

STUDY REPORT
2018-8337/18 23 00151

STERI-7 WORLDWIDE LTD

STERI-7 XTRA SURFACE DISINFECTANT

SUSPENSION TEST
ACCORDING TO EN 16615:2015
(Phase 2, step 2)

Chemical disinfectants and antiseptics - Quantitative test
method for the evaluation of bactericidal and yeasticidal activity
on non-porous surfaces with mechanical action employing wipes
in the medical area (4- field test) -
Test method and requirements (phase 2, step 2)

DECEMBER 2018

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STUDY REPORT 2018-8337/18 23 00151

TEST PRODUCT IDENTIFICATION

PRODUCT NAME	: STERI-7 XTRA SURFACE DISINFECTANT
PRODUCT TYPE	: Surface Disinfectant/ Pre Saturated Wipes
ACTIVE SUBSTANCES	: 0.147% w/w Didecyldimethylammonium chloride 0.086% w/w Benzalkonium chloride 0.0854% w/w Polyhexamethylene biguanide
APPEARANCE OF THE PRODUCT	: Wipes
STORAGE CONDITIONS	: Room temperature, darkness
TEST CONDITIONS	: Test conducted at 20°C ± 1 °C
LOT	: Not listed
METHOD	: EN 16615:2015
CONTACT TIME	: 5 minutes
DILUTION	: Undiluted
PRODUCT DILUENT	: Water
STUDY SPONSOR	: STERI-7 WORLDWIDE LTD
PRODUCT MANUFACTURER	: STERI-7 WORLDWIDE LTD
RECEIPT DATE	: 18/10/2018
STUDY PERIOD	: 27/11/2018 - 03/12/2018
LAB ID	: 2018-8337/18 23 00151

TEST OBJECTIVE

This European Standard specifies a carrier test for establishing whether a chemical disinfectant for use on surfaces administered with wipes has a bactericidal and yeasticidal activity in the fields described in the scope.

TEST METHOD

Chemical disinfectants and antiseptics - Quantitative test method for the evaluation of bactericidal and yeasticidal activity on non-porous surfaces with mechanical action employing wipes in the medical area (4- field test) - Test method and requirements (phase 2, step 2)

PRINCIPLE

A flooring is marked with 4 squares of 5 x 5 cm, the "test fields", in a row. Test field 1 on the flooring is inoculated with a test suspension of bacteria and yeasts in a solution of interfering substances. The inoculum is dried. A wipe is soaked with a sample of the product as delivered and/or diluted with hard water (for ready to use products: water). The flooring is wiped with the soaked wipe across the four marked test fields, starting in front of test field 1, turning immediately after test field 4 and wiped back to the starting point. In parallel a water control is performed: a wipe is soaked with water instead of the product and the exact same procedure is followed.

Temperature, soiling and contact time are employed as recommended by the manufacturer. At the end of the contact time, the test organisms are recovered from each test field with moistened cotton swabs. The swabs are brought into a tube containing broth and neutralizer and the test organisms are recovered from the swab by shaking. The numbers of surviving test organisms in each sample are determined, and the reduction is calculated by comparing the results of the drying control and the results obtained with the product. In parallel to the test with the product water is applied in the same way to ensure that the test organisms are spread on the 4 fields and their number reaches a certain level. The test is performed using *P. aeruginosa*, *S. aureus*, *E. hirae* and *C. albicans* as test organisms (minimum test conditions).

METHOD REQUIREMENTS - CRITERIA

The product shall be deemed to have passed the EN 16615 standard (bactericidal and yeasticidal activity) if it demonstrates in a valid test under the conditions defined by this standard within maximum 60 min contact time:

Bactericidal activity:

- a 5 lg reduction for *Staphylococcus aureus*, *Enterococcus hirae*, *Pseudomonas aeruginosa* on test field 1; and
- an average of equal or less than 50 cfu on test fields 2 to 4 for each test organism.

Yeasticidal activity:

- a 4 lg reduction for *Candida albicans* on test field 1; and
- an average of equal or less than 50 cfu on test fields 2 to 4 for each test organism.

The water control shall demonstrate in a valid test under the conditions defined by this standard for each test organism (bacterial and fungal) an average of equal or more than 10 cfu on the test fields 2 to 4.

ASSAY ACCEPTANCE CRITERIA

Basic limits for test microorganisms for bactericidal activity

1. Test Suspension N is between $1,5 \times 10^9$ cfu/ml and $5,0 \times 10^9$ cfu/ml ($9,17 \leq \lg N \leq 9,70$)
2. N_0 ($N/20$) is between $7,5 \times 10^7$ cfu/ml and $2,5 \times 10^8$ cfu/ml ($7,88 \leq \lg N_0 \leq 8,40$)
3. Validation Suspension= N_v is between $3,0 \times 10^2$ and $1,6 \times 10^3$.
4. N_{v0} ($N_v/10$) is between 30 cfu/ml and 160 cfu/ml ($3,0 \times 10^1$ and $1,6 \times 10^2$)
5. Neutralizer control(B) and Method Validation control(C) are equal to or greater than $0,5 \times N_{v0}$
6. Control of weighted mean counts - Quotient is not lower than 5 and not higher than 15.
7. D_{c0} represents the recovery immediately after drying. D_{c0} is between $7,5 \times 10^6$ cfu/ml and $2,5 \times 10^8$ cfu/ml ($6,88 \leq \lg T_0 \leq 8,40$).
8. D_{ct} represents the recovery after drying and the contact time t. D_{ct} is between $7,5 \times 10^6$ cfu/ml and $2,5 \times 10^8$ cfu/ml ($6,88 \leq \lg T_t \leq 8,40$).
9. N_w represents the test mixture in the water control from the surface. N_w is on average > 10 cfu/25 cm² on test fields 2 to 4.
10. N_a represents the bactericidal test mixture from the surface at the end of contact time.
11. Reduction (R) on test field 1 is expressed in logarithm. $\lg R = \lg D_{ct} - \lg N_a$

Basic limits for test microorganisms for yeasticidal activity

1. Test Suspension N is between $1,5 \times 10^8$ cfu/ml and $5,0 \times 10^8$ cfu/ml ($8,17 \leq \lg N \leq 8,70$)
2. N_0 ($N/20$) is between $7,5 \times 10^6$ cfu/ml and $2,5 \times 10^7$ cfu/ml ($6,88 \leq \lg N \leq 7,40$)
3. Validation Suspension= N_v is between $3,0 \times 10^2$ and $1,6 \times 10^3$.
4. N_{v0} ($N_v/10$) is between 30 cfu/ml and 160 cfu/ml ($3,0 \times 10^1$ and $1,6 \times 10^2$)
5. Neutralizer control(B) and Method Validation control(C) are equal to or greater than $0,5 \times N_{v0}$
6. Control of weighted mean counts - Quotient is not lower than 5 and not higher than 15.
7. D_{c0} represents the recovery immediately after drying. D_{c0} is between $7,5 \times 10^5$ cfu/ml and $2,5 \times 10^7$ cfu/ml ($5,88 \leq \lg N \leq 7,40$)
8. D_{ct} represents the recovery after drying and the contact time t. D_{ct} is between $7,5 \times 10^5$ cfu/ml and $2,5 \times 10^7$ cfu/ml ($5,88 \leq \lg N \leq 7,40$)
9. N_w represents the test mixture in the water control from the surface. N_w is on average > 10 cfu/25 cm² on test fields 2 to 4.
10. N_a represents the yeasticidal test mixture from the surface at the end of contact time.
11. Reduction (R) on test field 1 is expressed in logarithm. $\lg R = \lg D_{ct} - \lg N_a$

TEST MICROORGANISMS

The bactericidal activity is evaluated using the following four strains:

<i>Pseudomonas aeruginosa</i>	NCIMB 10421
<i>Staphylococcus aureus</i>	ATCC 6538
<i>Enterococcus hirae</i>	NCIMB 8192

The yeasticidal activity is evaluated using the following strain:

<i>Candida albicans</i>	ATCC 10231
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STUDY ENUMERATION METHOD

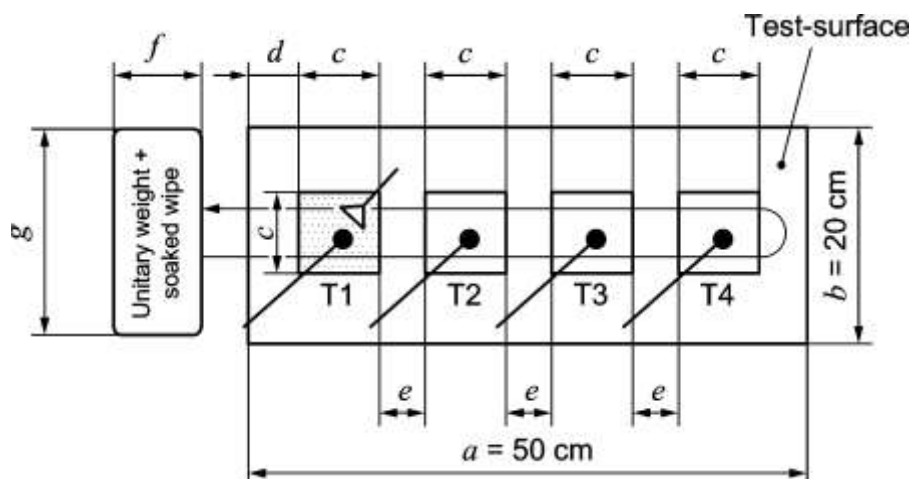
Dilution-Neutralization method followed, using plate count method/pour plate method.

NEUTRALIZER USED IN STUDY

Neutralizer: Lecithin 3 g/l, polysorbate 80 30 g/l, sodium thiosulphate 5 g/l, L-histidine 1 g/l, saponine 30g/l in phosphate buffer.

TEST SURFACE

PVC with PUR surface coating, thickness 2,5 mm; measuring 20 cm x 50 cm was used. The flooring was cleaned with 70% ethanol. The flooring was marked with a permanent marker four squares as test fields 1 to 4, each measuring 5 cm x 5 cm figuring a row at a distance of 5 cm from one another. The drying controls D_{C0} and D_{Ct} were performed on a smaller test surface measuring 7 cm x 13 cm - marked with two squares of 5 cm x 5 cm.



STUDY CONDITIONS

1. The following procedure was performed at 20 °C
2. The test product was tested for: 5 minutes contact time.
3. Interfering substance used: Dirty conditions (3g/L bovine albumin solution and 3ml/L sheep erythrocytes).
4. The sample was tested at one product concentration: undiluted. The product as received was used as one of the product test solutions. The wipes were soaked in 16ml of disinfectant solution as received by study sponsor (undiluted) for 30 minutes before the wiping procedure.
5. Incubation temperatures: Bacteria at 37°C ± 1°C, Yeast at 30°C ± 1°C.
6. Drying time: 30minutes for all microorganisms.

TEST RESULTS FOR *Pseudomonas aeruginosa*

Validation suspension (Nv0)		Neutralizer control (B)		Method validation (C) Product conc.: undiluted	
Vc 1	68	Vc 1	85	Vc 1	53
Vc 2	74	Vc 2	71	Vc 2	61
x mean	71	x mean	78	x mean	57
30 ≤ x mean of Nv0 ≤ 160?		x mean of B is ≥ 0,5*x mean of Nv0?		x mean of C is ≥ 0,5*x mean of Nv0?	
Yes		Yes		Yes	

Test suspension (N and No)				
N	Vc1	Vc2	X mean =	9.17 ≤ lgN ≤ 9.70 ?
10 ⁻⁷	231	245	248.18 x10 ⁻⁷	lgN= 9.39
10 ⁻⁸	34	36	No = N/20	lgNo= 8.09
7.88 ≤ lgNo ≤ 8.40 ?				Yes

Drying control (Dc0)				
Dc0	Vc1	Vc2	X mean =	lgT0= 7.24
10 ⁻⁵	156	192	173.64 x10 ⁻⁵	
10 ⁻⁶	14	20	6.88 ≤ lgT0 ≤ 8.40 ?	
				Yes

Drying control (Dct)				
Dct	Vc1	Vc2	X mean =	lgTt= 7.16
10 ⁻⁵	167	117	143.18 x10 ⁻⁵	
10 ⁻⁶	12	19	6.88 ≤ lgTt ≤ 8.40 ?	
				Yes

Test field 1 Reduction (Na)		Dilution step	Vc1	Vc2	Average of Vc1 and Vc2	Lg Na	Log Reduction lg Tt - lg Na	Criteria	Result
Product concentration (%)	Contact time (min)								
undiluted	5min	10 ⁰	< 14	< 14	< 14	< 1.15	> 6.01	≥5	PASS TEST
		10 ⁻¹	< 14	< 14					

Test fields 2-4 (cfu/25cm2)		Fields T2-T4 of Na			VT2to2 (= X or Xwm x 5) cfu/25cm ²	Contact time	Criteria x < 50
Product concentration (%)	Dilution step	Vc T2	Vc T3	Vc T4			
undiluted	10 ⁰	0	0	0	0	5min	Yes

Test fields 2-4 (cfu/25cm2)		Fields T2-T4 of Nw			VNw (= X or Xwm x 5) cfu/25cm ²	Contact time	Criteria Xwm > 10
Product concentration (%)	Dilution step	Vc T2	Vc T3	Vc T4			
undiluted	10 ⁰	246	256	283	1308	5min	Yes
	10 ⁻¹	24	19	31			

TEST RESULTS FOR *Staphylococcus aureus*

Validation suspension (Nv0)		Neutralizer control (B)		Method validation (C) Product conc.: undiluted	
Vc 1	46	Vc 1	39	Vc 1	28
Vc 2	36	Vc 2	40	Vc 2	35
x mean	41	x mean	39.5	x mean	31.5
30 ≤ x mean of Nv0 ≤ 160?		x mean of B is ≥ 0,5 × x mean of Nv0?		x mean of C is ≥ 0,5 × x mean of Nv0?	
Yes		Yes		Yes	

Test suspension (N and No)				
N	Vc1	Vc2	X mean =	9.17 ≤ lgN ≤ 9.70 ?
10 ⁻⁷	139	153	150.45 × 10 ⁻⁷	lgN = 9.18
10 ⁻⁸	18	21	No = N/20	lgNo = 7.88
7.88 ≤ lgNo ≤ 8.40 ?				Yes

Drying control (Dc0)				
Dc0	Vc1	Vc2	X mean =	lgT0 = 7.11
10 ⁻⁵	114	136	127.73 × 10 ⁻⁵	
10 ⁻⁶	15	16	6.88 ≤ lgT0 ≤ 8.40 ?	
				Yes

Drying control (Dct)				
Dct	Vc1	Vc2	X mean =	lgTt = 7.09
10 ⁻⁵	122	117	122.73 × 10 ⁻⁵	
10 ⁻⁶	12	19	6.88 ≤ lgTt ≤ 8.40 ?	
				Yes

Test field 1 Reduction (Na)		Dilution step	Vc1	Vc2	Average of Vc1 and Vc2	Lg Na	Log Reduction lg Tt - lg Na	Criteria	Result
Product concentration (%)	Contact time (min)								
undiluted	5min	10 ⁰	< 14	< 14	< 14	< 1.15	> 5.94	≥ 5	PASS TEST
		10 ⁻¹	< 14	< 14					

Test fields 2-4 (cfu/25cm ²)		Fields T2-T4 of Na			VT2to2 (= X or Xwm × 5) cfu/25cm ²	Contact time	Criteria x < 50
Product concentration (%)	Dilution step	Vc T2	Vc T3	Vc T4			
undiluted	10 ⁰	0	0	0	0	5min	Yes

Test fields 2-4 (cfu/25cm ²)		Fields T2-T4 of Nw			VNw (= X or Xwm × 5) cfu/25cm ²	Contact time	Criteria Xwm > 10
Product concentration (%)	Dilution step	Vc T2	Vc T3	Vc T4			
undiluted	10 ⁰	175	169	204	913	5min	Yes
	10 ⁻¹	21	18	22			

TEST RESULTS FOR *Enterococcus hirae*

Validation suspension (Nv0)		Neutralizer control (B)		Method validation (C) Product conc.: undiluted	
Vc 1	62	Vc 1	43	Vc 1	42
Vc 2	55	Vc 2	47	Vc 2	50
x mean	59	x mean	45	x mean	46
30 ≤ x mean of Nv0 ≤ 160? Yes		x mean of B is ≥ 0,5*x mean of Nv0? Yes		x mean of C is ≥ 0,5*x mean of Nv0? Yes	

Test suspension (N and No)				
N	Vc1	Vc2	X mean =	9.17 ≤ lgN ≤ 9.70 ?
10 ⁻⁷	189	194	191.82 x10 ⁻⁷	lgN= 9.28
10 ⁻⁸	16	23	No = N/20	lgNo= 7.98
7.88 ≤ lgNo ≤ 8.40 ?				Yes

Drying control (Dc0)				
Dc0	Vc1	Vc2	X mean =	
10 ⁻⁵	141	157	150.91 x10 ⁻⁵	lgT0= 7.18
10 ⁻⁶	18	16	6.88 ≤ lgT0 ≤ 8.40 ?	Yes

Drying control (Dct)				
Dct	Vc1	Vc2	X mean =	
10 ⁻⁵	95	104	102.73 x10 ⁻⁵	lgTt= 7.01
10 ⁻⁶	12	15	6.88 ≤ lgTt ≤ 8.40 ?	Yes

Test field 1 Reduction (Na)		Dilution step	Vc1	Vc2	Avarage of Vc1 and Vc2	Lg Na	Log Reduction lg Tt - lg Na	Criteria	Result
Product concentration (%)	Contact time (min)								
undiluted	5min	10 ⁰	< 14	< 14	< 14	< 1.15	> 5.87	≥5	PASS TEST
		10 ⁻¹	< 14	< 14					

Test fields 2-4 (cfu/25cm ²)		Fields T2-T4 of Na			VT2to2 (= X or Xwm x 5) cfu/25cm ²	Contact time	Criteria x < 50
Product concentration (%)	Dilution step	Vc T2	Vc T3	Vc T4			
undiluted	10 ⁰	0	0	0	0	5min	Yes

Test fields 2-4 (cfu/25cm ²)		Fields T2-T4 of Nw			VNw (= X or Xwm x 5) cfu/25cm ²	Contact time	Criteria Xwm > 10
Product concentration (%)	Dilution step	Vc T2	Vc T3	Vc T4			
undiluted	10 ⁰	231	189	234	1090	5min	Yes
	10 ⁻¹	23	16	22			

TEST RESULTS FOR *Candida albicans*

Validation suspension (Nv0)		Neutralizer control (B)		Method validation (C) Product conc.: undiluted	
Vc 1	38	Vc 1	29	Vc 1	33
Vc 2	40	Vc 2	35	Vc 2	30
x mean	39	x mean	32	x mean	31.5
30 ≤ x mean of Nv0 ≤ 160? Yes		x mean of B is ≥ 0,5*x mean of Nv0? Yes		x mean of C is ≥ 0,5*x mean of Nv0? Yes	

Test suspension (N and No)				
N	Vc1	Vc2	X mean =	8.17 ≤ lgN ≤ 8.70 ?
10 ⁻⁶	169	173	170.00 x10 ⁻⁶	lgN= 8.23
10 ⁻⁷	17	15	No = N/20	lgNo= 6.93
6.88 ≤ lgNo ≤ 7.40 ?				Yes

Drying control (Dc0)				
Dc0	Vc1	Vc2	X mean =	
10 ⁻⁴	98	82	88.64 x10 ⁻⁴	lgT0= 5.95
10 ⁻⁵	9	6	5.88 ≤ lgT0 ≤ 7.40 ?	Yes

Drying control (Dct)				
Dct	Vc1	Vc2	X mean =	
10 ⁻⁴	94	93	93.18 x10 ⁻⁴	lgTt= 5.97
10 ⁻⁵	10	8	5.88 ≤ lgT0 ≤ 7.40 ?	Yes

Test field 1 Reduction (Na)		Dilution step	Vc1	Vc2	Average of Vc1 and Vc2	Lg Na	Log Reduction lg Tt - lg Na	Criteria	Result
Product concentration (%)	Contact time (min)								
undiluted	5min	10 ⁰	< 14	< 14	< 14	< 1.15	> 4.82	≥ 4	PASS TEST
		10 ⁻¹	< 14	< 14					

Test fields 2-4 (cfu/25cm ²)		Fields T2-T4 of Na			VT2to2 (= X or Xwm x 5) cfu/25cm ²	Contact time	Criteria x < 50
Product concentration (%)	Dilution step	Vc T2	Vc T3	Vc T4			
undiluted	10 ⁰	0	0	0	0	5min	Yes

Test fields 2-4 (cfu/25cm ²)		Fields T2-T4 of Nw			VNw (= X or Xwm x 5) cfu/25cm ²	Contact time	Criteria Xwm > 10
Product concentration (%)	Dilution step	Vc T2	Vc T3	Vc T4			
undiluted	10 ⁰	154	89	139	637	5min	Yes
	10 ⁻¹	18	15	9			

CONCLUSION

TEST PRODUCT IDENTIFICATION

PRODUCT NAME	: STERI-7 XTRA SURFACE DISINFECTANT
PRODUCT TYPE	: Surface Disinfectant/ Pre Saturated Wipes
ACTIVE SUBSTANCES	: 0.147% w/w Didecyldimethylammonium chloride 0.086% w/w Benzalkonium chloride 0.0854% w/w Polyhexamethylene biguanide
APPEARANCE OF THE PRODUCT	: Wipes
STORAGE CONDITIONS	: Room temperature, darkness
TEST CONDITIONS	: Test conducted at 20°C ± 1 °C
LOT	: Not listed
METHOD	: EN 16615:2015
CONTACT TIME	: 5 minutes
DILUTION	: Undiluted
PRODUCT DILUENT	: Water
STUDY SPONSOR	: STERI-7 WORLDWIDE LTD
PRODUCT MANUFACTURER	: STERI-7 WORLDWIDE LTD
RECEIPT DATE	: 18/10/2018
STUDY PERIOD	: 27/11/2018 - 03/12/2018
LAB ID	: 2018-8337/18 23 00151

METHODOLOGY ABSTRACT

A test-surface is marked with 4 squares of 5 × 5 cm, the “test fields”, in a row. Test field 1 on the test surface is inoculated with a test suspension of bacteria or yeasts in a solution of interfering substances. The inoculum is dried. A wipe is soaked with a sample of the product as delivered (16ml for 30min). The test-surface is wiped with the soaked wipe across the four marked test fields, starting in front of test field 1, turning immediately after test field 4 and wiped back to the starting point. In parallel a water control is performed.

At the end of the contact time, the test organisms are recovered from each test field with moistened cotton swabs. The swabs are neutralized and the numbers of surviving test organisms in each sample are determined. The reduction is calculated by comparing the results of the drying control and the results obtained with the product. The test is performed using *P. aeruginosa*, *S. aureus*, *E. hirae* and *C. albicans* as test micro-organisms.

RESULT

The product under test: STERI-7 XTRA SURFACE DISINFECTANT demonstrated bactericidal activity for surface disinfection, according to the EN16615 standard under dirty conditions at 20 °C within 5 min for product dilution:

Neat (undiluted) for referenced strains *Pseudomonas aeruginosa*, *Staphylococcus aureus* and *Enterococcus hirae*.

The product under test: STERI-7 XTRA SURFACE DISINFECTANT demonstrated yeasticidal activity for surface disinfection, according to the EN16615 standard under dirty conditions at 20 °C within 5 min for product dilution:

Neat (undiluted) for referenced strains *Candida albicans*.

ARCHIVING

The laboratory book which contains all the information (raw data and results) regarding the study and the study reports are kept in the laboratory archives for 5 years.

Signature date: 05/12/2018

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Results refer to the sample as received and analyzed on the period specified above.

The test report shall not be reproduced except in full, without written approval of the laboratory.

The samples will be stored by the laboratory during 1 month from the end test date.

The study report and raw data will be stored by the laboratory for 5 years.

End of Test report