



BSI Standards Publication

**Chemical disinfectants and antiseptics —
Quantitative suspension test for the evaluation
of virucidal activity in the medical area — Test
method and requirements (Phase 2/Step 1)**

National foreword

This British Standard is the UK implementation of EN 14476:2025. It supersedes BS EN 14476:2013+A2:2019, which is withdrawn.

The UK participation in its preparation was entrusted to Technical Committee CH/216, Chemical disinfectants and antiseptics.

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

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European foreword

This document (EN 14476:2025) has been prepared by 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of an identical text or by endorsement, at the latest by April 2026, and conflicting national standards shall be withdrawn at the latest by April 2026.

Attention is drawn to the possibility that some of the elements of this document may be the subject of patent rights. CEN shall not be held responsible for identifying any or all such patent rights.

This document supersedes EN 14476:2013+A2:2019.

This document was revised to adapt it to the latest state of science, to correct errors and ambiguities, to harmonize the structure and wording with other existing tests of CEN/TC 216 or in preparation and to improve its readability and thereby make it more understandable.

EN 14476:2025 includes the following significant technical changes with respect to EN 14476:2013+A2:2019:

- de-harmonization of the standard (Annex ZA and references to MDR/MDD deleted);
- for textile disinfection and instrument disinfection a test for virucidal activity against enveloped viruses with vaccinia virus has been added;
- Glutaraldehyde has been added as an alternative reference substance to formaldehyde;
- for chemothermal disinfection e.g. textile disinfection peracetic acid has been added as reference substance;
- for the hygienic handrub and handwash claims, a test for virucidal activity against enveloped viruses with vaccinia virus was added with specific lg reduction requirement for handwash products;
- the calculation was shifted to the main text (harmonized with EN 13727);
- the LVP method was shifted to the main text;
- it was clarified that given cell line numbers are only examples;
- for hygienic handrubs, adenovirus shall be tested at $(25 \pm 1) ^\circ\text{C}$;
- spelling errors and incorrect references were corrected.

The changes of this revision have no impact on the test results obtained with reference to the version EN 14476:2013+A2:2019. Those results are still valid with the exception of test reports using gel filtration detoxification, which do not provide a parallel titration of the non-filtrated test mixture. Additional comparison between unfiltered and filtrated test mixture shall be added in the test report using gel filtration detoxification.

Test results for hygienic handrub products tested at $20 ^\circ\text{C}$ with adenovirus in accordance with the previous version are still valid.

Other methods to evaluate the efficacy of chemical disinfectants and antiseptics for different applications in the medical area are in preparation.

Any feedback and questions on this document should be directed to the users' national standards body. A complete listing of these bodies can be found on the CEN website.

According to the CEN-CENELEC Internal Regulations, the national standards organisations of the following countries are bound to implement this European Standard: Austria, Belgium, Bulgaria, Croatia, Cyprus, Czech Republic, Denmark, Estonia, Finland, France, Germany, Greece, Hungary, Iceland, Ireland, Italy, Latvia, Lithuania, Luxembourg, Malta, Netherlands, Norway, Poland, Portugal, Republic of North Macedonia, Romania, Serbia, Slovakia, Slovenia, Spain, Sweden, Switzerland, Türkiye and the United Kingdom.

Introduction

This document specifies a suspension test for establishing whether a chemical disinfectant or an antiseptic has a virucidal activity in the area and fields described in the scope.

This laboratory test takes into account practical conditions of application of the product including contact time, temperature, test organisms and interfering substances, i.e. conditions which can influence its action in practical situations. Each utilization concentration of the chemical disinfectant or antiseptic found by this test corresponds to the chosen experimental conditions.

1 Scope

This document specifies a test method and the minimum requirements for virucidal activity of chemical disinfectant and antiseptic products that form a homogeneous physically stable preparation when diluted with hard water – or in the case of ready-to-use products, i.e. products that are not diluted when applied, – with water. Ready-to-use-products can only be tested at a concentration up to 80 % (97 %, with a modified method for special cases) as some dilution is always produced by adding the test organisms and interfering substance.

This document is applicable to products that are used in the medical area in the fields of hygienic handrub, hygienic handwash, instrument disinfection by immersion, surface disinfection by wiping, spraying, flooding or other means and textile disinfection.

This document is applicable to 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 can occur in the workplace and in the home. It can also include services such as laundries and kitchens supplying products directly for the patient.

NOTE 1 The method described is intended to determine the activity of commercial formulations or active substances under the conditions in which they are used.

NOTE 2 This method corresponds to a phase 2, step 1 test.

NOTE 3 EN 14885 specifies in detail the relationship of the various tests to one another and to “use recommendations”.

2 Normative references

The following documents are referred to in the text in such a way that some or all of their content constitutes requirements of this document. For dated references, only the edition cited applies. For undated references, the latest edition of the referenced document (including any amendments) applies.

EN 12353,¹ *Chemical disinfectants and antiseptics — Preservation of test organisms used for the determination of bactericidal (including Legionella), mycobactericidal, sporicidal, fungicidal and virucidal (including bacteriophages) activity*

EN 14885,¹ *Chemical disinfectants and antiseptics — Application of European Standards for chemical disinfectants and antiseptics*

¹ EN 12353, and EN 14885 are updated on an ongoing basis as the standards to which they refer are developed and evolve. As the revision of both standards is much more frequent than the updating of this standard, the standards are not dated. Users should refer to the latest version of the referenced standards.

3 Terms and definitions

For the purposes of this document, the terms and definitions given in EN 14885 and the following apply.
ISO and IEC maintain terminology databases for use in standardization at the following addresses:

- IEC Electropedia: available at <https://www.electropedia.org/>
- ISO Online browsing platform: available at <https://www.iso.org/obp>

3.1

cytotoxicity

morphological alteration of cells and/or their destruction caused by the product

3.2

plaque forming units

PFU

number of infectious virus particles per unit volume

Note 1 to entry: The unit volume is given in ml.

3.3

reference test for virus inactivation

test with a defined reference product in parallel with a product under test for the internal control of the test and to determine the stability of the test organism

Note 1 to entry: As defined reference product e.g. glutaraldehyde or formaldehyde could be used.

3.4

TCID₅₀

50 % infecting dose of a virus suspension or that dilution of the virus suspension that induce a CPE (3.5) in 50 % of cell culture units

3.5

viral cytopathic effect

CPE

morphological alteration of cells and/or their destruction as a consequence of virus multiplication

3.6

viral plaque

area of lysis formed in a cell monolayer under semisolid medium due to infection by and multiplication of a single infectious virus particle

3.7

virus titre

amount of infectious virus per unit volume present in a cell culture lysate, cell culture supernatant or in a solution

Note 1 to entry: The unit volume is given in ml.

4 Requirements

The product shall demonstrate at least a decimal log (lg) reduction of 4 in virus titre (for hygienic handwash at least a 2 lg reduction) when tested in accordance with Table 1 and Clause 5.

Table 1 — Minimum and additional test conditions

Test conditions	Hygienic handrub	Hygienic handwash	Instrument disinfection	Surface disinfection	Textile disinfection
Minimum spectrum of test organisms	Virucidal activity poliovirus adenovirus murine norovirus		Virucidal activity poliovirus adenovirus murine norovirus when temperature is 40 °C or higher: only murine parvovirus	Virucidal activity poliovirus adenovirus murine norovirus	Virucidal activity murine parvovirus
	Limited spectrum virucidal activity^a adenovirus murine norovirus			Limited spectrum virucidal activity^a adenovirus murine norovirus	
	Virucidal activity against enveloped viruses^b vaccinia virus	Virucidal activity against enveloped viruses^b vaccinia virus	Virucidal activity against enveloped viruses^b (Pre-cleaning products with a combined cleaner/disinfectant) vaccinia virus	Virucidal activity against enveloped viruses^b vaccinia virus	Virucidal activity against enveloped viruses^b vaccinia virus
additional	Any relevant test organism				
Test temperature	according to the manufacturer's recommendation, but at / between				
	(20 ± 1) °C [alcohol dis. with adenovirus at (25 ± 1) °C] ^c	(20 ± 1) °C	20 °C and 70 °C ^d	4 °C and 30 °C	30 °C and 70 °C ^d
Contact time	according to the manufacturer's recommendation				
	but between 30 s and 120 s	but between 30 s and 120 s	but no longer than 60 min	but no longer than 60 min	but between 10 min and 20 min

Test conditions	Hygienic handrub	Hygienic handwash	Instrument disinfection	Surface disinfection	Textile disinfection
Interfering substance					
clean conditions	0,3 g/l bovine albumin solution (hygienic handrub)	–	0,3 g/l bovine albumin solution and/or	0,3 g/l bovine albumin solution and/or	0,3 g/l bovine albumin solution (for pre-washed process)
dirty conditions	–	3,0 g/l bovine albumin solution plus 3,0 ml/l erythrocytes (hygienic handwash)	3,0 g/l bovine albumin solution plus 3,0 ml/l erythrocytes	3,0 g/l bovine albumin solution plus 3,0 ml/l erythrocytes	3,0 g/l bovine albumin solution plus 3,0 ml/l erythrocytes (for non-pre-washed process)
Additional conditions	dirty any relevant substance	any relevant substance	any relevant substance	any relevant substance	any relevant substance
^a The test for “limited spectrum virucidal activity” will cover all enveloped viruses (Annex A) and norovirus, rotavirus and adenovirus. ^b The test for “virucidal activity against enveloped viruses” will cover all enveloped viruses only (Annex A). ^c For hand rubs, adenovirus shall be tested only at $(25 \pm 1) ^\circ\text{C}$. It has long been known that adenoviruses, unlike other non-enveloped viruses (polio + enteroviruses), exhibit a temperature effect in alcoholic formulations. [16], [17] This is most likely due to the special structure with antenna-like fibre proteins, which could cause precipitation of the virus, which then dissolves again at $(37 \pm 1) ^\circ\text{C}$. The viruses are then released into the solution. Thus, the hand disinfectant appears to be false-negative ineffective, although only a few degrees difference makes the viruses capable of inactivation again in this artificial suspension test. $25 ^\circ\text{C}$ is still in the range of the hand skin temperature. ^d For further information regarding validity at elevated temperatures see EN 14885.					

5 Test methods

5.1 Principle

5.1.1 A sample of the product as delivered and/or diluted with hard water (or water for ready to use products) is added to a test virus suspension in a solution of an interfering substance. The mixture is maintained at one of the temperatures and the contact times specified in Clause 4 and 5.5.1.1. At the end of this contact time, an aliquot is taken; the virucidal action in this portion is immediately suppressed by a validated method (dilution of the sample in ice-cold cell maintenance medium). The dilutions are transferred into cell culture units i.e. wells of microtitre plates either using monolayer or cell suspension. Infectivity tests are done either by plaque test or quantal tests. After incubation, the titres of infectivity are calculated according to Spearman and Kärber (quantal tests, 5.6.2.1), by plaque counting (plaque test, 5.6.2.5) or large-volume-plating (LVP) method (5.6.2.6) and evaluated. Reduction of virus infectivity is calculated from differences of lg virus titres without (virus control) and after treatment with the product.

Handwash products are always prediluted with hard water (5.2.2.7). The resulting solution is regarded as a ready-to-use product (5.4.2).

5.1.2 The test is performed using the test organisms as specified in Clause 4, Table 1.

5.1.3 Other contact times and temperatures within the limits specified in Clause 4, Table 1 may be used. Additional interfering substances and test organisms may be used.

5.2 Materials and reagents, including cell cultures

5.2.1 Test viruses

The virucidal activity shall be evaluated using the following strains as test organisms selected according to Clause 4, Table 1. Virus strains shall be obtained from a national or international culture collection²:

a) non-enveloped RNA virus³:

1) poliovirus type 1, LSc 2ab (Picornavirus);

Regarding poliovirus only virus material that passed the requirements for the production of oral polio vaccine of the competent authority shall be used (Other stocks derived from LSc-2ab cannot be used any longer).

NOTE 1 See the list of vaccines of the World Health Organisation (WHO).

NOTE 2 See also WHO Global Action Plan for Poliovirus Containment.

2) murine norovirus, strain S99 Berlin;

b) non-enveloped DNA virus:

1) adenovirus type 5, strain Adenoid 75, ATCC VR-5;

2) murine parvovirus, minute virus of mice, strain Crawford, ATCC VR-1346;

c) enveloped DNA virus:

1) modified vaccinia virus Ankara (MVA), ATCC VR-1508, or vaccinia virus strain Elstree, ATCC VR-1549.

The required incubation temperature for these test organisms is $(36 \pm 1) ^\circ\text{C}$ or $(37 \pm 1) ^\circ\text{C}$ (5.3.1.3). The same temperature (either $36 ^\circ\text{C}$ or $37 ^\circ\text{C}$) shall be used for all incubations performed during a test and its control and validation.

If additional test organisms are used, they shall be kept and used under optimum growth conditions (temperature, time, atmosphere, media, susceptible cell line) noted in the test report. If these additional test organisms (viruses and cell line) are not classified at a reference centre, their identification characteristics shall be stated. In addition, they shall be held by the testing laboratory or national culture collection under a reference for five years.

² The ATCC numbers are the collection numbers of strains supplied by these culture collections. This information is given for the convenience of users of this document and does not constitute an endorsement by CEN.

³ Poliovirus type 1, LSc-2ab can be obtained either from NIBSC (<https://nibsc.org/>) or Friedrich-Loeffler-Federal Research Institute for Animal Health, Hauptsitz Insel Riems Südufer 10, 17493 Greifswald-Insel Riems; phone: +49 38351 7-0; fax: +49 38351 7-121. <http://www.fli.de/>. Murine norovirus can be obtained from Friedrich-Loeffler-Federal Research Institute for Animal Health. This information is given for the convenience of users of this document and does not constitute an endorsement by CEN.

5.2.2 Culture media, reagents and cell cultures

5.2.2.1 General

All weights of chemical substances given in this document refer to the anhydrous salts. Hydrated forms may be used as an alternative, but the weights required shall be adjusted to allow for consequent molecular weight differences.

The reagents shall be of analytical grade and/or appropriate for microbiological purposes. They shall be free from substances that are toxic or inhibitory to the test organisms.

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

For each culture medium and reagent, a time limitation for use should be fixed.

All specified pH values are measured at $(20 \pm 1) ^\circ\text{C}$.

5.2.2.2 Water

The water shall be freshly glass-distilled water or demineralized water, which meets the requirements of ASTM D 1193 (Type I or II, see Table 2) or use water for injections (see bibliographic reference [1]).

Sterilize in the autoclave [5.3.1.1 a)]. Sterilization is not necessary if the water is used e.g. for preparation of culture media and subsequently sterilized. Alternatively, cell culture grade water is suitable for preparation of cell culture media and other solutions used in cell culture.

Table 2 — Consolidated classification of water qualities

Characteristic	Unit	Type I (ultrapure water)	Type II (purified water)
Resistance	$(\text{M/cm})\Omega$	> 18	> 1
Conductivity	$\mu\text{S/cm}$	< 0,056	< 1
Silicates(SiO_4)	ppb or $\mu\text{g/L}$	3	3
TOC	ppb or $\mu\text{g/l}$	50	50
Bacteria	CFU/ml	< 1	< 100
Endotoxin	EU/ml	< 0,001	0,03
Type I	Required for critical laboratory applications (e.g. for HPLC, GC, MS etc.). Also, for the production of buffers for cell culture, as well as for applications in molecular biology.		
Type II	Used for buffers and microbiological culture media. Used as system water for laboratory automats and for dissolving controls and reagents		

See 5.2.2.7 for the procedure to prepare hard water.

5.2.2.3 Phosphate buffered saline (PBS)

Sodium chloride (NaCl)	8,00 g
Potassium chloride (KCl)	0,20 g
Disodium hydrogen phosphate, 12-hydrate ($\text{Na}_2\text{HPO}_4 \times 12\text{H}_2\text{O}$)	2,89 g
Potassium phosphate, monobasic (KH_2PO_4)	0,20 g
Water (5.2.2.2)	to 1 000,0 ml

5.2.2.4 Neutral Red (1:1 000 solution)

Prepare neutral red (e.g. Sigma N7005⁴) stock solution at 0,1 mg/ml in water (5.2.2.2). Filter through a 0,45 µm pore size filter and store at $(4 \pm 1)^\circ\text{C}$ in the dark.

5.2.2.5 Foetal calf serum (FCS)

FCS shall be certified free of viruses and mycoplasma. Extraneous viruses and mycoplasma may interfere with cell and virus growth resulting in false results.

For RAW 264.7 cells, low endotoxin FCS shall be used due to the cells' high sensitivity to endotoxins.

5.2.2.6 Trichloroacetic acid (10 % solution) (TCA)

Dissolve 10 g of TCA crystals in 80 ml of water (5.2.2.2), then adjust the volume to 100 ml with water. Stir to complete solution.

5.2.2.7 Hard water for dilution of products

For the preparation of 1 l of hard water, the procedure is as follows:

- prepare solution A: dissolve 19,84 g magnesium chloride (MgCl_2) and 46,24 g calcium chloride (CaCl_2) in water (5.2.2.2) and dilute to 1 000 ml. Sterilize by membrane filtration (5.3.1.7) or in the autoclave [5.3.1.1 a)]. Autoclaving – if used - may cause a loss of liquid. In this case make up to 1 000 ml with water (5.2.2.2) under aseptic conditions. Store the solution in the refrigerator (5.3.1.8) for no longer than one month;
- prepare solution B: dissolve 35,02 g sodium bicarbonate (NaHCO_3) in water (5.2.2.2) and dilute to 1000 ml. Sterilize by membrane filtration (5.3.1.7). Store the solution in the refrigerator (5.3.1.8) for no longer than one week;
- place 600 ml to 700 ml of water (5.2.2.2) in a 1 000 ml volumetric flask (5.3.1.12) and add 6,0 ml (5.3.1.9) of solution A, then 8,0 ml of solution B. Mix and dilute to 1 000 ml with water (5.2.2.2). The pH (5.3.1.4) of the hard water shall be $7,0 \pm 0,2$ (5.3.1.4). If necessary, adjust the pH by using a solution of approximately 40 g/l (about 1 mol/l) of sodium hydroxide (NaOH) or approximately 36,5 g/l (about 1 mol/l) of hydrochloric acid (HCl).

The hard water shall be freshly prepared under aseptic conditions and used within 12 h.

NOTE When preparing the product test solutions (5.4.2), the addition of the product to the hard water produces different final water hardness in each test tube. In any case, the final hardness in the test tube expressed as calcium carbonate (CaCO_3) is lower than 375 mg/l.

⁴ Sigma N 7005 is an example of a suitable product available commercially. This information is given for the convenience of users of this document and does not constitute an endorsement by CEN of this product.

5.2.2.8 Interfering substance

5.2.2.8.1 General

The interfering substance shall be chosen according to the conditions of use laid down for the product.

The interfering substance shall be sterile and prepared at 10 times its final concentration in the test (50 times in the case of the modified method, 5.2.2.8.4).

The ionic composition (e.g. pH, calcium and/or magnesium hardness) and chemical composition (e.g. mineral substances, protein, carbohydrates, lipids and detergents) shall be specified.

NOTE 1 “Diluent” is generally used in the other European Standards in the medical area to prepare the interfering substance. Since there is no experience in virucidal testing with diluent, water (5.2.2.2) is used instead.

NOTE 2 The term “interfering substance” is used even if it contains more than one substance.

5.2.2.8.2 Clean conditions (bovine albumin solution – low concentration)

Dissolve 0,30 g of bovine albumin fraction V (suitable for microbiological purposes) in 100 ml of water (5.2.2.2).

Sterilize by membrane filtration (5.3.1.7), keep in a refrigerator (5.3.1.8) and use within one month.

The final concentration of the bovine albumin in the test procedure (5.5) shall be 0,3 g/l.

5.2.2.8.3 Dirty conditions (Mixture of bovine albumin solution – high concentration with sheep erythrocytes)

Dissolve 3,00 g of bovine albumin fraction V (suitable for microbiological purposes) in 97 ml of water (5.2.2.2).

Sterilize by membrane filtration (5.3.1.7).

Prepare at least 8,0 ml fresh defibrinated sheep blood (5.2.2.9). Centrifuge the erythrocytes at 800 g_N for 10 min (5.3.1.13). After discarding the supernatant, resuspend erythrocytes in PBS (5.2.2.3). Repeat this procedure at least 3 times, until the supernatant is colourless.

Resuspend 3 ml of the packed sheep erythrocytes in the 97 ml of sterilized bovine albumin solution (see above). To avoid later contamination this mixture should be split in portions probably needed per day. Keep the mixture in separate containers for a maximum of 7 d in a refrigerator (5.3.1.8).

The final concentration of bovine albumin and sheep erythrocytes in the test procedure (5.5) shall be 3 g/l and 3 ml/l respectively.

5.2.2.8.4 Clean and dirty conditions for the modified method for ready-to-use products (5.5.3)

Follow the procedures for preparation according to 5.2.2.8.2 and 5.2.2.8.3, but prepare the interfering substance in fivefold higher concentrations, for the dirty conditions maximum 50 ml to avoid problems with the filtration.

- a) Clean conditions (5.2.2.8.2) – dissolve 1,50 g bovine albumin (instead of 0,3 g) in 100 ml of water (5.2.2.2);
- b) dirty conditions (5.2.2.8.3) – dissolve 7,5 g bovine albumin (instead of 1,5 g) in 42,5 ml (instead of 48,5 ml) of water (5.2.2.2). Prepare at least 20 ml (instead of 4,0 ml) sheep blood. Resuspend 7,5 ml (instead of 1,5 ml) of the packed sheep erythrocytes in 42,5 ml of sterilized bovine albumin solution to obtain 50 ml.

5.2.2.9 Defibrinated sheep blood

The defibrinated sheep blood shall be sterile (aseptic blood-letting and preparation). The defibrinated sheep blood can be pooled from more than one sheep and can be acquired from a commercial supplier.

5.2.2.10 Medium for cell cultures

Eagle's minimal essential medium (MEM) or equivalent, supplemented with FCS (5.2.2.5), antibiotics, and other growth factors as needed shall be used.

- a) A growth medium for cell multiplication is generally supplemented with 10 % FCS. Add 10 parts of FCS (5.2.2.5) to 90 parts of MEM.
- b) A maintenance medium to maintain the cell culture metabolism without stimulation of cell proliferation is generally supplemented with 2 % FCS. Add 2 parts of FCS (5.2.2.5) to 98 parts of MEM.

Other media may be used if appropriate for certain cell lines.

See EN 12353 for a detailed description.

5.2.2.11 Cell cultures

Before virus inoculation, cell monolayers shall be at appropriate level of confluence based on the specific virus (see also EN 12353). Cell lines are selected in accordance with their sensitivity to the test organisms (5.2.1). Cells for virus titration, if used as suspensions in quantal tests, shall be added to the dilutions of the test mixture (5.5.2) in such a density as to enable the formation of a monolayer in at least two days in the cell control. Cell cultures can be used as cell monolayers or in suspensions for quantal tests. For details of cell lines see 5.5.1.1 e).

5.2.2.12 Reference substances

5.2.2.12.1 Glutaraldehyde (Glutaral, 1,5-Pentanedial) CAS Number 111-30-8

Required chemical and physical parameters for use as reference standard for testing disinfectant preparations are specified in Table 3.

Table 3 — Required chemical and physical parameters

Parameters	Specifications	
	Solution 1 %	Solution 25 %
Appearance ^a	clear liquid	clear liquid
pH-value	3,1 to 4,5 *	3,1 to 4,5
Concentration of glutaraldehyde (by titration)	50,0 % to 52,0 %	25,0 % to 26,0 %
Stability (ambient conditions)	12 months	12 months
* The pH value of the 1 % solution was in a ring trial $3,78 \pm 0,5$.		
^a Yellowish colour indicates a beginning polymerization.		

The specification above (see Table 3) should be checked on the certificate of analyses. The pH values shall be tested and confirmed regularly by the laboratory. If the pH values and / or appearance are out of specification the glutaraldehyde shall not be used.