TEST REPORT

Report No.: ZC200322330/PHY

EN 14885:2018

Chemical disinfectants and antiseptics- Application of European Standards for chemical disinfectants and antiseptics

Applicant	:	Ningbo Chenxing Daily Necessities Co., Ltd.
Address	:	No.66, Dongfeng Road, Fenghua District, Ningbo City, Zhejiang Province, China
Product(s)	:	Antibacterial wet wipes
Model(s)	:	CXKJ01
Standard(s)	:	EN 14885-2018 EN 13704:2018

TEST REPORT FOR COMPLIANCE WITH

EN 14885:2018

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Model / Specification	CXKJ01	
Test Report No.	ZC200322330/PHY	
Standards Compliance	EN 14885:2018 EN 13704:2018	
Date of Testing	2020.2.29-2020.3.22	
Testing Laboratory	Zuoce Certification and Testing Center. ROOM 318 Building 6, No.26 Hexuan Road, Jiading District, Shanghai, China, 201803 Tel: 086-21-39922156 Email: info@zuoce.org	
Tested by	**Stone Lee**	Stone Lee
Approved by	**Jack Yang**	Jack Yang

ZUOCE CERTIFICATION & TESTING CENTER CHINA BRANCH

Clause	Requirement-Test	Result-Remark	Verdict
4	**Procedures for claiming activity**		
4.1	**Category of tests**		
	The tests are categorized on a modular basis as follows:		
	— **Phase 1 tests** are quantitative suspension tests to establish that active substances or products under development have bactericidal, fungicidal or sporicidal activity without regard to specific areas of application. Phase 1 tests cannot be used for any product claim.		N/A
	— **Phase 2** comprises two steps:		
	a) **Phase 2, step 1 tests** are quantitative suspension tests to establish that a product has bactericidal, fungicidal, yeasticidal, mycobactericidal, tuberculocidal, sporicidal or virucidal activity under simulated practical conditions appropriate to its intended use;	See Appendix 1	P
	b) **Phase 2, step 2 tests** are quantitative laboratory tests to establish that a product has bactericidal, fungicidal, yeasticidal, mycobactericidal, tuberculocidal, sporicidal or virucidal activity when applied to a surface or skin under simulated practical conditions (e.g. surface,instrument, handwash and handrub tests);		N/A
	— **Phase 3 tests** are field tests under practical conditions. Applicable methodologies for this type of test are not yet available, but may be developed in the future. Guidance on the design of phase 3 tests and the use of data from phase 3 tests is provided in Annex C.		N/A
	NOTE In the following phase 2, step 1 is mostly shortened to “2,1” or “2/1” and phase 2, step 2 to “2,2” or “2/2”.		

	The phase 2, step 1 tests prove the irreversible inactivation of microorganisms. This test design provides relevant information about the activity of the product against microorganisms in suspension.Desiccated microorganisms may be stressed and may offer different challenges.		P
	Phase 2, step 2 tests provide information about the activity against desiccated microorganisms on inanimate surfaces or on living tissues or against non-desiccated microorganisms on living tissues.		N/A
4.2	**General**		
4.2.1	In order to determine that an active substance or a product under development has microbicidal properties, it shall be tested in accordance with and shall conform to the relevant test conditions and requirements of the European phase 1 standards.		P
4.2.2	For the medical area see 4.3, for the veterinary area see 4.4, for the food, industrial, domestic and institutional areas see 4.5. The standards specified in 4.3, 4.4 or 4.5 may be used to support product claims of activity/conformity to this European Standard on the basis of criteria specified in those standards (minimum requirements, obligatory and/or specified additional conditions).	The food, industrial, domestic and institutional areas.	P
4.2.3	When recommendations for use are made based on the standards referenced in EN 14885 these shall be supported by test results relevant for this recommendation, e.g. a result for 30 min contact time does not allow a claim for 10 min (but a result for 10 min allows a claim for 30 min if the same product concentration is recommended for use). It is not possible to extend or shorten the time for		P

	use beyond the limits (i.e. the minimum and maximum additional contact times in the medical, veterinary, food,industrial, domestic and Ainstitutional areas) specified in standards referred to in EN 14885.		
4.2.4	The product marketed shall be equivalent to the one tested. Equivalent means that it contains the same active substances in the same quantity and that only substances of no proven impact on the product's activity such as fragrance or colouring are non-identical.		P
4.2.5	Where there is no appropriate standard for an application within a specific area, a standard from another area may be recommended for use. If later on an appropriate standard is published, this new standard shall be used.		P
4.2.6	Where in EN 14885 no standard exists for a specific activity in an area (e.g. medical), a standard from another area (e.g. veterinary) may be used and test conditions modified for relevance to the area of application to match the specific application. In certain cases it may be necessary or recommendable to modify even the test organism(s) to match the requirements of the area. These choices shall be scientifically justified taking into account the field of application and the intended use of the product. In the test report the European Standard shall be referenced as modified; details of and the reasons for the modification shall be reported and highlighted. Conformity to the standard used shall not be claimed,but it should be stated that the product was tested in accordance with the standard.		P
4.2.7	Where in EN 14885 there is no intention to develop a test for specific product activity, the		P

	methodology in a standard specified in EN 14885 may be used and test conditions modified to match the required activity. These choices shall be scientifically justified taking into account the field of application and the intended use of the product. In the test report the European Standard shall be referenced as Amodified; details of and the reasons for the modification shall be reported and highlighted. Conformity to the standard used shall not be claimed, but it should be stated that the product was tested in accordance with the standard.		
4.2.8	Where in EN 14885 no standard exists that specifies the use conditions for a specific product activity in an area (e.g. activity at a temperature or contact time not specified in the obligatory or additional test conditions), a standard may be used with the relevant test condition modified for relevance to the area of application. In the test report the European Standard shall be referenced as modified; details of and the reasons for the modification shall be reported and highlighted. Conformity to the standard used shall not be claimed, but it should be stated that the product was tested in accordance with the standard.		P
4.2.9	The reduction of the number of test organisms caused by a product is generally expressed as decimal logarithm (lg) with two significant figures after the comma.		P
4.5	**Chemical disinfectants and antiseptics for use in food, industrial, domestic and institutional areas**		P
4.5.1	In order to make a claim that a product has disinfectant properties, suitable for use in food, industrial, domestic and institutional areas, the product shall be tested in accordance with and		P

	shall conform to the relevant European Standards as given in Table 11 as specified for the particular type of product and its claimed spectrum of activity (e.g. bactericidal, fungicidal etc.). A summary of the test conditions and requirements for the relevant phase 2, step 1 and phase 2, step 2 tests is given in Tables 12 to 22.		
4.5.2	Tests shall be carried out under the obligatory conditions as specified in the standards. According to the claimed use of the product, tests under additional conditions (test organisms, contact time, temperatures, diluents and interfering substances) shall be carried out as specified in the standard. Additional claims which can be made are given in Tables 11, 12 to 22.		P
7	**Minimum information for the user including labelling regarding efficacy claims and use recommendations**		
	The manufacturer shall provide at least the following information:		
	a) the type and/or purpose of the product (hygienic handwash, chemical disinfectant for surfaces etc.);	Suitable for skin antibacterial action	P
	b) the area and field of application:		
	1) the area of application (medical, veterinary etc.);	Institutional and domestic area	P
	2) the field of application (hygienic handrub, hard surfaces etc.);	Hygienic handrub	P
	c) the spectrum of activity (e.g. bactericidal, fungicidal); a general “microbicidal activity” cannot be claimed;	The product can kill staphylococcus aureus, escherichia coil, candida albicans,etc	P

	d) reference of the European Standards to which conformity is claimed (e.g. bactericidal (EN xx), fungicidal (EN xx));	EN 14885-2018 EN 13704:2018	P
	e) the recommended method(s) of application (use concentration(s), product diluent(s), volume to be applied, application procedure, contact time(s), temperature(s));		P
	The information a) to c) should be on the label. The other information may be given in an accompanying use instruction.		P

Remark:

--N/A (Not Applicable)

--P (Pass)

--F (Fail)

Appendix 1

<table>
<tr><th>Clause</th><th>EN 13704-2018
Chemical disinfectants – Quantitative suspension test for the evaluation of sporicidal activity of chemical disinfectants used in food, industrial, domestic and institutional areas – Test method and requirements (phase 2, step 1)</th></tr>
<tr><td>4</td><td>Requirements</td></tr>
<tr><td></td><td>The product shall demonstrate at least 3 decimal log (lg) reduction, when tested in accordance with Table 1 here below and Clause 5.</td></tr>
<tr><td></td><td>Table 1 — Minimum and additional test conditions
<table><tr><th colspan="2">Test Conditions for Surface disinfection</th></tr><tr><td>Minimum spectrum of test organisms</td><td>Bacillus subtilis</td></tr><tr><td>Additional sporicidal activity vs anaerobes for specific uses</td><td>Clostridium sporogenes</td></tr><tr><td>Additional sporicidal activity vs aerobes for specific uses</td><td>Bacillus cereus</td></tr><tr><td>Required reduction</td><td>≥ 3 lg</td></tr><tr><td>Test temperature</td><td>according to the manufacturer's recommendation, but between 4 °C and 75 °C</td></tr><tr><td>Contact time (in minutes)</td><td>according to the manufacturer's recommendation, but between 1 min and 60 min (only contact times of 1, 5, 10, 15, 20, 25, 30, 35, 40, 45, 50, 55 and 60 min are allowed in this range)</td></tr><tr><td>Interfering substance</td><td>Clean conditions:
0,3 g/l bovine albumin solution
or
Dirty conditions:
3,0 g/l bovine albumin solution</td></tr><tr><td>Additional interfering substance for dairies</td><td>10,0 g / l of reconstituted milk</td></tr></table>Other additional strains and additional test conditions may be tested according to product claim.</td></tr>
<tr><td>5</td><td>Test method</td></tr>
<tr><td>5.1</td><td>Principle</td></tr>
<tr><td></td><td>A test suspension of bacterial spores in a solution of interfering substance, simulating clean and/or dirty conditions, is added to a prepared sample of the product under test diluted in hard water (in water for ready-to-use products). The mixture is maintained at specific test temperature ± 1 °C for the specific test contact (time ± 10) s (required test conditions). In case the contact time is 1 min, the tolerance allowed shall be ± 5 s.</td></tr>
<tr><td></td><td>At this contact time, an aliquot is taken; the sporicidal action in this portion is immediately neutralized or suppressed by a validated method.The method of choice is dilution-neutralization. If a suitable neutralizer cannot be found, membrane filtration is used. The number of surviving bacterial spores in each sample are determined and the reduction in viable counts is calculated.</td></tr>
</table>

5.2	**Materials and reagents**
5.2.1	**Test organisms**
	The sporicidal activity shall be evaluated by using spores of the following strain :
	— *Bacillus subtilis* ATCC 6633 [1)].
	If required for specific applications or products, additional strains may be chosen from, for example :
	— *Bacillus cereus* CIP 105151; — *Clostridium sporogenes* ATCC 19404, CIP 79.3 [1)].
	NOTE 1 See Annex F for corresponding strain numbers in some other culture collections. NOTE 2 See Annex C for particular culture and handling conditions for *Clostridium sporogenes*. NOTE 3 It has been noted that different sources of *Bacillus cereus* strain can lead to different sporulation behaviour, in particular CIP 105151 strain seems to sporulate better.
	If additional strains are used, they shall be incubated under optimum growth conditions (temperature, time, atmosphere) and noted in the test report.
	If the additional strains selected do not correspond to the specified strains, their suitability for supplying inocula of sufficient concentration shall be verified. If the additional strains tested 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 under a reference for 5 years.
5.2.2	**Culture media and reagents**
5.2.2.1	**General**
	The reagents shall be of analytical grade and/or appropriate for microbiological purposes.
5.2.2.2	**Water**
	The water shall be free from substances that are toxic or inhibiting to the bacterial spores or to the bacteria. It shall be freshly glass distilled water and not demineralized water.
	Sterilize in the autoclave (5.3.2.1). Sterilization is not necessary if the water is used e.g. for preparation of culture media and subsequently sterilized. If distilled water of adequate quality is not available, water for injectable preparation can be used.
	See 5.2.2.6 for preparation of hard water.
5.2.2.3	**Tryptone Soja Agar (TSA)**

	For counting of viable *Bacillus* spores :
	Tryptone, pancreatic digest of casein 15,0 g
	Soya peptone, papaic digest of Soybean meal 5,0 g
	Sodium Chloride (NaCl) 5,0 g
	Agar 15,0 g
	Water (see 5.2.2.2) 1 000,0 ml
	Sterilize in the autoclave (5.3.2.1). After sterilization the pH of the medium shall be equivalent to 7,2 ± 0,2 measured at (20 ± 1) °C
5.2.2.4	**Neutralizer**
	The neutralizer shall be validated for the product under test in accordance with Annex D. The neutralizer shall be sterile.
	NOTE Information on neutralizers that have been found to be suitable for some categories of products is given in Annex D.
5.2.2.5	**Rinsing liquid (for membrane filtration)**
	The liquid shall be sterile, compatible with the filter membrane and capable of filtration through the filter membrane under the test conditions described in Annex B.
	NOTE Information on rinsing liquids that have been found to be suitable for some categories of products is given in Annex D.
5.2.2.6	**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 (MgCl2) and 46,24 g calcium chloride (CaCl2) in water (5.2.2.2) and dilute to 1000 ml. Sterilize by membrane filtration (5.3.2.7) or in the autoclave (5.3.2.1 a)). Autoclaving – if used - may cause a loss of liquid. In this case make up to 1000 ml with water (5.2.2.2) under aseptic conditions. Store the solution in the refrigerator at 5 °C ± 3 °C (according 5.3.2.15) for no longer than four weeks;
	— prepare solution B: dissolve 35,02 g sodium bicarbonate (NaHCO3) in water (5.2.2.2) and dilute to 1000 ml. Sterilize by membrane filtration (5.3.2.7). Store the solution in the refrigerator at 5 °C ± 3 °C (according 5.3.2.15) for no longer than one week;
	— place 600 ml to 700 ml of water (5.2.2.2) in a 1000 ml volumetric flask (5.3.2.12) and add 6,0 ml(5.3.2.9) of solution A, then 8,0 ml of solution B. Mix and dilute to 1000 ml with

	water (5.2.2.2). The pH of the hard water shall be (7,0 ± 0,2), when measured at (20 ± 1) °C (5.3.2.4). 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 a different final water hardness in each test tube. In any case the final hardness expressed as calcium carbonate (CaCO3) is in the test tube lower than 375 mg/l.
5.2.2.7	**Interfering substance**
5.2.2.7.1	**General**
	The interfering substance shall be sterile and prepared at 10 times its final concentration in the test.
5.2.2.7.2	**Clean conditions**
	Bovine albumin solution for the test conditions shall be prepared as follows:
	— dissolve 0,30 g of bovine albumin fraction V (suitable for microbiological purposes) in 100 ml of water (see 5.2.2.2) ;
	— sterilize by membrane filtration (5.3.2.7).
	The final concentration of the bovine albumin in the test procedure (5.5.2) is 0,3 g/l.
5.2.2.7.3	**Dirty conditions**
	Dissolve 3,00 g of bovine albumin fraction V (suitable for microbiological purposes) in 100 ml of water (5.2.2.2).
	Sterilize by membrane filtration (5.3.2.7).
	The final concentration of bovine albumin in the test procedure (5.5.2) shall be 3,0 g/l.
5.2.2.7.4	**Additional interfering substance for dairies**
	Skimmed milk, guaranteed free of antibiotics and additives and reconstituted at a rate of 100 g powder per litre of water (5.2.2.2), shall be prepared as follows:
	—prepare a solution of 100 g milk-powder in 1 000 ml water (5.2.2.2). Heat for 30 min at (105 ± 3) °C [or 5 min at (121 ± 3) °C].
	The final concentration of reconstituted milk in the test procedure (5.5.1,c)) is 10,0 g/l of reconstituted milk.
5.3	**Apparatus and glassware**

5.3.1	**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 which are supplied sterile, by one of the following methods :
	a) in the autoclave (see 5.3.2.1);
	b) in the dry heat sterilizer (see 5.3.2.1).
5.3.2	**Usual microbiological laboratory equipment and, in particular, the following**
5.3.2.1	**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 a minimum holding time of 2 h.
5.3.2.2	**Water baths**, capable of being controlled at (20 ± 1) °C, (45 ± 1) °C, (75 ± 1) °C and at test temperatures ± 1 °C (see 5.5.1).
5.3.2.3	**Incubator**, capable of being controlled at (30 ± 1) °C and (36 ± 1) °C or (37 ± 1) °C.
5.3.2.4	**pH-meter**, having an accuracy of calibration of ± 0,1 pH units at 20 °C or equivalent.
5.3.2.5	**Stopwatch**
5.3.2.6	**Vortex mixer (electromechanical agitator, i.e. Vortex® mixer** 3**))**
5.3.2.7	**Membrane filtration apparatus** (if this method is used), constructed of a material compatible with the product under test, with a filter holder which shall have a usable volume 50 ml minimum, and suitable for use with filters of diameter 47 mm to 50 mm, of 0,45 µm pore size.
	The vacuum source used shall give an even filtration flow rate. In order to obtain a uniform distribution of the microorganisms over the membrane and in order to prevent overlong filtration, the device shall be set so as to obtain the filtration of 100 ml of rinsing liquid in 20 s to 40 s.
5.3.2.8	**Containers :** Test tubes or flasks of suitable capacity.
5.3.2.9	**Graduated pipettes of nominal capacities** 10 ml and 1 ml and 0,1 ml. Calibrated automatic pipettes may be used.
5.3.2.10	**Petri dishes** of size 90 mm to 100 mm.
5.3.2.11	**Glass beads** (Diameter : 3 mm to 4 mm).

5.3.2.12	**Volumetric flasks.**
5.3.2.13	**Glass Roux bottles** with straight neck.
5.3.2.14	**Microscope**, preferably, a phase-contrast type, with magnification of at least x 400.
5.3.2.15	**Fridge**, capable of being controlled at (5 ± 3) °C.
5.3.2.16	**Jars** for anaerobiosis with oxygen removal system or any other system suitable for generating anaerobiosis.
5.3.2.17	**Centrifuge** capable of 10 000 *g* acceleration.
5.4	**Preparation of spore test suspension and test solutions**
5.4.1	**Spore suspensions**
5.4.1.1	**Stock spore suspension of test organism**
	The *Bacillus subtilis* ATCC 6633 CIP 52.62 spore stock suspension shall be prepared according to Annex A. Check the viability and the susceptibility of each spore batch after at least 4 weeks storage at 2°C to 8°C for *Bacillus* spores. Check the viability and the susceptibility of each spore batch after at least 12 month storage at 2°C to 8°C for Bacillus spores. The *Bacillus* spore suspensions may be stored for a maximum of 5 years if periodically checked. Use glutaraldehyde and peracetic acid at set concentrations. Perform the tests according to the Dilution-neutralization method (5.5.2.2) and use water (5.2.2.2) instead of an interfering substance (5.2.2.7). The susceptibility validation has to be performed with a suspension adjusted to 1,5 –5,0 × 106 cfu/ml (colony forming units).
	Pre-examination studies agreed that at (20 ± 1) °C:
	With *Bacillus subtilis* ATCC 6633 without interfering substance:
	— 3,0 % (v/v) – 30 min: Glutaraldehyde solution should achieve a lg reduction of < 3 lg
	— 10,0 % (v/v) – 30 min: Glutaraldehyde solution should achieve a lg reduction of ≥ 3 lg.
	— 0,001 % (v/v) – 30 min: Peracetic acid solution should achieve a lg reduction of < 3 lg.
	— 0,05 % (v/v) – 30 min: Peracetic acid solution should achieve a lg reduction of ≥ 3 lg
	With *Bacillus cereus* without interfering substance:
	— 0,5 % (v/v) – 15 min: Glutaraldehyde solution should achieve a lg reduction of < 3 lg
	— 3,0 % (v/v) – 15 min: Glutaraldehyde solution should achieve a lg reduction of ≥ 3 lg.

	— 0,05 % (v/v) –30 min: Peracetic acid solution should achieve a lg reduction of < 3 lg.
	— 0,50 % (v/v) – 30 min: Peracetic acid solution should achieve a lg reduction of ≥ 3 lg
	Glutaraldehyde 50 % shall be used with pH between 3,1 and 4,5.
	For the validation a specific Standard-Biocide should be used. Appropriate substances are e.g.: Glutaraldehyd – Product name: BIOBANTM GA 50 Antimicrobial4, DOW Chemical Company Ltd, Diamond House, Lotus Park, Kingsbury Crascent, TW18 3 AG Staines, Middlesex, United Kingdom. Peracetic acid – Product name: PES 5/25 (mixture of 5 % peracetic acid and 25 % hydrogen peroxide), Stockmeier Chemie Eilenburg GmbH and Co. KG, Gustav-Adolf-Ring 5, D-04838 Eilenburg. Stored at 2 °C to 8 °C.
	For the preparation of the stock spore suspensions of additional strains (see 5.2.1) refer to :
	— Annex A for *Bacillus cereus*; CIP 105.151
	— Annex C for *Clostridium sporogenes* ATCC 19404, CIP 79.3.
	For *Clostridium sporogenes* no susceptibility check shall be done as there are no data available in order to define a susceptibility range. The spore suspension may be stored for a maximum of 12 months.
5.4.1.2	**Spore test suspension**
	To prepare the spore test suspension, dilute the spore stock suspension (see 5.4.1.1) with water (see 5.2.2.2). The number of spores in the test suspension shall be adjusted to 1,5 × 106 to 5 × 106 cfu/ml, estimating the number of units by any suitable mean.
	Maintain the suspension test in the water bath at (20 ± 1) °C and use within 2 h.
	Microscopic examination under 400 × magnification shall be carried out immediately after the preparation of the spore test suspension and just before the test, to show the absence of vegetative cells and germinative spores.
	If there is any evidence of spore germination, the suspension shall be discarded.
	For counting of the spore test suspension prepare 10−4 and 10−5 dilutions of the test suspension (see 5.4.1.3) using water (see 5.2.2.2). Mix (see 5.3.2.6). Take a sample of 1,0 ml of each dilution in duplicate and transfer each 1,0 ml sample into separate Petri dishes (see 5.3.2.10) and add 12 ml to 15 ml melted TSA (see 5.2.2.3), cooled to (45 ± 1) °C.
5.4.1.3	**Counting of spore test suspension**
	Incubate the Petri dishes at (30 ± 1) °C (see 5.3.2.3) for 3 days. Determine the highest

	number of colonies Vc for each plate. Calculate the number of cfu/ml 5) (see 5.6) in the test suspension (*N*) using the method given in 5.6.1.2.
5.4.1.4	**Validation suspension ("Nv", "Nv0")**
	a) To prepare the validation suspension ("Nv"), dilute the test suspension (5.4.1.2) with the water (5.2.2.2) to obtain 3,0 x 102 cfu/ml to 1,6 x 103 cfu/ml [about one fourth (1+3) of the 10−3 dilution].
	b)Maintain and use this validation suspension (*NV*) the same way as the test suspension (5.4.1.2).
	For counting Nv prepare a 10−1 dilution with water (5.2.2.2), mix and take a sample of 1,0 ml in duplicate and inoculate using the pour plate technique or spread plate technique (only Bacillus) (5.4.1.4).
	For incubation and counting see 5.5.2.2.3.
	Nv is the number of cells per ml in the validation suspension. It is tenfold higher than the counts in terms of *Vc* values due to the dilution step of 10−1 [5.4.1.4b)].
	*Nv*0 is the number of cells per ml in the mixtures A, B and C at the beginning of the contact time.
5.4.2	**Product test solution**
	Details of samples of the product as received shall be recorded.
	Solutions of the test product shall be prepared in hard water (see 5.2.2.6) or distilled water (5.2.2.2) in the case of ready-to-use products at three different concentrations to include one concentration in the active range and one concentration in the non-active range. The concentration of the product test solution shall be 1,25 times the required test concentration.
	For solid products, dissolve the product as received by weighing at least 1,0 g ± 10 mg of the product in a volumetric flask and filling up with hard water (see 5.2.2.6). Subsequent dilutions shall be prepared in volumetric flasks (see 5.3.2.12) on a volume/volume basis in hard water (see 5.2.2.6).
	For liquid products, dilutions of the product shall be prepared in hard water (see 5.2.2.6) on a volume/volume basis using volumetric flasks (5.3.2.12).
	For products supplied in a ready to use state, water (see 5.2.2.2) shall be used to prepare the second and third dilutions.
	When the product is diluted in hard water (or in water, see 5.2.2.2) it shall give a physically homogeneous stable preparation. If precipitate or flocculation appears during the assay, it shall be mentioned in the test report.

	The product test solutions and dilutions of it shall be prepared freshly and used within 2h.
	If the product is of low stability this period should be shortened.
	The concentration of the product stated in the test report shall be the test concentration. Record the test concentration in terms of mass per volume or volume per volume.
5.5	**Procedure**
5.5.1	**Choice of experimental conditions**
	The experimental conditions may be selected according to the practical use considered for the product (Clause 4):
	a) temperature θ (in °C):
	The temperatures to be tested are specified in Clause 4, Table 1. The allowed deviation for each chosen temperature is ± 1 °C.
	b) contact time t (in min):
	The contact times to be tested are specified in Clause 4, Table 1. The allowed deviation for each chosen contact time is ± 10 s (±5 s when the contact time is 1 min).
	c) interfering substance:
	The interfering substance to be tested is 0,30 g/l bovine albumin (5.2.2.7.2) under clean conditions or 3,0 g/l bovine albumin (5.2.2.7.3) under dirty conditions – according to Clause 4, Table 1 and practical applications. Additional interfering substance may be tested according to the specific intended uses of the product. The product shall not cause the formation of any precipitate in the experimental conditions used.
	Each selected experimental condition (*θ*, *t*, strains) shall be validated in accordance with Annex B.
	The longest contact time and the highest concentration shall be validated.
5.5.2	**Test procedure for assessing the sporicidal effect of the product**
5.5.2.1	**General**
	The method of choice is the dilution-neutralization method. To determine a suitable neutralizer the following procedure shall be adopted. Carry out the validation of the dilution neutralization method (B.4.1) using a suitable neutralizer, chosen according to laboratory experience and published data.
	If this neutralizer is unsuitable, repeat the validation test with another neutralizer taking into account the information given in Annex D.

	If neither of the two neutralizers is considered valid, the membrane filtration method (5.5.2.3) may be used. The inactivation of the sporicidal activity of the product shall be validated for each of the tested strains and for each of the chosen experimental conditions (see 5.5.1).
5.5.2.2	**Dilution-neutralization method**
5.5.2.2.1	**General**
	a) if the test temperature is lower or equal to 40 °C ± 1 °C:
	Prior to testing, equilibrate all reagents (product test solutions, spore test suspension, interfering substance) to the test temperature of*θ* °C ± 1 °C using the water bath (see 5.3.2.2) controlled at*θ* °C ± 1 °C. Check that the temperature of the reagents is stabilized at*θ* °C ± 1 °C. The neutralizer and water (see 5.2.2.2) shall be equilibrated at a temperature of 20 °C ± 1 °C.
	b) if the test temperature is higher than 40 °C ± 1 °C:
	Prior to testing, equilibrate the product test solutions to the test temperature of*θ* °C ± 1 °C using the water bath (see 5.3.2.2) controlled at*θ* °C ± 1 °C. Check that the temperature of the reagents is stabilized at *θ* °C ± 1 °C. The neutralizer, the spore test suspension, the interfering substance and the water (see 5.2.2.2) shall be equilibrated at a temperature of 20 °C ± 1 °C.
5.5.2.2.2	**Test procedure for sporicidal activity of products**
	Pipette 1,0 ml of interfering substance (see 5.2.2.7) into a test tube. Add 1,0 ml of the spore test suspension containing 1,5 x 106 to 5 x 106 cfu/ml 6) (see 5.4.1.2).
	Start the stopwatch immediately, mix (see 5.3.2.6) and place the test tube in the water bath at *θ* °C ± 1 °C for 2 min ± 10 s. At the end of the contact time, add 8,0 ml of each of the product test solutions. Restart the stopwatch immediately, mix (see 5.3.2.6) and place the test tube in a water bath controlled at *θ* °C ± 1°C for the appropriate contact time (*t* ± 10) s (±5 s when the contact time is 1 min).
	When adding spore suspension, care should be taken to avoid touching the upper part of the test tube sides.
	Just before the end of the contact time, mix (see 5.3.2.6). At the end of the contact time pipette 1,0 ml of the test mixture into a tube containing 8,0 ml neutralizer (see 5.2.2.4) and 1,0 ml water (see 5.2.2.2). Mix (see 5.3.2.6) and place in a water bath controlled at (20 ± 1) °C.
	After a neutralization time of 5 min ± 10 s, immediately take a sample of 1,0 ml of neutralized mixture (neutralizer, product test solution, interfering substance, spore test suspension) in duplicate and transfer each 1,0 ml sample into separate Petri dishes (see

	5.3.2.10) and quickly add 12 ml to 15 ml melted TSA (see 5.2.2.3), cooled to (45 ± 1) °C.
5.5.2.2.3	**Incubation and counting of the test mixture**
	Incubate the Petri dishes at (30 ± 1) °C (see 5.3.2.3) for 3 days.
	Determine the highest number of colonies Vc for each plate.
	Calculate the number of cfu/ml in the test mixture (*Na*) using the method given in 5.6.2.
	For calculating the viable count of the test mixture, the dilution factor is 1:10. The text mixture *Na* and two 1:10 dilutions are performed and counted.
5.5.2.3	**Membrane filtration method**
5.5.2.3.1	**General**
	Prior to testing, equilibrate all reagents (product test solutions, spore test suspension, interfering substance) to the test temperature of (θ± 1) °C using the water bath (see 5.3.2.2) controlled at (θ± 1) °C. Check that the temperature of the reagents is stabilized at (θ± 1) °C. The rinsing liquid (see 5.2.2.5) and water (5.2.2.2) shall be equilibrated at a temperature of (20 ± 1) °C.
	a) if the test temperature is lower or equal to 40 °C ± 1 °C:
	Prior to testing, equilibrate all reagents (product test solutions, spore test suspension, interfering substance) to the test temperature of (θ ± 1) °C using the water bath (see 5.3.2.2) controlled at (θ± 1) °C. Check that the temperature of the reagents is stabilized at (θ± 1) °C. The rinsing liquid (see 5.2.2.5) and water (5.2.2.2) shall be equilibrated at a temperature of (20 ± 1) °C.
	b) if the test temperature is higher than (40 ± 1) °C:
	Prior to testing, equilibrate the product test solutions to the test temperature of (θ± 1)°C using the water bath (see 5.3.2.2) controlled at (θ± 1) °C. Check that the temperature of the reagents is stabilized at (θ± 1)°C. The rinsing liquid (see 5.2.2.5), the spore test suspension, the interfering substance and the water (see 5.2.2.2) shall be equilibrated at a temperature of (20± 1) °C.
5.5.2.3.2	**Test procedure for sporicidal activity of products**
	Pipette 1,0 ml of interfering substance (see 5.2.2.7) into a test tube. Add 1,0 ml of the spore test suspension(see 5.4.1.3).
	Start the stopwatch immediately, mix (see 5.3.2.6) and place the test tube in the water bath at (θ ± 1) °C for 2 min ± 10 s. At the end of the contact time, add 8,0 ml of each of the product test solutions. Restart the stopwatch immediately, mix (see 5.3.2.6) and place the test tube in a water bath controlled at (θ ± 1) °C for the appropriate contact time

	(t ± 10) s or (t ± 5) s in the case of a contact time of 1 min.
	Just before the end of the chosen contact time, mix (see 5.3.2.6). At the chosen contact time pipette two samples of 0,1 ml of the test mixture and transfer each sample into a separate membrane filtration apparatus equipped with a membrane and containing 50 ml of the rinsing liquid (see 5.2.2.5). Filter immediately. The time required for transfer and filtration should not exceed 1 min. If greater than 1 min, this time shall be recorded in the test report. Rinse with at least 150 ml but not more than 500 ml of rinsing liquid (see 5.2.2.5). Filter and rinse with 50 ml of water (see 5.2.2.2) and then transfer the membranes to the surface of two separate TSA plates (see 5.2.2.3).
	When transferring, care should be taken to ensure that the spores are on the upper side of the membrane when placed on the TSA and to avoid trapping air between the membrane and agar surface.
	Perform this procedure using the other product test solutions.
5.5.2.3.3	**Incubation and counting of test mixture**
	Incubate the Petri dishes at (30 ± 1) °C (see 5.3.2.3) for 72 h ± 6 h.
	Determine the higher number of colonies Vc for each plate.
	Calculate the number of cfu/ml in the test mixture (*Na*) using the method given in 5.6.2.
5.5.3	**Validation of dilution neutralization and membrane filtration method**
	The dilution-neutralization and membrane filtration methods shall be validated for each of the test organisms according to Annex B.
	The validation test (see Annex B) shall also be carried out at the same time as the test procedure (see 5.5) using only the highest concentration, the longest contact time and the same conditions (spore test suspension, product test solution and neutralizer or rinsing liquid) as used in the test (see 5.5.2.2 or 5.5.2.3).
5.6	**Calculation and expression of results**
5.6.1	**Overview of the different suspensions and test mixtures**
5.6.1.1	**General**
	N, N0 and Nv represent the spore suspensions, Na represents the sporicidal test mixture, A (experimental conditions control), B (neutralizer or filtration control), C (method validation) represent the different control test mixtures (see Table 2).

Table 2 — Number of cells counted per ml in the different test mixtures

N, N_V, N_0, N_a, A, B and C represent the number of cells counted per ml in the different test mixtures in accordance with Table 1.	Number of cells per ml in the spore suspensions	Number of cells per ml in the test mixtures at the beginning of the contact time (*time* 0)	Number of survivors per ml in the test mixtures at the end of the contact time t (A) or 5 min (B) or 30 min (C)
Test	N Test suspension	N_0 (= N/10)	N_a (after neutralization or filtration)
Controls	Nv Validation suspension	Nv_0 (= Nv/10) Validation suspension	A, B, C

5.6.1.2	***Vc*-values**
	All experimental data are reported as *Vc*-values:
	— in the dilution-neutralization method (test and controls), a *Vc*-value is the number of cfu counted per 1,0 ml sample;
	— in the membrane filtration method, a *Vc*-value is the number of cfu counted per 0,1 ml sample of test mixture *Na,* of filtration control (B) and method validation (C) and per 1,0 ml sample in the experimental condition control A.
5.6.2	**Calculation**
5.6.2.1	**General**
	The first step in the calculation is the determination of the *Vc*-values, the second the calculation of *N*, *N*0, *Na*, *Nv* *Nv*0, *A*, *B* and *C*. The third step is the calculation of the reduction *R* (5.8).
5.6.2.2	Determination of *Vc*-values
	The *Vc*-values are determined as follows.
	a) The usual limits for counting bacteria on agar plates are between 15 and 300. In this European Standard a deviation of 10 % is accepted, so the limits are 14 and 330. On membranes, the usual upper limits are different: 150, i.e. with the 10 % deviation: 165.
	NOTE The lower limit (14) is based on the fact that the variability increases the smaller the number counted in the sample (1 ml or 0,1 ml) is and therefore subsequent calculations can lead to wrong results. The lower limit refers only to the sample (and not necessarily to the counting on one plate), e.g. three plates per 1 ml sample with 3 cfu, 8 cfu and 5 cfu give a Vc value of 16. The upper limits (330, 165) reflect the imprecision of counting confluent colonies and growth inhibition due to nutriment depletion. They refer

	only to the counting on one plate and not necessarily to the sample.
	b) For counting the test suspension N (5.4.1.3, Annex A), the validation suspension Nv (5.4.1.3, Annex A) and for all countings of the dilution-neutralization method (5.5.2.2.2 and 5.6.1.2, Annex B), determine and record the Vc values according to the number of plates used per 1 ml sample (5.6.1.2).
	If more than one plate per 1 ml sample has been used to determine the Vc value, the countings per plate should be noted.
	c) If the count on one plate is higher than 330, report the number as ">330". If more than one plate per 1 ml sample has been used and at least one of them shows a number higher than 330, report this Vc value as "more than sum of the counts," e.g. for ">330, 310, 302", report " > 942".
	d) If a Vc value is lower than 14, report the number (but substitute by "<14" for further calculations in the case of Na).
	e) For the membrane-filtration method (5.5.2.3.2), the countings on the membranes are the Vc values (5.6.1.2). Report the Vc values below the lower limit (14) or above the upper limit (165) as described above.
	f) Only Vc values within the counting limits are taken into account for further calculation, except in the case of Na (5.6.2.4).
5.6.2.3	**Calculation of *N* and *N*0**
	N is the number of cells per ml in the test suspension (5.4.1.3; 5.6.1.2).
	Since two dilutions of the test suspension (5.4.1.3) are evaluated, calculate the number of cfu/ml as the weighted mean count using the following formula: $N = \frac{c}{(n_1 + 0{,}1\, n_2)\, 10^{-4}}$
	where *c* is the sum of *Vc* values taken into account; *n*1 is the number of *Vc* values taken into account in the lower dilution, i.e. 10−4; *n*2 is the number of *Vc* values taken into account in the higher dilution, i.e. 10−5; 10^{-4} is the dilution factor corresponding to the lower dilution.
	Round off the results calculated to two significant figures. For this, if the last figure is below 5, the preceding figure is not modified; if the last figure is more than 5, the preceding figure is increased by one unit; if the last figure is equal to 5, round off the preceding figure to the next nearest even figure. Proceed stepwise until two significant figures are obtained. As a result, the number of cfu/ml is expressed by a number between 1,0 and 9,9 multiplied by the appropriate power of 10.

5.6.2.4	**Calculation of *Na***
	Na is the number of survivors per ml in the test mixture [5.5.2.2.2 or 5.5.2.3.2] at the end of the contact time and after neutralization or membrane filtration. It is tenfold higher than the *Vc* values due to the addition of neutralizer and water [5.5.2.2.2] or the sample volume of 0,1 ml [5.5.2.3.2] in the membrane filtration method.
	a) Calculate the mean for each dilution step Na^0, Na^{-1} and Na^{-2} using the following formula.
	$Na^0, Na^{-1}, Na^{-2} = 10\ c / n$
	where *c* is the sum of *Vc* values taken into account; *n* is the number of *Vc* values taken into account.
	If one or both of the duplicate *Vc* values are either below the lower or above the upper limit, express the results as “less than” or “more than”.
	b) For calculation of *Na* use only *Na0, Na−1, Na−2* results, where one or both Vc values are within the counting limits. Exceptions and rules for special cases are explained below.
	b1 If all subsequent dilutions of *Na* show mean values of „more than”, take only the highest dilution (10−1) as result for *Na*.
	b2 If all subsequent dilutions of *N*a show mean values of „less than”, take only the lowest dilution (10°) as result for *N*a.
	b3 If one or both duplicate *VC*-values in only one dilution of *N*a are within the counting limits, use this result as *N*a.
	b4 If the higher dilution in two subsequent dilutions of *N*a shows a mean value of „less than” and the lower dilution shows a mean value of „more than”, take only the lower dilution as *N*a value.
	c) Use maximum 2 subsequent dilutions for calculating *Na* as a weighted mean. Exceptions and rules for special cases are explained below.
	c1 If one or both duplicate Vc values in three subsequent dilutions of *Na* (including *Na*0) are within the counting limits (e.g. *Na*−2: 17, 23; *Na*−1: 120, 135; *Na0*: 308, > 330) the whole test is invalid (5.7.1).
	c2 If two subsequent dilutions of *Na* show duplicate Vc values within the counting limits calculate *Na* as the weighted mean using the Formula (3): $Na = \frac{c \times 10}{2{,}2 \times 10^{Z}}$
	where *c* is the sum of Vc values taken into account;

	Z is the dilution factor corresponding to the lower dilution, e.g. Na0 is the lower dilution in comparison with Na−1
	c3 If in two subsequent dilutions of *Na* both *Vc* values of the higher dilution are within the counting limits and one *Vc* value of the lower dilution is „more than", calculate *Na* as the weighted mean, using the Formula (3), see c 2.
	c4 If in two subsequent dilutions of *N*a one of the higher dilution duplicate values shows„ < 14", take only the lower dilution as result for *N*a.
5.6.2.5	**Calculation of *Nv*, *Nv*0**
	Nv is the number of cells per ml in the validation suspension [Annex A]. It is tenfold higher than the counts in terms of *Vc* values due to the dilution step of 10^{-1} [Annex A].
	Calculate *Nv,* using the following formula: $Nv = 10\ c / n$
	where *c* is the sum of Vc values taken into account; *n* is the number of Vc values taken into account
5.6.2.6	**Calculation of *A*, *B* and *C***
	A, *B* and *C* are the numbers of survivors in the experimental conditions control A (Annex B), neutralizer control B (Annex B) or filtration control (Annex B) and method validation C (Annex B) at the end of the contact time *t* (A) or the defined times of 5 min (B) and 30 min (C). They correspond to the mean of the *Vc* values of the mixtures A, B and C taken into account.
	Calculate *A*, *B* and *C* using the following formula: $A, B, C = c / n$
	where *c* is the sum of Vc values taken into account; *n* is the number of Vc values taken into account
5.7	**Verification of methodology**
5.7.1	**General**
	A test is valid if:
	— all results meet the criteria of 5.7.3 and
	— it is not invalidated by a result described under 5.6.2.4 c) first special case (c1).
5.7.2	**Control of weighted mean counts**
	For results calculated by weighted mean of two subsequent dilutions (e.g. "*N*"), the quotient of the means of the two results shall be not higher than 15 and not lower than 5.

	Results below the lower limit are taken as lower limit number (14). Results above the respective upper limit [5.6.2.3] are taken as the upper limit number.
	NOTE When the counts obtained on plates are out of limits fixed for the determination of Vc values, check for the weighted mean as mentioned above but use only the Vc values within the counting limits for the calculation of N.
5.7.3	**Basic limits**
	For each test organism check that:
	a) N is between 1,5 x 10^6 and 5,0 x 10^6 (6,17 ≤ lg N ≤ 6,70) N_0 is between 1,5 x 10^5 and 5,0 x 10^5 (5,17 ≤ lgN0 ≤ 5,70) b) N_{V0} is between 30 and 160 (3,0 x 10^1 and 1,6 x 10^2) N_V is between 3,0 x 10^2 and 1,6 x 10^3 *A*, *B*, *C* are equal to or greater than 0,5 x N_{V0}
	Control of weighted mean counts (5.7.2): quotient is not lower than 5 and not higher than 15.
5.7.4	**Expression of results**
	For the test organism record the number of cfu/ml in the spore test suspension (*N*) (see 5.4.1.3) and after the test procedure for sporicidal activity of the product (*Na*) (see 5.5.2.2.2 or 5.5.2.3.2). Calculate *N0 (*5.6.2.3*).*
	For the validation of neutralization (see Annex B) record the number of cfu/ml (*Nv*) in the spore suspension (see B.2).
	For validation of the dilution neutralization method (see B.4.1) record the number of cfu/ml in the neutralizer toxicity control (B), the dilution neutralization control (C) and the experimental conditions control (A).
	For validation of the membrane filtration method (see B.4.2), record the number of cfu/ml in the filtration control(B), the filtration test control (C) and the experimental conditions control (A).
	The reduction (*R* = *N0/Na*) is expressed in logarithm.
	For each product concentration and each experimental condition, calculate and record the decimal log reduction (lg) separately using the formula:
	lg R = lg N_0 − lg Na
5.8	**Conclusion**
	Sporicidal activity for general purposes is characterized by the concentration of the tested product for which criteria 5.7.3 are met and for which a 3 lg or more reduction in viability is demonstrated under the chosen test conditions (see Clause 4), and when the test organisms are spores of *Bacillus subtilis.*

Test results (sporicidal quantitative suspension test)

Product-name: Hand Sanitizer

Production date: 2020/02/19

Dilution neutralization method ☑

Pour plate ☑ ☐ Number of plates 2 / ml

Neutralizer: Lecithin 3,0 g/l in diluent

Membrane filtration method ☐

Rinsing liquid: Test temperature: 20 °C

Interfering substance: Bovine albumin: 0,3 g/l

Test organism: Bacillus subtilis ATCC 6633

Incubation temperature: 30°C

Diluent used for product test solutions: hard water

Appearance of the product test solutions: clear

Date of testing: 2020-03-24

Testing Laboratory: Zuoce Certification and Testing Center.

ROOM 318 Building 6, No.26 Hexuan Road, Jiading District, Shanghai, China, 201803

Tel: 086-21-39922156 Email: info@zuoce.org

Responsible person: Stone Lee Stone Lee

Signature: Jack Yang Jack Yang

ZUOCE CERTIFICATION & TESTING CENTER CHINA BRANCH

Validation and controls

Validation suspension (Nvo)			Experimental conditions control (A)			Neutralizer or filtration control (B)			Method validation (C) Product conc.: 10 ml/l		
VC1	86 (40 + 46)	$\bar{x}$=	VC1	79 (43 + 36)	$\bar{x}$ =	VC1	86 (42 + 44)	$\bar{x}$=	VC1	75 (35 + 40)	$\bar{x}$=
VC2	92 (47 + 45)	89	VC2	84 (39 + 45)	81,5	VC2	91 (43 + 48)	88,5	VC2	87 (41 + 46)	81
30 ≤ $\bar{x}$ of Nvo ≤ 160 ? ☑yes ☐no			$\bar{x}$ of A is ≥ 0,5x x of Nvo ? ☑yes ☐no			$\bar{x}$ of B is ≥ 0,5x x of Nv0 ☑yes ☐no			$\bar{x}$ of C is ≥ 0,5x x of Nvo? ☑yes ☐no		

Test suspension and Test

Test-suspension (N and N0):	N	VC1	VC2	$\bar{x}_{wm}$ = 193,64 x 10^4 ; lgN = 6,29
	10^{-4}	168	213	N0 = N/10 ; lgN0 = 5,29
	10^{-5}	20	25	5,17 ≤ lgN0 ≤ 5,70? ☑yes ☐no

Concentration of the product %	Dilution step	VC1	VC2	Na (= $\bar{x}$ or $\bar{x}$ wm x 10)	lg *N*a	lg *R* (lg*N*o = 6,29)	Contact time (min)
0,5	10°	> 330	> 330	8318	3,92	1,37	60
	10^{-1}	77	85				
	10^{-2}	7	8				
0,75	10°	122	154	1380	3,14	2,15	60
	10^{-1}	14	17				
	10^{-2}	1	2				
1,00	10°	7	0	< 141	< 2,15	> 3,14	60
	10^{-1}	0	0				
	10^{-2}	0	0				

Explanations:

Vc = count per ml (one plate or more) $\bar{x}$ = average of VC1 and VC2 (1. + 2. duplicate)

$\bar{x}$ wm = weighted mean of $\bar{x}$ R = reduction (lg R = lgN0 – lgNa)

The conclusion: The above test results demonstrated at least 3 decimal log (lg) reduction, when the product was tested in accordance with Table 1 and Clause 5.

The end of report