

Chemical disinfectants and antiseptics — Hygienic handrub — Test method and requirements (phase 2/step 2)

The European Standard EN 1500:1997 has the status of a
British Standard

ICS 11.080

National foreword

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The UK participation in its preparation was entrusted to Technical Committee IH/57, Chemical disinfectants and antiseptics, which has the responsibility to:

- aid enquirers to understand the text;
- present to the responsible European committee any enquiries on the interpretation, or proposals for change, and keep the UK interests informed;
- monitor related international and European developments and promulgate them in the UK.

A list of organizations represented on this committee can be obtained on request to its secretary.

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Summary of pages

This document comprises a front cover, an inside front cover, pages i and ii, the EN title page, pages 2 to 16 and a back cover.

This standard has been updated (see copyright date) and may have had amendments incorporated. This will be indicated in the amendment table on the inside front cover.

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Amendments issued since publication

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ICS 11.080

Descriptors: Disinfectants, chemical compounds, hygiene, hand (anatomy), tests, contamination, antibacterial activity, neutralizing, counting, micro-organisms

English version

Chemical disinfectants and antiseptics — Hygienic handrub — Test method and requirements (phase 2/step 2)

Antiseptiques et désinfectants chimiques — Traitement hygiénique des mains par frictions — Méthode d'essai et prescriptions (phase 2/étape 2)

Chemische Desinfektionsmittel und Antiseptika — Hygienische Händedesinfektion — Prüfverfahren und Anforderungen (Phase 2/Stufe 2)

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CEN

European Committee for Standardization
Comité Européen de Normalisation
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Foreword

This European Standard has been prepared by the Technical Committee CEN/TC 216, Chemical disinfectants and antiseptics, the secretariat of which is held by AFNOR.

This European Standard shall be given the status of a national standard, either by publication of an identical text or by endorsement, at the latest by January 1998, and conflicting national standards shall be withdrawn at the latest by January 1998.

According to the CEN/CENELEC Internal Regulations, the national standards organizations of the following countries are bound to implement this European Standard: Austria, Belgium, Czech Republic, Denmark, Finland, France, Germany, Greece, Iceland, Ireland, Italy, Luxembourg, Netherlands, Norway, Portugal, Spain, Sweden, Switzerland and the United Kingdom.

Annex A is normative. Annex B, Annex C, Annex D and Annex E are informative.

NOTE Attention is drawn to the fact that tests on human volunteers are the subject of legal provisions in certain European countries/regions.

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1 Scope

This European Standard specifies a method of test simulating practical conditions for establishing whether a product for hygienic handrub reduces the release of transient flora according to the requirements when rubbed onto the artificially contaminated hands of volunteers.

This European Standard applies to products for hygienic handrub for use in areas and situations where disinfection is medically indicated. Such indications occur in patient care, for example:

- in hospitals, in community medical facilities and in dental institutions;
- in clinics of schools, of kindergartens and of nursing homes.

and may occur in the workplace and in the home. It may also include services such as laundries and kitchens supplying products directly for the patient.

2 Normative references

This European Standard incorporates by dated or undated reference, provisions from other publications. These normative references are cited at the appropriate places in the text and the publications are listed hereafter. For dated references, subsequent amendments to or revisions of any of these publications apply to this European Standard only when incorporated in it by amendment or revision. For undated references the latest edition of the publication referred to applies.

prEN 12054, *Chemical disinfectants and antiseptics — Products for hygienic and surgical handrub and handwash — Bactericidal activity — Test method and requirements (phase 2/step 1)*.

3 Definitions

For the purposes of this standard, the following definitions apply:

3.1

product (for chemical disinfection and/or antiseptics)

chemical agent or formulation used as a chemical disinfectant and/or antiseptic (EN 1040)

3.2

hygienic handrub

postcontamination treatment procedure that involves rubbing hands without the addition of water, using a bactericidal preparation directed against transient microorganisms to prevent their transmission regardless of the resident skin flora

3.3

prevalue

number of colony forming units (cfu) sampled from hands before treatment

3.4

postvalue

number of colony forming units (cfu) sampled from hands after treatment

3.5

reduction factor (RF)

ratio of prevalues and postvalues, generally expressed by decimal logarithms:

$$\log_{10} \text{RF} = \log_{10} \text{prevalue} - \log_{10} \text{postvalue}$$

4 Requirements

When tested in accordance with clause 5, the mean reduction of the release of test organisms achieved by the hygienic handrub product shall not be significantly smaller than that achieved by a reference handrub (R) (see 5.6.4.2) with propan-2-ol 60 % (V/V).

Products under test shall at least have a bactericidal activity as specified in prEN 12054.

5 Test method

5.1 Principle

The number of test organisms released from the fingertips of artificially contaminated hands is assessed before and after the hygienic handrub. The ratio of the two resulting values is called the reduction factor. It represents a measure of the antimicrobial activity of the hygienic handrub product tested. The necessary precision is achieved by repeating the test on 12 to 15 subjects. To compensate for extraneous influences it is compared with the reduction factor obtained with a parallel reference handrub procedure (R) which is performed with the same subjects, on the same day and under comparable environmental conditions.

5.2 Experimental design

For testing one product at a time a cross-over design is used. The subjects are randomly divided into two groups of approximately the same size. The test is first performed with group 1 using the reference handrub procedure (see 5.6.4.2) and group 2 using the handrub procedure with the test product (see 5.6.4.3).

The test is then repeated on the same day with group 1 using the handrub procedure with the test product and group 2 using the reference handrub procedure. Before every reference handrub procedure and every handrub procedure with the test product, the procedure described in 5.6.2 and 5.6.3 shall be carried out.

For testing more than one product at a time, a Latin-square design is used with as many groups of subjects and as many experimental runs as there are handrub products (including the reference propan-2-ol). In each run all handrub procedures are employed in parallel. At the end of the whole series of runs every subject shall have used each handrub product once.

5.3 Subjects

The test shall be performed on 12 to 15 healthy persons who have hands with healthy skin, without cuts or abrasions, and with short and clean fingernails. Although, in general, age is not a limiting factor, subjects should be at least 18 years of age.

5.4 Materials

5.4.1 Test organism

Escherichia coli K12 NCTC¹⁾ 10538.

NOTE This test organism has been specifically chosen to meet health and safety guidance and ethical committee considerations. It is a K12 strain of *E. coli* of normal flora origin internationally recognized as being non-pathogenic. According to the UK catalogue of the National Collections of Industrial & Marine Bacteria (see [1] in Annex D), NCIMB strain 10083 is identical to NCTC 10538 and classified as a risk group 1 organism. The German Safety Ordinance on Gene Technology [2] also assigns the K12 strain to group 1. Directive 93/88/EEC [3] (Annex III to Directive 90/679/EEC [4]) explicitly states that non-pathogenic strains of *Escherichia coli* are excluded from the group 2 assignment.

5.4.2 Media and reagents

5.4.2.1 General

The reagents shall be of analytical grade and/or appropriate for microbiological purposes.

NOTE To improve reproducibility, it is recommended that commercially available dehydrated material is used for the preparation of culture media. The manufacturer's instructions relating to the preparation of these products should be rigorously followed.

5.4.2.2 Water

The water shall be free from substances that are toxic or inhibiting to the bacteria. It shall be freshly glass distilled and not demineralized water.

Sterilize in the autoclave (see 5.5.2.1).

NOTE 1 If the water is sterilized during the sterilization of the reagents, this is not necessary.

NOTE 2 If distilled water of adequate quality is not available, water for injectable preparations (see European Pharmacopoeia) can be used.

¹⁾ NCTC 10538 is the collection number of strain supplied by the National Collection of Type Cultures. This information is given for the convenience of users of this standard and does not constitute an endorsement by CEN of the product named. Corresponding strains supplied by another culture collection may be used if they can be shown to lead the same results.

5.4.2.3 Tryptone soya agar (TSA)

For maintenance of bacterial strains and performance of viable counts.

Tryptone, pancreatic digest of casein	15,0 g
Soya peptone, papaic digest of Soybean meal	5,0 g
NaCl	5,0 g
Agar	15,0 g
Water (see 5.4.2.2)	to 1000,0 ml

Sterilize in the autoclave (see 5.5.2.1). After sterilization the pH of the medium shall be equivalent to $7,2 \pm 0,2$ when measured at 20 °C.

For quantitative cultures, TSA with added sodium-desoxycholate (0,5 g/l) shall be used to inhibit the growth of skin staphylococci.

5.4.2.4 Tryptone soya broth (TSB)

For preparation of the contamination fluid.

Tryptone, pancreatic digest of casein	15,0 g
Soya peptone, papaic digest of Soybean meal	5,0 g
NaCl	5,0 g
Water (see 5.4.2.2)	to 1000,0 ml

Sterilize in the autoclave (see 5.5.2.1). After sterilization the pH of the medium shall be equivalent to $7,2 \pm 0,2$ when measured at 20 °C.

5.4.2.5 Neutralizer

A neutralizer validated according to prEN 12054 shall be incorporated in the sampling fluids and diluents (TSB, see 5.4.2.4) for the assessment of postvalues in both test and reference procedure, but neither in those for the prevalues nor in the counting plates.

NOTE A list of neutralizers which can be used is given in Annex B.

5.4.2.6 Soft soap, 200 g/L

Linseed oil	50 parts ^a
Potassium hydroxide	9,5 parts
Ethanol	7 parts
Distilled water	as needed

^a By weight.

Add linseed oil to a solution of potassium hydroxyde in 15 parts distilled water and heat up to approximately 70 °C while constantly stirring. Add the ethanol and continue heating while stirring until the saponification process is completed and a sample dissolves clearly in water and almost clearly in alcohol. The weight of the soft soap is then brought up to 100 parts by addition of hot distilled water. Take 200 g of the soft soap in 1 l of distilled water and sterilize in the autoclave (see 5.5.2.1).

5.4.2.7 Propan-2-ol 60 % (V/V) (see European Pharmacopoeia)**5.5 Apparatus and glassware****5.5.1 General**

Sterilize all glassware and parts of the apparatus that will come into contact with the culture media and reagents or the sample, except those that are supplied sterile, by one of the following methods:

- in the autoclave (see 5.5.2.1) by maintaining it at 121^{+3}_0 °C for a minimum holding time of 15 min;
- in the dry heat sterilizer (see 5.5.2.1) by maintaining it at 180 °C for a minimum holding time of 30 min, at 170 °C for a minimum holding time of 1 h or at 160 °C for a minimum holding time of 2 h.

5.5.2 Usual microbiological laboratory equipment²⁾ and, in particular, the following.

5.5.2.1 Apparatus for sterilization

- a) For moist heat sterilization an autoclave capable of being maintained at 121^{+3}_0 °C for a minimum holding time of 15 min;
- b) For dry heat sterilization a hot air oven capable of being maintained at 180 °C for a minimum holding time of 30 min, at 170 °C for a minimum holding time of 1 h or at 160 °C for a minimum holding time of 2 h.

5.5.2.2 Incubator, capable of being controlled at (36 ± 1) °C.

5.5.2.3 pH-meter, having an accuracy of calibration of $\pm 0,1$ pH units at 25 °C.

5.5.2.4 Stopwatch

5.5.2.5 Graduated pipettes of nominal capacities 10 ml and 1,0 ml and 0,1 ml. Calibrated automatic pipettes may be used.

5.5.2.6 Petri dishes, of diameter 90 mm.

5.5.2.7 Container of sufficient capacity to take both hands immersed vertically up to the mid-metacarpals.

5.5.2.8 Bottles of at least 1 l capacity.

5.6 Procedure

5.6.1 Preparation of the contamination fluid

Grow the *E. coli* (see 5.4.1) in two tubes each containing 5 ml of TSB (see 5.4.2.4) for 18 h to 24 h at (36 ± 1) °C. Inoculate these cultures into two bottles (see 5.5.2.8) with 1 l TSB (see 5.4.2.4) each and incubate for 18 h to 24 h at (36 ± 1) °C. Pool the resulting bacterial suspension.

This contamination fluid shall contain between 2×10^8 cfu/ml and 2×10^9 cfu/ml which shall be confirmed by a standard quantitative culture technique in accordance with prEN 12054.

5.6.2 Application of the contamination fluid

Prepare the hands by washing for 1 min with soft soap (see 5.4.2.6) to remove natural transients. Dry them thoroughly on paper towels. Pour the contamination fluid into the container (see 5.5.2.7) and immerse the hands up to the mid-metacarpals for 5 s with fingers spread apart. Carefully allow surplus liquid to drain back into the container.

NOTE During this procedure care should be taken to avoid contamination of the immediate work area.

Allow the hands to dry in the air for 3 min, holding them in a horizontal position with the fingers spread out and rotating them to and fro to avoid the formation of droplets.

One batch of contamination fluid shall be used not longer than 3 h after the first subject's hands have been contaminated. Additionally, it shall be ensured that, in a test, all subject's hands shall be treated with the same batch of contamination fluid, even if various products are tested against the reference product.

5.6.3 Prevalues

Immediately after drying, rub the fingertips (including that of the thumb) for 1 min on the base of a Petri dish (see 5.5.2.6) containing 10 ml of TSB (see 5.4.2.4) without neutralizer, in order to assess the release of test organisms before treatment of the hands (prevalues). Use a separate Petri dish for each hand.

Prepare dilutions of these sampling fluids of 10^{-3} and 10^{-4} in TSB (see 5.4.2.4). For each dilution, spread 0,1 ml over the surface of a TSA plate (see 5.4.2.3) using glass spreaders. The interval between sampling and plating shall not exceed 30 min.

5.6.4 Hygienic handrub procedure

5.6.4.1 General

Immediately after sampling for the prevalues and without recontaminating the hands, the groups shall perform the handrub in accordance with either 5.6.4.2 or 5.6.4.3, as applicable (see 5.2).

5.6.4.2 Reference handrub procedure R

Pour 3 ml of propan-2-ol 60 % (V/V) (see 5.4.2.7) into the cupped dry hands and rub vigorously for 30 s onto the skin up to the wrists in accordance with the standard handrub procedure shown Figure A.1, to ensure total coverage of the hands.

²⁾ Disposable equipment is an acceptable alternative to reusable equipment.

This comprises five strokes backwards and forwards, palm to palm, right palm over left dorsum and left palm over right dorsum, palm to palm with fingers interlaced, back of fingers to opposing palms with fingers interlocked, rotational rubbing of right thumb clasped in left palm and left thumb clasped in right palm, rotational rubbing with clasped fingers of right hand in palm of left hand and clasped fingers of left hand in palm of right hand.

Repeat with a further 3 ml propan-2-ol, to give a total rubbing time of 60 s.

The reference procedure is completed by a 5 s rinse of the fingers under running tap water; excess water is shaken off.

5.6.4.3 Handrub procedure with test product (P)

This procedure is always performed according to information provided by the manufacturer, which shall include volume of product and frequency of application. The total rubbing time is limited to either 30 s or 60 s.

The procedure with the test product is completed by a 5 s rinse of the fingers under running tap water; excess water is shaken off.

5.6.5 Postvalues

Immediately afterwards, rub the fingertips and thumbtips on the base of a Petri dish (see 5.5.2.6) containing 10 ml TSB (see 5.4.2.4) containing neutralizer for 1 min. Use a separate Petri dish for each hand.

Spread 1,0 ml and 0,1 ml samples of the undiluted sampling fluid and 0,1 ml of a 10^{-1} dilution in TSB (see 5.4.2.4) containing neutralizer over the surface of TSA plates (see 5.4.2.3) using glass spreaders. If necessary, repeat with 0,1 ml of a 10^{-2} dilution. The interval between sampling and plating shall not exceed 30 min.

5.6.6 Incubation

Incubate all plates aerobically at $(36 \pm 1)^\circ\text{C}$ for 18 h to 24 h. Count the colony forming units and reincubate for a further 24 h to detect any slow growing colonies.

5.7 Calculation

Record the number of colony forming units (cfu) per plate for each dilution step. Calculate the dilution factor by multiplying the sample dilution and the sample volume (in millilitres). Calculate the number of cfu per millilitre of sampling fluid by multiplying the plate count (cfu) by the dilution factor.

NOTE 1 Whenever possible, the counts should be obtained from plates showing 15 to 300 colonies. With very efficient handrubs some counting plates for postvalues can show fewer than 15 colonies or no growth at all even if inoculated with 1 ml of undiluted sampling fluid (TSB, see 5.4.2.4). These values can then be accepted.

If suitable counts are obtained from two adjacent dilution steps, e.g. 299 colonies from 10^{-1} dilution and 31 from 10^{-2} dilution, the weighted arithmetic mean of both is calculated as follows:

$$\frac{(299 + 31) \text{ cfu}}{1,1 \times 10^{-1}} = 3\,000 \text{ cfu/ml undiluted sampling fluid}$$

NOTE 2 If colony counts of different dilution steps are grossly disproportional, insufficient neutralization of the antimicrobial agent should be suspected.

All viable counts per millilitre sampling fluid are transformed to decimal logarithms. For computational reasons values of 0 ($\log 0 = -\infty$) have to be set 1 ($\log 1 = 0$).

NOTE 3 Since 0-values should be found only among postvalues and should occur only with the most active products, this adjustment can, at the worst, introduce a conservative bias of underestimating the antimicrobial efficiency of a product.

For both reference and test procedure the log counts from right and left hands of each subject shall be averaged separately for prevalues and postvalues.

NOTE 4 This double weighting increases the precision of the measurement.

From the difference between this individual combined log prevalue and the log postvalue a log reduction factor is established for each subject.

Then, the two arithmetic means of all individual log reduction factors are calculated for both the reference and the test procedure.

If the data conforms to 5.8, compare the results for both procedures, P and R.

5.8 Test validation

The results of a test shall be accepted for further evaluation if they conform to the following criteria, otherwise the test shall be repeated.

Requirements for acceptance of test results are:

- a) All results from at least 12 subjects shall be available.
- b) The overall mean of the log prevalues for reference and test procedure(s) shall be at least 5,00.
- c) In each procedure, R and P, not more than three individual log reduction factors fewer than 3,00 shall occur.

5.9 Evaluation of P

If the quality of the data has been found acceptable (see 5.8), they shall be used for the evaluation of the antimicrobial efficacy of the product(s) under test by applying the following pass criterion:

- For any product, the mean log reduction factor obtained shall not be significantly smaller than that obtained for the reference propan-2-ol;
- If the mean log reduction factor of a test product is smaller than that of the reference propan-2-ol, the difference shall be tested for statistical significance (see 5.10);
- If the mean log reduction factor is significantly smaller than that obtained with the reference propan-2-ol, the test product does not conform to this standard.

5.10 Significance testing

NOTE Examples of significance testing are given in Table C.1 to Table C.5.

For testing the mean log RF of P against that of R, the Wilcoxon matched-pairs signed-ranks test shall be used (see Table C.4 and Table C.5).

For testing the data obtained in a Latin-square design experiment, the (k-1) sign test by Rhyne and Steel [5] shall be used, comparing means of more than one test procedure with that of the reference in a pairwise manner.

Because of the confirmative nature of the test in this application, the level of significance is set at $p = 0,1$. The test is to be used one-sided. The discrimination efficiency of the test procedure described has been set to detect a difference between the two mean log reduction factors of approximately 0,6 log at a power of 95 %.

5.11 Test report

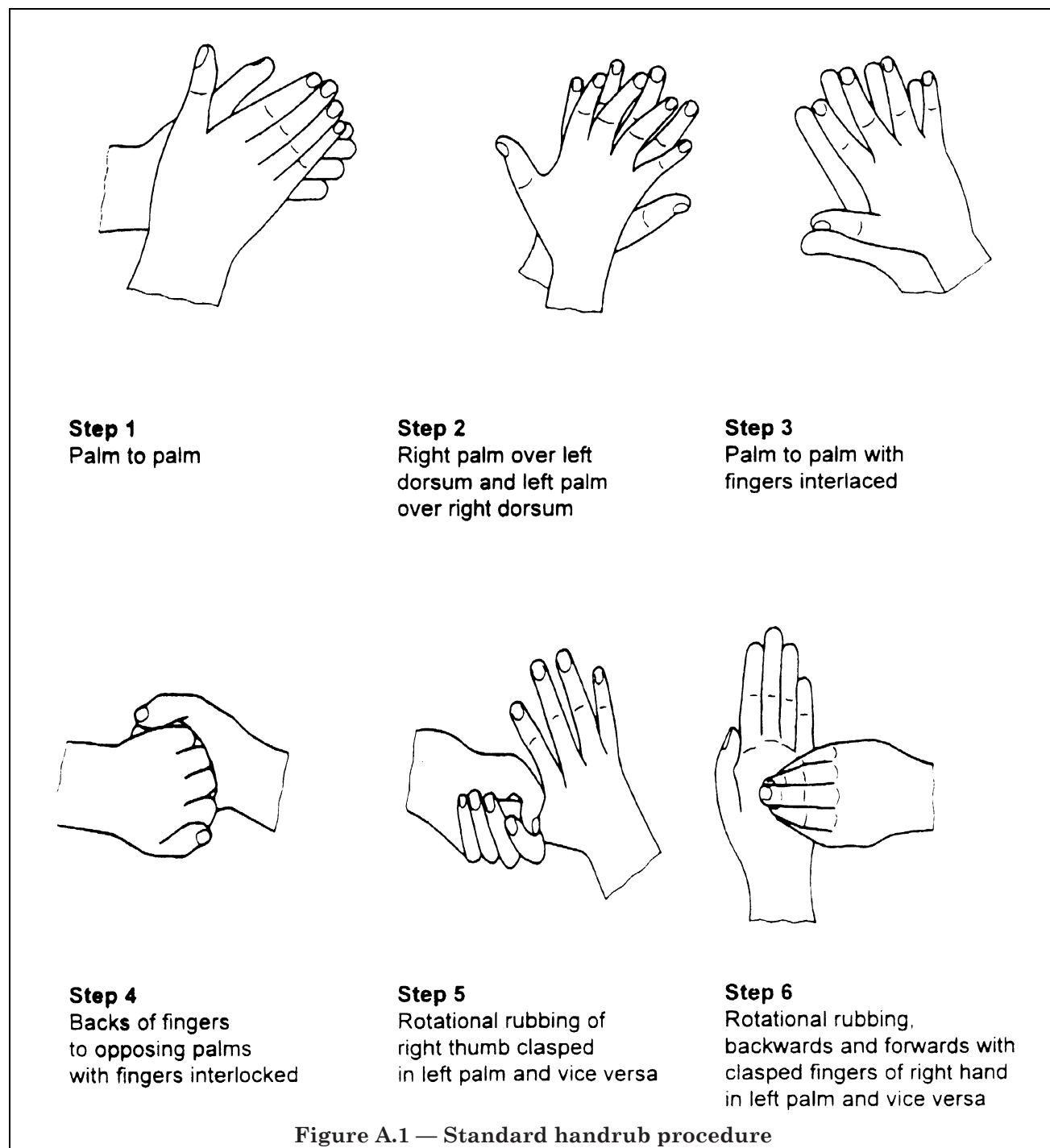
The report shall contain the following items:

- a) a reference to this European Standard;
- b) an exact description of the mode of application of P as detailed in 5.6.4.3 (volume, contact time, frequency of application);
- c) lists of experimental results for R and P (see Table C.1 and Table C.2) containing the colony counts found on the plates in relation to the respective dilution of the sampling fluid together with labels indicating which of the colony counts have been used for further calculation;
- d) a list of the processed log values, i.e. logarithms (see Table C.3) of left-right averaged and, when applicable, weighted viable counts per millilitre of sampling fluid as derived from the marked colony counts. This list contains the individual log prevalues and log postvalues and the log reduction factors for each subject separately for the reference and the test procedure as well as the overall means and standard deviations;
- e) a list comparing the individual log reduction factors of the reference procedure with those of the test procedure for an intra-individual comparison if significance testing is necessary, including the other components of Wilcoxon's matched pairs signed-ranks test such as the intra-individual differences of both log reduction factors, their rank and sign (+ or -) as well as both rank sums, T+ and T- (see Table C.5);
- f) a viable count of the contamination fluid;
- g) the composition of the neutralizer and results of its validation in the phase 2/step 1 suspension test method (see prEN 12054);
- h) a statement in conclusion to indicate whether the product conforms to this European Standard (see clause 4).

Annex A (normative)

Standard handrub procedure

Pour appropriate volume of handrub product, R or P, into the cupped dry hands and rub hands during 30 s in accordance with the standard handrub shown below to ensure total coverage of the hands. The action in each step is repeated five times before proceeding to the next step. After concluding step 6, recommence the series of steps as appropriate to complete the washing time as specified in 5.6.4.2 and 5.6.4.3.



Annex B (informative)

Examples of neutralizers

Table B.1 — Examples of neutralizers which can be incorporated in sampling fluids and diluents for the assessment of postvalues (see 5.6.5)

Disinfectant	Neutralizer (concentration per litre of sampling fluid or diluent)
chlorhexidine gluconate	polysorbate 80 ^a (30 ml) + lecithin from eggs (3 g) + histidine (1 g) (higher concentrations, up to five times, can be necessary for complete neutralization)
povidone iodine and chlorine compounds	polysorbate 80 ^a (30 ml) + lecithin from eggs (3 g) + histidine (1 g) + sodium thiosulfate (5 g) + bovine serum albumin, lyophilized (1 g)
phenolic compounds	polysorbate 80 ^a (30 ml) + lecithin from eggs (3 g) + histidine (1 g) + sodium thiosulfate (5 g)
quaternary ammonium compounds	polysorbate 80 ^a (30 ml) + saponin (30 g) + histidine (1 g) + cysteine (1 g)
^a Analytical grade, non-hydrolyzed in accordance with European Pharmacopoeia volume 1. TWEEN 80 [®] is an example of a suitable product available commercially. This information is given for the convenience of users of this standard and does not constitute an endorsement by CEN of this product.	

Annex C (informative)

Examples of reporting of results and significance testing

Table C.1 — Reference handrub procedure — experimental results

Preparation:		R [propan-2-ol 60 % (V/V)]				
Application:		rub-in 3 ml/30 s, repeat once				
Date of experiment:		15 October 1995				
Test organism:		E. coli K 12 NCTC 10538				
Suspension:		$2,14 \times 10^8$ cfu/ml				
No.	Subject Hand left or right	Number of cfu per plate from dilution 10^x				
		Prevalues		Postvalues		
		10^{-4}	10^{-5}	10^0	10^{-1}	10^{-2}
1	l	<u>66</u>	7	> 300	<u>47</u>	7
	r	<u>181*</u>	<u>25*</u>	<u>165</u>	13	2
2	l	> 300	<u>42</u>	<u>3</u>	0	0
	r	> 300	<u>59</u>	<u>1</u>	0	0
3	l	<u>174*</u>	<u>17*</u>	<u>269*</u>	<u>39*</u>	3
	r	<u>151*</u>	<u>17*</u>	<u>37</u>	5	0
4	l	> 300	<u>46</u>	<u>175*</u>	<u>25*</u>	2
	r	> 300	<u>34</u>	> 300	<u>45</u>	4
5	l	> 300	<u>46</u>	<u>93*</u>	<u>16*</u>	0
	r	> 300	<u>66</u>	<u>71</u>	7	1
6	l	<u>160</u>	13	> 300	<u>106</u>	12
	r	<u>67</u>	6	> 300	<u>47</u>	6
7	l	> 300	<u>50</u>	<u>71</u>	14	1
	r	> 300	<u>50</u>	<u>110</u>	13	0
8	l	<u>130</u>	7	<u>276*</u>	<u>25*</u>	2
	r	<u>148</u>	12	<u>85</u>	11	0
9	l	<u>78</u>	13	<u>260*</u>	<u>35*</u>	7
	r	<u>134</u>	9	> 300	40	3
10	l	<u>200*</u>	<u>21*</u>	<u>148*</u>	<u>25*</u>	4
	r	<u>38</u>	6	<u>189*</u>	<u>23*</u>	3
11	l	<u>151*</u>	<u>17*</u>	<u>5</u>	0	0
	r	<u>147</u>	9	<u>3</u>	0	0
12	l	<u>124*</u>	<u>17*</u>	> 300	<u>117</u>	8
	r	<u>163</u>	13	> 300	<u>40</u>	5
13	l	<u>9</u>	0	<u>77</u>	10	0
	r	<u>10</u>	0	<u>30</u>	6	2
14	l	<u>133*</u>	<u>18*</u>	<u>64</u>	12	0
	r	<u>163</u>	10	<u>12</u>	2	0
15	l	> 300	<u>38</u>	<u>14</u>	1	1
	r	<u>268*</u>	<u>18*</u>	> 300	<u>63</u>	0
underlined: count used for further computation						
*: use weighted mean						

Table C.2 — Handrub procedure with the test product — experimental results

Preparation:		P				
Application:		rub-in 3 ml/30 s, repeat once				
Date of experiment:		24 October 1995				
Test organism:		E. coli K 12 NCTC 10538				
Suspension:		$2,14 \times 10^8$ cfu/ml				
No.	Subject Hand left or right	Number of cfu per plate from dilution 10^x				
		Prevalues		Postvalues		
		10^{-4}	10^{-5}	10^0	10^{-1}	10^{-2}
1	l	> 300	<u>60</u>	> 300	<u>40</u>	2
	r	> 300	<u>33</u>	<u>69</u>	14	1
2	l	<u>230*</u>	<u>30*</u>	<u>17</u>	0	0
	r	<u>290*</u>	<u>42*</u>	<u>8</u>	0	0
3	l	<u>22</u>	2	<u>118</u>	14	1
	r	<u>27</u>	3	<u>90</u>	8	0
4	l	<u>279*</u>	<u>25*</u>	> 300	<u>40</u>	1
	r	<u>126*</u>	<u>22*</u>	> 300	<u>107</u>	14
5	l	<u>260*</u>	<u>23*</u>	<u>126*</u>	<u>28*</u>	4
	r	<u>214*</u>	<u>20*</u>	<u>166*</u>	<u>23*</u>	2
6	l	<u>137</u>	13	<u>95</u>	12	1
	r	<u>95</u>	10	<u>165*</u>	<u>18*</u>	1
7	l	<u>140*</u>	<u>20*</u>	<u>216*</u>	<u>28*</u>	4
	r	<u>150*</u>	<u>25*</u>	<u>180*</u>	<u>22*</u>	2
8	l	<u>292</u>	<u>29*</u>	> 300	<u>35</u>	4
	r	> 300	<u>55</u>	<u>278*</u>	<u>25*</u>	1
9	l	<u>238*</u>	<u>29*</u>	> 300	<u>113*</u>	<u>18*</u>
	r	> 300	<u>69</u>	n.a.	<u>245*</u>	<u>20*</u>
10	l	> 300	<u>81</u>	> 300	<u>85</u>	8
	r	> 300	<u>75</u>	> 300	<u>86</u>	14
11	l	> 300	<u>80</u>	<u>53</u>	8	1
	r	> 300	<u>45</u>	<u>37</u>	4	0
12	l	<u>174*</u>	<u>22*</u>	> 300	<u>144</u>	<u>15*</u>
	r	<u>171*</u>	<u>16*</u>	n.a.	> 300	<u>46</u>
13	l	<u>47</u>	4	<u>200*</u>	<u>34*</u>	0
	r	<u>56</u>	4	<u>205*</u>	<u>42*</u>	4
14	l	<u>133</u>	14	<u>211*</u>	<u>21*</u>	4
	r	<u>149*</u>	<u>19*</u>	<u>154</u>	14	1
15	l	> 300	<u>31</u>	<u>41</u>	4	0
	r	<u>189*</u>	<u>26*</u>	> 300	<u>39</u>	6
underlined: count used for further computation						
*: use weighted mean						
n.a.: not available						

Table C.3 — List of computed log values (means of left and right hand) and log reduction factors of the experimental results (Table C.1 and Table C.2)

Subject	Reference disinfection procedure (R) [propan-2-ol 60 % (V/V)]			Disinfection procedure with test product (P)		
	log x	log y	log z	log x	log y	log z
1	6,05	2,45	3,60	6,65	2,22	4,43
2	6,70	0,24	6,46	6,43	1,07	5,36
3	6,21	2,01	4,20	5,39	2,01	3,38
4	6,60	2,46	4,14	6,29	2,82	3,47
5	6,74	1,93	4,81	6,37	2,20	4,17
6	6,02	2,85	3,17	6,06	2,10	3,96
7	6,70	1,95	4,75	6,18	2,31	3,87
8	6,14	2,19	3,95	6,61	2,49	4,12
9	6,01	2,52	3,49	6,62	3,23	3,39
10	5,94	2,25	3,69	6,90	2,94	3,96
11	6,18	0,59	5,59	6,78	1,65	5,13
12	6,16	2,84	3,32	6,24	3,41	2,83
13	4,98	1,69	3,29	5,71	2,34	3,37
14	6,18	1,45	4,73	6,15	2,26	3,89
15	6,39	1,97	4,42	6,39	2,10	4,29
$\bar{x}$	6,20	1,94	4,24	6,32	2,34	3,98
s	0,43	0,74	0,92	0,40	0,59	0,67
N	15	15	15	15	15	15
log x: log prevalue $\bar{x}$: overall mean of log x, log y and log z log y: log postvalue s : standard deviation log z: log reduction factor N: number of values (= subjects) in each column						

**Table C.4 — Statistical comparison of values as obtained with R and P
(WILCOXON's matched-pairs signed-ranks test)**

Subject	log RF derived from		Difference R – P	Rank of difference	
	R	P		without sign	with sign
1	3,60	4,43	– 0,83	12	– 12
2	6,46	5,36	1,10	15	+ 15
3	4,20	3,38	0,82	11	+ 11
4	4,14	3,47	0,67	9	+ 9
5	4,81	4,17	0,64	8	+ 8
6	3,17	3,96	– 0,79	10	– 10
7	4,75	3,87	0,88	14	+ 14
8	3,95	4,12	– 0,17	4	– 4
9	3,49	3,39	0,10	2	+ 2
10	3,69	3,96	– 0,27	5	– 5
11	5,59	5,13	0,46	6	+ 6
12	3,32	2,83	0,49	7	+ 7
13	3,29	3,37	– 0,08	1	– 1
14	4,73	3,89	0,84	13	+ 13
15	4,42	4,29	0,13	3	+ 3
RF: reduction factor Sum of ranks (+): 88 Sum of ranks (–): 32					

Compare smaller sum of ranks (here 32) with tabulated values from the WILCOXON table (see Table C.5) for $n = 15$ at level of significance $p = 0,1$ ($= 36$).

If calculated smaller sum of ranks (here 32) ≤ 36 , then P has to be rejected as significantly less effective.

Table C.5 — WILCOXON's matched-pairs signed-ranks test: critical values of the lower of both sums of ranks with (+) or (–) sign at different significance levels

n (number of pairs with difference $\neq 0$)	Level of significance (directional test)		
	0,1	0,05	0,01
12	12	17	9
13	26	21	12
14	31	25	15
15	36	30	19

The difference is significant at the indicated level if the calculated value equals the tabulated value or is lower.

Annex D (informative)

Bibliography

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Annex E (informative)

Information on the application and interpretation of European Standards on chemical disinfectants and antiseptics

CEN/TC 216 would like to draw the attention of the reader of this standard to the agreements which were reached concerning the relationship between this standard and future standards.

This information should be followed when using the European Standards on chemical disinfectants and antiseptics:

E.1 General guidelines for the application and interpretation of test methods in accordance with European Standards for chemical disinfectants and antiseptics

a) All use recommendations for chemical disinfectant and antiseptic products should be supported by results of bactericidal, fungicidal, sporicidal and virucidal EN tests which are appropriate to the intended field and method of application.

b) To achieve this, chemical disinfectant and antiseptic products should be subjected to a specified programme of testing which will include phase 1, phase 2/step 1 and phase 2/step 2 tests, except for situations as given in points e), f) and g).

c) Use recommendations may be supported by results of phase 3 tests which are appropriate to the intended field and method of application.

d) The various phases and steps are defined as follows:

- phase 1 suspension test for the basic activity of the product;
- phase 2/step 1 suspension test under conditions representative of practical use;
- phase 2/step 2 other laboratory tests e.g. handwash, handrub and surface tests simulating practical conditions;
- phase 3 field tests under practical conditions.

e) It is accepted that for certain applications, the phase 2/step 1 and phase 2/step 2 tests may provide sufficient information for the particular application and that additional phase 1 tests may not be relevant.

For applications where phase 2/step 1 and phase 2/step 2 tests without phase 1 tests are used to support use recommendations, the justification for omitting phase 1 tests should be given. Such applications will be indicated either in the standard itself or in the additional standard which specifies guidelines for the application and interpretation of the tests.

f) It is accepted that for certain applications, the phase 2/step 1 suspension tests may provide sufficient information for the particular application and that additional phase 2/step 2 tests may not be relevant.

For applications where phase 2/step 1 tests without phase 2/step 2 tests are used to support use recommendations, the justification for omitting phase 2/step 2 tests should be given. Such applications will be indicated either in the standard itself or in the additional standard which specifies guidelines for the application and interpretation of the tests.

g) It is accepted that for certain applications the phase 2/step 2 together with phase 1 tests may provide sufficient information for the particular application and that additional phase 2/step 1 tests may not be relevant.

For applications where phase 2/step 2 tests without phase 2/step 1 tests are used to support product claims, the justification for omitting phase 2/step 1 tests should be given. Such applications may be indicated either in the standard method or in the standard for the application and interpretation of the tests.

h) All bactericidal, fungicidal and sporicidal claims for bioactive substances should be supported by appropriate phase 1 tests.

E.2 Guide to the interpretation of tests for chemical disinfectants and antiseptics

A separate standard (or standards) which will be used as a Guide to interpretation of tests for chemical disinfectants and antiseptics will be prepared after the standard test methods have been agreed; the purpose of this standard will be to specify in detail the relationship of the various tests to one another and to use recommendations.

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