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Chemical disinfectants –
Quantitative suspension test
for evaluation of sporadicidal activity (phase 1)
(EN 14347)

Chemical disinfectants –
Quantitative suspension test
for evaluation of fungicidal activity
for chemical disinfection products (phase 2/ step 1)
(EN 1650)

Chemical disinfectants and antiseptics –
Quantitative suspension test
for the evaluation of bactericidal activity
of chemical disinfectants and antiseptics used in food,
industrial, domestic, and institutional areas -
test methods and requirements
(phase 2 / step 1) (EN 1276)

Chemical disinfectants – Quantitative suspension test for evaluation of sporadicidal activity (phase 1) (EN 14347)

Scope

This draft European Standard describes a suspension test method for establishing whether a product proposed as a chemical disinfectant has or does not have a sporadicidal activity under the laboratory conditions defined by this European Standard. This standard specifies a test method (phase 1) and the minimum requirements for sporadicidal activity of chemical disinfectant products that form a homogeneous physically stable preparation in water.

Requirements

The product, when tested in simulated conditions with the test protocol described above, shall demonstrate at least a 10^4 log reduction in viable counts when tested according to its practical applications and under required test conditions (20°C, 60 min) when the test organisms according to EN is Bacillus subtilis.

Test Method

The sporadicidal activity is evaluated using the following strain of spores commericially available:

Bacillus subtilis ATCC 6633

Product test solutions shall be prepared in hard water at three different concentrations to include at least in concentrations in the active range.

Dilution-neutralization method is the method of choice. Prior to testing all reagents are equilibrated to the test temperature of 20°C using the water bath.
Pipette 8 ml of product to be tested and 1,0 ml water substance into a container of suitable capacity. Add 1ml spore suspension containing $3{,}0 \times 10^8$ - 1×10^9 cfu/ml. Immediately start the stopwatch, mix and place the container in the water bath at 20°C for for the appropriate contact time (60 min).

NOTES

NOTES

At the chosen contact time, pipette 0.1ml of the test mixture into a tube containing 10 ml neutralizer (30g/l polysorbate 80 + 3g/l lecithine). Mix and incubate in the water bath for 30 minutes. After neutralization take a 1ml sample in duplicate and transfer on TSA plates. Incubate the plates in 36°C for 24 hours. Count the plates and determine the number of colony forming units for each plate.

Results

For each test organism record the number of cfu/ml in the sporadicidal test suspension (N) and after the test procedure for sporadicidal activity of the product (Na).

$$\text{Reduction in viability} = R = \frac{Nw}{Na} \quad \lg R = \lg Nw - \lg Na$$

Conclusion

The product shall be deemed to have passed the test if it demonstrates a 10^4 or more reduction in viability within 60 min or less at 20°C under the clean test conditions when the test organism *Bacillus subtilis* spores.

Test report

European draft Standard EN 14347
«Determination of the sporadicidal activity of chemical disinfectants»

Test laboratory:	Laboratory of Hygiene and Microbiology, Dept. of Public Health, University of Helsinki
Name of the products:	1. MultiDez 2. Industrial Teflex
Manufacturer:	ZAO Soft Protector, 195030, Himikov 28, Saint Petersburg, Russia, INN 7825341974 Tel. (812) 527-65-26 http:// www.teflex.net e-mail: admin@teflex.net
Date of delivery:	March 2010
Storage conditions:	room temperature
Test method and validation:	Dilution-neutralization method Polysorbate 80+lecithin neutralizer
Period of analysis:	week 15 2010
Product test concentrations:	1. MultiDez 0.005%, 0.05% and 0.4% 2. Industrial Teflex 0.4% and 1%
Test temperature:	20° ± 1° C
Product diluent:	hard water
Contact times:	60 min
Interfering substances:	0.3 g/l bovine serum albumine
Bacterial strain used:	*Bacilus subtilis* ATCC 6633

Industrial TEFLEX

Test microbe	colony	Forming units	/ ml
1.0%	Bacterial test suspensio (N)	Bacterial test mixture (N_a)	Reduction in viability
Escherichia coli	1.11×10^8	$< 1.5 \times 10^2$	$> 10^5$
Staphylococcus aureus	1.50×10^8	$< 1.5 \times 10^2$	$> 10^5$
Pseudomonas aeruginosa	1.42×10^8	$< 1.5 \times 10^2$	$> 10^5$
Enterococcus hirae	1.04×10^8	$< 1.5 \times 10^2$	$> 10^5$

Conclusion:
According to EN 1276 products tested possess good bacterical activity for all microbial strains tested.

Helsinki 19.4.2010

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MultiDez

Test microbe	colony	Forming units	/ ml
0.4%	Bacterial test suspensio (N)	Bacterial test mixture (N_a)	Reduction in viability
Escherichia coli	1.11×10^8	$< 1.5 \times 10^2$	$> 10^5$
Staphylococcus aureus	1.50×10^8	$< 1.5 \times 10^2$	$> 10^5$
Pseudomonas aeruginosa	1.42×10^8	$< 1.5 \times 10^2$	$> 10^5$
Enterococcus hirae	1.04×10^8	$< 1.5 \times 10^2$	$> 10^5$

Conclusion:
According to EN 1276 products tested possess good bacterical activity for all microbial strains tested, except at concentration 0.005% against Enterococcus hirae.

Test results: all concentrations in clean and dirty conditions

Industrial TEFLEX

Test microbe	colony	Forming units	/ ml
0.4%	Bacterial test suspensio (N)	Bacterial test mixture (N_a)	Reduction in viability
Escherichia coli	1.11×10^8	$< 1.5 \times 10^2$	$> 10^5$
Staphylococcus aureus	1.50×10^8	$< 1.5 \times 10^2$	$> 10^5$
Pseudomonas aeruginosa	1.42×10^8	$< 1.5 \times 10^2$	$> 10^5$
Enterococcus hirae	1.04×10^8	$< 1.5 \times 10^2$	$> 10^5$

Test verification and validation of the method done simultaneously with the test. Validation results were all acceptable.

MultiDez

Bacillus subtilis	Bacterial test suspension	Bacterial test mixture	Reduction in viability at test conc.
0.005%	1.12×10^8	$>1 \times 10^4$	$< 1x10^4$
0.05%	1.12×10^8	$>1 \times 10^4$	$< 1x10^4$
0.4%	1.12×1^{08}	2.82×10^3	$8.38x10^3$

Conclusion:
According to EN 14347 test product possesses borderline sporicidal activity at concentration 0.4% against *Bacillus subtilis* ATCC 6633.

Industrial Teflex

Bacillus subtilis	**Bacterial test suspension**	**Bacterial test mixture**	**Reduction in viability at test conc.**
0.4%	1.12×10^8	1.03×10^3	$9x10^3$
1%	1.12×10^8	4.8×10^2	$6.4x10^4$

Conclusion:
According to EN 14347 test products possesses good sporicidal activity at concentration 1% and borderline sporicidal activity at concentration 0.4% against Bacillus subtilis ATCC 6633.

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Chemical disinfectants – Quantitative suspension test for evaluation of fungicidal activity for chemical disinfection products (phase 2/ step 1) (EN 1650)

Scope

This draft European Standard describes a suspension test method for establishing whether a product proposed as a chemical disinfectant has or does not have a fungicidal activity under the laboratory conditions defined by this European Standard. This standard specifies a test method and the minimum requirements for fungicidal activity of chemical disinfectant products that form a homogeneous physically stable preparation in water.

Requirements

The product, when tested in simulated clean or dirty conditions with the test protocol described above, shall demonstrate at least a 104 log reduction in viable counts when tested according to its practical applications and under required test conditions (20°C, 15 min) when the test organisms are *Candida albicans* and *Aspergillus niger*.

Test Method

The fungicidal activity is evaluated using the following two strains:

Candida albicans	ATCC 10231
Aspergillus niger	ATCC 16404

In order to prepare the working culture of *Candida albicans*, subculture from the stock culture by streaking onto MEA agar and incubate overnight. Make a second subculture and incubate as earlier. The second subculture is the working culture. Candidal test suspensions are prepared by taking 10 ml of diluent (Tryptone Sodium Chloride solution) in a 100 ml flask with 10g of glass beads. From the working culture a loopfull of candida cells is transferred into the diluent and suspended. Flask is shaken 3 minutes using a mechanical shaker.

Test temperature: 20° + 1° C

Contact times: 5 min

Temperature of incubation: 37° + 1° C

Test verification and validation of the method done simultaneously with the test. Validation results were all acceptable.

Test results: all concentrations in clean and dirty conditions

MultiDez

Test microbe	colony	Forming units	/ ml
0.005%	Bacterial test suspensio (N)	Bacterial test mixture (N_a)	Reduction in viability
Escherichia coli	1.11×10^8	$< 1.5 \times 10^2$	$> 10^5$
Staphylococcus aureus	1.50×10^8	$< 1.5 \times 10^2$	$> 10^5$
Pseudomonas aeruginosa	1.42×10^8	$< 1.5 \times 10^2$	$> 10^5$
Enterococcus hirae	1.04×10^8	6.94×10^3	3.46×10^3

MultiDez

Test microbe	colony	Forming units	/ ml
0.05%	Bacterial test suspensio (N)	Bacterial test mixture (N_a)	Reduction in viability
Escherichia coli	1.11×10^8	$< 1.5 \times 10^2$	$> 10^5$
Staphylococcus aureus	1.50×10^8	$< 1.5 \times 10^2$	$> 10^5$
Pseudomonas aeruginosa	1.42×10^8	$< 1.5 \times 10^2$	$> 10^5$
Enterococcus hirae	1.04×10^8	$< 1.5 \times 10^2$	$> 10^5$

Test report

European Standard EN 1276

Test laboratory:	Laboratory of Hygiene and Microbiology, Dept. of Public Health, University of Helsinki
Name of the products:	1.MultiDez 2. IndustrialTeflex
Batch number:	
Manufacturer:	ZAO Soft Protector, 195030, Himikov 28, Saint Petersburg, Russia, INN 7825341974 Tel. (812) 527-65-26 http:// www.teflex.net e-mail: admin@teflex.net
Date of delivery:	March 2010
Storage conditions:	room temperature
Active substances:	PHMG
Test method and validation:	Dilution-neutralization method Polysorbate 80+lecithin neutralizer
Product test concentrations:	1. MultiDez 0.005%, 0.05% and 0.4% 2. Industrial Teflex 0.4% and 1%
Test microbes:	*Pseudomonas aeruginosa* ATCC 15442 *Staphylococcus aureus* ATCC 6538 *Escherichia coli* ATCC 10536 *Enterococcus hirae* ATCC 8043
Period of analysis:	week 14 2010

The number of cells in the suspension is adjusted to 1,5x107 cfu/ml to 5x107 cfu/ml using diluent by turbidimetry. Always check the cell count by dilution method.
For *Aspergillus niger* use only the first subculture grown on MEA in Roux bottles and incubate 7 days. No further sulbculturing is needed. Take the working culture and suspend the cells in 10 ml of sterile 0.05% polysorbate 80 solution in water. Using a sterile spatula detach the conidiospores. The suspension is gently shaken with glass beads and filtered through a fritted filter. Suspension is examined microscopically for absence of mycelia and spore germination. If mycelia or spores are present the suspension is washed again. The conidiospore suspension is the adjusted to $1,5x10^7$ cfu/ml to $5x10^7$ cfu/ml using the diluent. To determine the cell count the Malassaz chamber is used.

Product test solutions shall be prepared in hard water at three different concentrations to include at least in concentrations in the active range.

Dilution-neutralization method is the method of choice. Prior to testing all reagents are equilibrated to the test temperature of 20°C using the water bath.
Pipette 1,0 ml the interfering substance(s) into a container of suitable capacity. Add 1ml fungal suspension containing $1,5x10^7$ cfu/ml to $5x10^7$ cfu/ml. Immediately start the stopwatch, mix and place the container in the water bath at 20°C for 2 min. Pipette 8 ml of the test products and place the container again in the waterbath for the appropriate contact time (minimum 15 min). At the chosen contact time, pipette 1ml of the test mixture into a tube containing 8ml neutralizer (30g/l polysorbate 80 + 3g/l lecithine)
and 1ml of water. Mix and incubate in the water bath for 5 minutes. After neutralization take a 1ml sample in duplicate and transfer on MEA plates. Incubate the plates in 36°C for 24 to 48 hours. Count the plates and determine the number of colony forming units for each plate.

Results

For each test organism record the number of cfu/ml in the fungicidal test suspension (N) and after the test procedure for fungicidal activity of the product (N_a).

$$\text{Reduction in viability} = \frac{N \times 10^{-1}}{N_a}$$

Conclusion

The product shall be deemed to have passed the test if it demonstrates a 10^4 or more reduction in viability within 15 min or less at 20°C under the clean and/or dirty test conditions when the test organisms are *Candida albicans* and *Aspergillus niger*.

The number of cells in the suspension is adjusted to $1{,}5 \times 10^8$ cfu/ml to 5×10^8 cfu/ml using diluent by turbidimetry. Always check the cell count by dilution method.

Product test solutions shall be prepared at three different concentrations to include at least two concentrations in the active range.
Dilution-neutralization method is the method of choice. Prior to testing all reagents are equilibrated to the test temperature of 20°C using the water bath.
Pipette 8 ml of the test products in to container of suitable capacity and add 1ml of water. Add 1ml bacterial suspension containing $1{,}5 \times 10^8$ cfu/ml to 5x108 cfu/ml and 1ml of intefering subsrance (0.3/3g/l BSA) after incubating these two 2 minutes. Immediately start the stopwatch, mix and place the container in the water bath at 20°C. The activity of the product shall be determined for a contact time chosen from one of the following: 1min, 5min, 15min, 30min, 45min, 60min. At the chosen contact time, pipette 1ml of the test mixture into a tube containing 8ml neutralizer (30g/l polysorbate 80 + 3g/l lecithine)
and 1ml of water. Mix and incubate in the water bath for 5 minutes. After neutralization take a 1ml sample in duplicate and transfer on TSA plates. Incubate the plates in 36°C for 24 hours. Count the plates and determine the number of colony forming units for each plate.

Results

For each test organism record the number of cfu/ml in the bactericidal test suspension (N) and after the test procedure for bactericidal activity of the product (N_a).

$$\text{Reduction in viability} = \frac{N \times 10^{-1}}{N_a}$$

Conclusion

The product shall be deemed to have passed the test if it demonstrates a 10^5 or more reduction in viability within 60 min or less at 20°C.

Chemical disinfectants and antiseptics – Quantitative suspension test for the evaluation of bactericidal activity of chemical disinfectants and antiseptics used in food, industrial, domestic, and institutional areas - test methods and requirements (phase 2 / step 1) (EN 1276)

Scope

This European Standard describes a suspension test method and the minimum requirements for bactericidal activity of chemical disinfectant or antiseptic products that form a homogeneous physically stable preparation in hard water and are used in food, industrial, domestic, and institutional areas.

Requirements

The product, when tested in accordance with the test protocol described above, shall demonstrate at least a 10^5 log reduction in viable conts when the test organisms are *Pseudomonas aeruginosa*, *Staphylococcus aureus*, *Escherichia coli* and *Enterococcus hirae*.

Test Method

The bactericidal activity is evaluated using the following two strains:

Pseudomonas aeruginosa	ATCC 15442
Staphylococcus aureus	ATCC 6538
Enterococcus hirae	ATCC 8043
Escherichia coli	ATCC 10536

In order to prepare the working culture of bacterial strains, subculture from the stock culture by streaking onto TSA agar and incubate overnight. Make a second subculture and incubate as earlier. The second subculture is the working culture. Bacterial test suspensions are prepared by taking 10 ml of diluent (Tryptone Sodium Chloride solution) in a 100 ml flask with 5g of glass beads. From the working culture a loopfull of bacterial cells is transferred into the diluent and suspended. Flask is shaken 3 minutes using a mechanical shaker.

Test report

European Standard EN 1650

Test laboratory:	Laboratory of Hygiene and Microbiology, Dept. of Public Health, University of Helsinki
Name of the products:	1. MultiDez 2. Industrial Teflex
Batch number:	
Manufacturer:	ZAO Soft Protector, 195030, Himikov 28, Saint Petersburg, Russia, INN 7825341974 Tel. (812) 527-65-26 http:// www.teflex.net e-mail: admin@teflex.net
Date of delivery:	March 2010
Storage conditions:	room temperature
Active substances:	Polyhexamethyleneguanidine
Test method and validation:	Dilution-neutralization method Polysorbate 80+lecithin neutralizer
Period of analysis:	week 15
Product test concentrations:	1. MultiDez 0.4% and 1% 2. Industrial Teflex 0.4% and 1%
Test temperature:	20° ± 1° C
Product diluent:	hard water
Contact times:	60 min

Interfering substances: 0.3g/l BSA (clean conditions) and 3g/l BSA (dirty conditions)

Temperature of incubation: 37° ± 1° C

Test verification and validation of the method done simultaneously with the test. Validation results were all acceptable.

Test results in clean and dirty conditions

MultiDez

Test organisms	viable	counts	cfu/ml
Candida albicans	Fungicidal test suspension	Fungicidal test mixture	Reduction in viability at test conc.
0.4%	$5.21x10^7$	$<5x10^2$	$>10^4$
1%	$5.21x10^7$	$<5x10^2$	$>10^4$
Aspergillus niger			
0.4%	$5.64x10^7$	$>10^4$	$<10^4$
1%	$5.64x10^7$	$< 5x10^2$	$>10^4$

Conclusion: Concentration 0.4% showed good fungicidal activity against Candida albicans. Concentrationn 1% fullfills the standard requirements against Aspergillus niger.

Test results in clean and dirty conditions

Industrial Teflex

Test organisms	viable	counts	cfu/ml
Candida albicans	Fungicidal test suspension	Fungicidal test mixture	Reduction in viability at test conc.
0.4%	$5.21x10^7$	$1.14x10^1$	$>10^4$
1%	$5.21x10^7$	$<1x10^1$	$>10^4$
Aspergillus niger			
0.4%	$5.64x10^7$	$>10^4$	$<10^4$
1%	$5.64x10^7$	$<5x10^2$	$>10^4$

Conclusion: Concentrations 0.4% and 1% showed good fungicidal activity against *Candida albicans*. Concentration 1% fullfills the standard requirements against *Aspergillus niger*.

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