the reference product (0.776). Similarly, interlaboratory reproducibility was consistent, with values of 0.612 for the test product and 0.730 for the reference product (Table II).

The statistical analysis focusses on the influence of the volunteers. The $\log_{10}$ reduction of the test product compared with the reference product is significantly different for all laboratories except for one laboratory (laboratory 11). No significant difference in the overall standard deviation (see repeatability) could be found (*P* value: 0.769). The Levene test revealed no significant difference in the variance in the individual laboratories.

Non-inferiority testing was conducted according to Hodges and Lehmann. The bactericidal efficacy of the test product was found to be non-inferior to that of the reference procedure in one of the 14 laboratories. In the remaining 13 laboratories, the test product did not meet the predefined criteria for non-inferiority (Table III). Despite this, the z-scores derived from the Hodges and Lehmann confidence intervals indicated satisfactory analytical performance across all 14 laboratories (Figure 1). An overview of compliance with the defined acceptance criteria in all participating laboratories is presented in Table III. Each laboratory included a minimum of 18 test volunteers, and the mean pretreatment values for both the reference alcohol and the test product exceeded 5.0 $\log_{10}$. All laboratories met the requirements of having not more than three individual $\log_{10}$ reductions below 3.0 for the reference procedure. Furthermore, the absolute difference in mean differences remained <2.0 in all laboratories. The concentration of the test organism in the contamination fluid was within the specified range of 1.5–5.0 x 10^8 cfu/mL across all laboratories. Additional acceptance criteria including the test organism concentrations in the validation suspensions Nv (300–1600 cfu/mL) and Nv0 (30–160 cfu/mL), neutralizer toxicity B (≥0.0005 x NvB), and method validation C (≥0.5 x Nv0) were fulfilled by almost all laboratories. In 2 of the 14 laboratories, the NvB values were outside the acceptance criteria (30,000–160,000 cfu/mL). Since the two laboratories did not respond to verify whether the submitted values were correct, the values were considered outliers (Table III).

Hand size was documented for all 275 study volunteers. Of these, 146 (52.9%) volunteers had hand size M, 52 (20.7%) had hand size S, 63 (22.8%) had hand size L, and 10 (3.6%) had hand size XL. One participant (0.4%) had hand size XS; due to the insufficient sample size, this participant was excluded from the statistical analysis. While a trend towards decreasing mean $\log_{10}$ reductions with increasing hand size (S to XL) was observed, this difference did not reach statistical significance for either the reference procedure ($P = 0.077$; Kruskal–Wallis test) or the test product ($P = 0.073$; Kruskal–Wallis test).

Discussion

Over the past decades, the recommended duration for hygienic hand antisepsis has significantly decreased. Earlier guidelines from the 1980s recommended application times of one to three minutes [19,20], primarily due to the limited efficacy and skin tolerability of the products available at the time. With the introduction of ABHR, the standard rub-in time was reduced to 30 s [21,22], as reflected in the European standard EN 1500 [4] and subsequently adopted by the WHO in its 2009 guidelines [1]. Since time pressure and workload are recognized barriers to compliance with hand antisepsis [23], it was investigated whether the same efficacy can be achieved with the ABHR within 15 s. Under controlled laboratory conditions, a 15-second hand rub was found to be as effective as a 30-second application in reducing microbial load [8,10,11,24]. Similarly, clinical studies demonstrated comparable antimicrobial efficacy for 15- and 30-second rub-in times [9,25]; however, the actual extent of microbial reduction could not be determined. Furthermore, shorter exposure durations of 15 s have been shown to achieve equivalent hand surface coverage (wettability) [26] and are associated with significantly improved compliance with hand hygiene protocols among healthcare workers [9,25].

Therefore, some recommendations now accept a 15-second rub-in time under specific conditions [12–15]. For example, USA guidelines recommend ensuring full hand-surface coverage during rubbing for at least 15 s when assessing hand hygiene technique in healthcare workers [13].

Nevertheless, the European standard EN 1500 still requires an exposure time of at least 30 s [4]. Consequently, there is increasing interest in testing protocols with shorter contact times that can lead to the same level of safety. While previous studies have consistently shown that 15-second hand rubs can achieve similar microbial reductions as 30-second applications, the present study primarily focussed on evaluating the feasibility and reproducibility of this shortened protocol across multiple laboratories.

This study presents the method and results of a multi-centre ring trial to evaluate the validity of a shortened hand antisepsis protocol according to EN 1500. The objective was to determine whether the reduced rub-in time of 15 s yields reproducible efficacy outcomes across different laboratories, thereby contributing to the scientific basis for a potential adaptation of the current efficacy testing standard in line with clinical practice.

In 13 out of 14 laboratories, the average reductions achieved with the test product (60% v/v propan-2-ol, 1 x 3 mL for 15 s) were significantly lower than those achieved with the

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Table III
Results from 14 laboratories on the EN 1500 acceptance criteria

Laboratory	Number of volunteers (≥18)	Mean pretreatment values reference product (≥5.0)	Mean pretreatment values test product (≥5.0)	Fourth lowest log_{10} reduction of reference product (≥3.0)	Absolute difference of mean differences (<2.0)	Test suspension (range: 1.5–5.0 x 10^8 cfu)	Validation suspension Nv (range: 300–1600 cfu)	Validation suspension Nv0 (range: 30–160 cfu)	Validation suspension NvB (range: 30,000–160,000 cfu)	Neutralizer toxicity B (≥0.0005 x NvB)	Method validation C (≥0.5 x Nv0)
1	20	6.83	6.99	4.67	0.75	4.47 x 10^8	870	87	88,500	86.5	82.5
2	20	5.72	5.54	3.03	0.10	4.68 x 10^8	1230	123	84,000	73.5	82.5
3	20	5.88	5.91	3.03	0.34	5.00 x 10^8	1300	130	130,000	115	105
4	20	5.99	6.02	4.02	0.01	4.24 x 10^8	1430	142.5	139,500	130.5	151
5	20	5.78	5.58	3.17	0.43	5.00 x 10^8	895	89.5	90,000	92	87
6	18	6.00	6.00	4.20	0.16	2.80 x 10^8	890	89	98,500	97	103
7	20	5.83	5.79	3.45	0.47	2.14 x 10^8	550	55	51.5[a]	48.5	39,5
8	20	5.93	6.03	2.49	0.32	1.58 x 10^8	320	32	36,500	30.5	31,5
9	19	5.87	5.96	4.82	0.72	2.86 x 10^8	710	71	109[a]	89	151
10	20	6.45	6.38	4.67	0.11	4.79 x 10^8	1140	114	116,500	96	94
11	18	6.23	6.24	4.22	0.23	4.90 x 10^8	1340	131.5	140,000	143	123,5
12	20	6.35	6.48	4.09	0.25	2.04 x 10^8	1041	104.1	108,000	123	151
13	20	6.25	6.43	3.14	0.23	3.47 x 10^8	1500	150	140,000	150	110
14	20	5.17	5.13	4.24	0.30	3.00 x 10^8	1080	108	127,000	112	109

Nv, number of cells/mL in the validation suspension; Nv0, number of cells/mL in the mixture of the neutralizer and the validation suspension at the beginning of the contact time; NvB, colony-forming unit/mL in the suspension used for the neutralizer control.
[a] outlier (the laboratories did not respond to verify if the submitted values are correct).

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reference product (60% v/v propan-2-ol, 2 x 3 mL for 60 s). Nevertheless, the variance of the results was not significantly different when tested with a shorter contact time of 15 s compared with the reference with 30 s contact time. This suggests that while performance varied between the two products, the method itself is statistically reliable and repeatable. In addition, no statistically significant association was observed between hand size and $\log_{10}$ reduction in our study. This is consistent with our methodological findings but differs from some previous reports, where hand size was found to influence antimicrobial efficacy [27]. Our results highlight the critical role of interlaboratory tests in developing standardized methods and emphasize the necessity of conducting experiments under practical conditions (phase 2/step 2) rather than relying solely on in-vitro studies (phase 2/step 1). Some volunteers described challenges in applying the full EN 1500 six-step technique within such a short timeframe. This suggests that the shortened rub-in time may require targeted training to ensure proper technique and hand coverage. With appropriate instruction, however, these challenges could likely be overcome. While the present study focussed on the standard EN 1500 protocol, it should be noted that alternative, simplified rub-in procedures have been reported to achieve comparable log reductions while potentially improving compliance compared with the full six-step method [28,29]. Indeed, real-world observations indicate that healthcare personnel rarely perform all the six steps [28,30], further supporting that simplified approaches could be a feasible option to explore in future assessments of the EN 1500 protocol.

Given the positive methodical results and the alignment with updated international recommendations, the question now arises whether the EN 1500 should be revised to permit a 15-second rub-in time under clearly defined conditions. Any such revision must be approached with caution to ensure that antimicrobial efficacy is not compromised. Total hand coverage and strict adherence to application technique must continue to be ensured as incomplete application significantly reduces the efficacy of the hand rub [31].

Any concerns regarding the efficacy of the shortened protocol must be thoroughly dispelled through further validations to guarantee its safe and effective use in clinical settings. A recommendation to limit hand rubbing to 15 s may lead to assume that smaller volumes of ABHRs, such as 1 or 2 mL, are adequate. However, studies have clearly demonstrated that volumes of 2 mL or less are insufficient to achieve the required bactericidal efficacy [32,33]. Nevertheless, at least one study reported that $\log_{10}$ reductions achieved with 2 mL of 60% v/v propan-2-ol were not significantly lower than those obtained with 3 mL under specific experimental conditions [34]. Therefore, the potential role of reduced application volumes may warrant consideration in future reassessments of the EN 1500 protocol, provided that efficacy and reproducibility can be reliably ensured. Likewise, the use of larger volumes of ABHRs that may result in sufficient efficacy within 15 s should be critically evaluated. Some products need more than 3 mL to pass the EN 1500. Such volumes typically require significantly more than 15 s for drying [31,34]. To be able to claim products for hygienic hand antisepsis with an exposure time of 15 s and thus guaranteeing the safety of the products for users, the permitted exposure time must also be reduced in the BPR requirements [16]. This is based on the specifications of EN 1500, which currently stipulates a minimum rub-in time of 30 s. If the 15 s are included in the EN 1500 standard, it is likely that the shortened rub-in time will also be facilitated in the corresponding ECHA guidance document [16], paving the way for this claim. The results of this study support the implementation of a 15-s rub-in time to EN 1500.

In conclusion, the results of this ring trial demonstrate that a modified EN 1500 protocol with a rub-in time of 15 s is statistically and methodically feasible. These findings provide a robust scientific basis for considering an update of EN 1500 that aligns more closely with real-world clinical workflows while continuing to uphold high standards of infection prevention. In addition, this modification to EN 1500 would give manufacturers the opportunity to prove the efficacy of their products in 15 s, thereby ensuring user compliance and product safety in 15 s.

CRediT authorship contribution statement

K-M. Roesch: Writing — review & editing, Writing — original draft, Project administration, Methodology, Investigation, Formal analysis, Data curation, Conceptualization. **J. Gebel:** Writing — review & editing, Funding acquisition, Formal analysis, Conceptualization. **A. Bolten:** Writing — review & editing, Investigation, Conceptualization. **M. Cavalleri:** Investigation. **B. Christiansen:** Writing — review & editing, Methodology, Formal analysis, Conceptualization. **F. Droop:** Validation. **B. Eilts:** Investigation. **M. Exner:** Writing — review & editing, Methodology, Conceptualization. **H. Gabriel:** Investigation. **C. Hildebrandt:** Investigation. **T. Koburger-Janssen:** Investigation. **K. Konrat:** Writing — review & editing, Methodology, Conceptualization. **C.S. Lee:** Investigation. **J. Lenz:** Writing — review & editing, Methodology, Conceptualization. **H. Martiny:** Writing — review & editing, Methodology, Conceptualization. **M. Meckel:** Investigation. **N.T. Mutters:** Writing — review & editing, Methodology, Conceptualization. **S. Pahl:** Writing — review & editing, Methodology, Conceptualization. **L. Paßvogel:** Writing — review & editing, Methodology, Conceptualization. **C. Schartner:** Investigation. **F. Seyringer:** Investigation. **K. Steinhauer:** Investigation. **L.J. Vecchio:** Investigation. **L. Vossebein:** Writing — review & editing, Methodology, Conceptualization. **A. Wille:** Investigation. **A. Kramer:** Writing — review & editing, Writing — original draft, Supervision, Methodology, Investigation, Conceptualization. **M. Suchomel:** Writing — review & editing, Writing — original draft, Supervision, Methodology, Investigation, Conceptualization.

Ethics statement

The study protocol was approved by the Steering committee for interlaboratory tests and the 4+4 Working Group of the Association for Applied Hygiene (VAH), Germany and the Industrial Association Hygiene and Surface Protection (IHO), Frankfurt, Germany.

Data availability

The datasets used and/or analysed during the current study are available from the first author on reasonable request.

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Conflict of interest statement

The authors declare no conflict of interest. The views expressed here are those of the authors and do not necessarily reflect those of the universities/companies they are affiliated with.

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Appendix A. Supplementary data

Supplementary data to this article can be found online at https://doi.org/10.1016/j.jhin.2026.01.027.

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