

EVERBRITE

TYPICAL PARAMETERS:

Features	Specification
Colour	Colourless
Odour	Slight, characteristic
Aspect	Clear liquid
Specific Gravity	1.06
pH (100%)	12.9
pH (10% aqueous solution)	11.8
pH (1% aqueous solution)	11.2
Surface tension (1 % aqueous solution)	29 mN/m
Viscosity at 23°C (spindle 1, 10 rpm, Brookfield)	30 mPa·s
Refraction index at 20°C	1.385

EVERBRITE

(NP 1679 - DR25a)

SUMMARY OF Bactericidal, Virucidal and Fungicidal Test Results

1- TEST RESULTS ON FRAME FORMULATION DR25a:

1.1 BACTERICIDAL / FUNGICIDAL PERFORMANCE:

1.1.1. Tested according to European Norms:

EN 1040

Bactericidal result, Test strains: P. aeruginosa and S. aureus

Result: **0.1 % 5 min.**

Certificate: Laboratory OPC-E, Switz, 19. February 2004

EN 1276

Bactericidal results in presence of organic load (Albumin)

Test strains: P. aeruginosa, S. aureus, E. coli and E. hirae

Results: **0.5 % 0.3 g/l Albumin 5 min.**

1.5 % 3.0 g/l Albumin 5 min.

Certificate: Dr. H. Brill, Hamburg, 12. Nov 2001

EN 1276

Bactericidal results in presence of organic load (Albumin)

Test strains: Legionella pneumophila Serogroup 1

Results: **1.0 % 5 min.**

Certificate: IMSL Laboratory, UK, 3 May 2006

prEN 13623

Bactericidal results in presence of organic load (yeast) and buffered ferrous hard water

Test strains: Legionella pneumophila Serogroup 1

Results: **1.0 % 60 min.**

Certificate: IMSL Laboratory, UK, 3 May 2006

EN 13697 (Surface test)

Bactericidal result in presence of organic load (Albumin)

Test strains: P. aeruginosa, S. aureus, E. coli and E. hirae

Result: **2.0 % 3.0 g/l Albumin 5 min.**

Fungicidal result in presence of organic load (Albumin)

Test strains: A. niger and C. albicans

Result: **4.0 % 3.0 g/l Albumin 15 min.**

Certificates: Laboratory OPC-E, Switz., 19. October 2004

EN 1650

Fungicidal results in presence of organic load (Albumin)

Test strains: A. niger and C. albicans

Results: **0.5 % 0.3 g/l Albumin 15 min.**

1.0 % 3.0 g/l Albumin 15 min.

Certificate: Dr. H. Brill, Hamburg, 14. Nov 2001

Everbrite

Test results, continued...

EN 1275

Fungicidal result, Test strains: Aspergillus niger and Candida albicans

Result: 2.0% 15 min

Certificate: Laboratory OPC-E, Switz, 22 July 2005

1.1.2. Tested according other procedures

Tuberculocidal test

Test method: Qualitative suspension test

Test organism: Mycobacterium terrae ATCC 15755

Result: **1:27 (3.7%) diluted in deionized water - 5 min.**

Certificate: MICROBIOTEST Inc., Sterling, Virginia, USA, Study 163-230, 11 April 2002

1.1.3. Tested according to DGHM: (German Society for Hygiene and Microbiology), now VAH

Surface disinfection:

Mechanical disinfection of non porous hard surfaces in hospital and general practice:

Test strains: P. aeruginosa, S. aureus, E. hirae and C. albicans

Results: Low organic load (0.3% alb.)	Increased organic load (3% alb.)
2.0% 15 and 30 minutes	2.0% 15, 30 and 60 minutes
1.0% 60 minutes	1.0% 240 minutes
0.5% 240 minutes	

Certificates: Prof. Dr. R. Schubert, Frankfurt (M), 29. Dec 2002

Prof. Dr. H.-P. Werner, Schwerin, 04. Oct 2002

Salmonellacidal efficacy :

Test method: DGHM qualitative suspension test (without organic load)

Test organism: Salmonella typhimunium

Results: **0.25% 5 minutes**
0.05% 15, 30, 60 minutes

Certificate: Prof. Dr. H.-P. Werner, Schwerin, 31. Jan 2003

Tuberculocidal efficacy :

Test method: DGHM, surface disinfection (low organic load)

Test organism: Mycobacterium terrae

Results: **5.0% 30 minutes**
3.0% 60 minutes
2.0% 240 minutes

Certificate: Prof. Dr. R. Schubert, Frankfurt (M), 8. April 2005

1.1.4 Tested according to DVG Food sector : (German Veterinary Medical Society)

Disinfectants for handling / processing area regarding animal based food.

Test strains: P. aeruginosa, S. aureus, E. faecium, P. mirabilis and C. albicans

Sector A: 10% Bovine serum as protein load

Sector A / B	°C	Use concentration in volume percent (V-%) for 30 and 60 minutes							
		Low organic load				Increased organic load			
		Bacteria		Fungi		Bacteria		Fungi	
		30'	60'	30'	60'	30'	60'	30'	60'
4	5	6a	6b	7a	7b	8a	8b	9a	9b
A	20°C	0.2%	0.2%	0.1%	0.1%	1.5%	1.5%	1.0%	0.2%
A	10°C	1.0%	1.0%	0.1%	0.1%	3.0%	2.5%	1.0%	0.5%

Certificates: Prof. Dr. R. Böhm, Stuttgart, 8 August 2005

Prof. Dr. R. Schubert, Frankfurt (M), 6 May 2006

Test results, continued...

1.2 VIRUCIDAL PERFORMANCE:

1.2.1. Tested according to BGA (now RKI)/ DVV

Polio virus type 1 (Picorna, non env.)

Quantitative suspension test (for surgical instruments) -Results according BGA and DVV

With soil load (2% albumin and 10% bovine serum)

5.0 % 15 min.

4.0 % 60 min.

German Society for the control of Virus Diseases

Certificate: Dr. J. Steinmann, Bremen, 15. Feb 2002

ECBO virus (Enteric Cytopathogenic Bovine Orphan)- Veterinary field

Quantitative suspension test - Results according BGA and DVV requirements

With soil load (2% albumin and 10% bovine serum)

5.0 % 30 min.

3.0 % 60 min.

German Society for the control of Virus Diseases

Certificate: Dr. J. Steinmann, Bremen, 21. Aug 2002

Adeno virus

Result according BGA (now RKI) and DVV

With soil load **4.0 % 30 min.**

Certificate: Dr. J. Steinmann, Bremen, 24. May 2005

Noro (Norwalk) virus

Feline calici virus (FCV) was used as surrogate

Result according BGA (now RKI) and DVV

With soil load **4.0 % 30 min.**

Certificate: Dr. J. Steinmann, Bremen, 25. May 2005

Rota virus

Result according BGA (now RKI) and DVV

Without soil load **3.0 % 15 min**

Certificate: Dr. J. Steinmann, Bremen, 8 June 2005

Vaccinia virus

Result according BGA (now RKI) and DVV

With soil load **2.0 % 5 min**

Certificate: Dr. J. Steinmann, Bremen, 30 July 2005

Polyoma virus SV 40 (formely Papova virus)

Result according BGA (now RKI) and DVV

With soil load **2.0 % 30 min**

Certificate: Dr. J. Steinmann, Bremen, 9 March 2006

Summary of Dr. J. Steinmann, Mikrolab Bremen:

The surface disinfectant DR-25a fulfils the requirement "virucidal" defined by a working group "Viruzidie" of the Robert Koch-Institute (RKI), the expert group of DVV (German Association for the Control of Virus Diseases) "virus disinfection" and the disinfectant commission of DGHM (now VAH). Following this recommendation a disinfectant can be declared "virucidal", if it is able to inactivate following four test viruses (Polio-, Adeno-, Vaccinia- and Polyoma virus) in a quantitative suspension test under defined conditions.

Thus, the following concentrations and exposure times are necessary for inactivation of above mentioned four test viruses:

4.0% 60 minutes

5.0% 15 minutes

Everbrite

Test results, continued...

1.2.2 Tested according to DVG (German Veterinary Medical Society)

Animal husbandry

Enveloped viruses (column 7b, 20°C, limited virucidal performance)

Test virus: Newcastle disease, Vaccinia virus

Result: **3% 120 minutes**

Certificate: Prof Dr. E. F. Kaleta, Frankfurt (M), 30 October 2003

1.2.3. Tested according to EN 14476: 2005

Polio virus

Clean conditions: 4.0 % 30 min

Dirty conditions: 6.0 % 120 min

Certificate: Dr. J. Steinmann, Mikrolab Bremen, 24 December 2005

Adeno Virus

Clean conditions: 2.0 % 60 min

4.0 % 30 min

Dirty conditions: 2.0 % 60 min

4.0 % 30 min

Certificate: Dr. J. Steinmann, Mikrolab Bremen, 20 December 2005

Summary (Polio- and Adeno virus) of Dr. J. Steinmann, MikroLab Bremen

The following concentrations and exposure times are necessary for inactivation of the two test viruses (Polio- and Adeno virus):

4.0% 30 minutes (clean)

6.0% 120 minutes (dirty)

In order to achieve a four log₁₀ reduction (inactivation ≥ 99.99%) in a quantitative suspension test according to the EN 14476 under clean and dirty conditions.

After evaluation with Polio virus and Adeno virus the surface disinfectant DR-25a can be declared as having “virucidal” properties according to EN 14476:2005.

Therefore, after successful experiments with the above mentioned non-enveloped viruses the surface disinfectant **DR-25a is also effective against enveloped viruses including HBC, HCV and HIV.**

Avian influenza virus (H₃N₂ / H₅N₁)

Result according EN 14476:2005

Influenza virus A/duck/Ukraine/1/63 (H₃N₂) was incorporated as surrogate of Avian influenza virus (H₅N₁) due to bio safety reasons.

Clean conditions 0

1

Dirty conditions 0

1

Certificate: Dr. J. Steinmann, MikroLab Bremen, 13 February 2006

1.2.4. Tested according other procedures

Canine parvovirus (type-2)

With soil load (5% minimum organic load)

1:35 (2.86%) diluted in deionized water 10 min

1:35 (2.86%) diluted in 400 ppm AOAC hard water 10 min.

Everbrite

Certificate: MICROBIOTEST Inc., Sterling, Virginia, USA, Study 163-238, 03. Jan 2003

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Everbrite

Test results, continued...

1.3. REGISTRATIONS (formulation DR25a):

- France:** Registered with Ministry of Agriculture, Paris
N° 2020122, 4 September 2003
Dosage against bacteria 1.5%
Dosage against fungi 4.0%
- Spain:** Registered with Ministry of Agriculture, Madrid
Veterinary application, Registration N° 0946-P, 31 May 2006
Valid until 2 February 2011
- Switzerland:** Registered with Federal Health Office, Bern
BAG E N° 1510, 25 May 2004
BAG T N° 105525, Toxicity class N° 4, 12 August 2004
- Food Industry** (Ingredients according French Positive List (Matériaux au contact des denrées alimentaires produits de nettoyage de ces matériaux, 1997))

II - TESTS CARRIED OUT AT: NCH EUROPEAN TECHNICAL CENTRE, RIPLEY, UK:

Bactericidal Activity according to EN 1276, 1997:

August 2005:

Listeria monocytogenes	0.1% dilution, 5 min contact time, hard water conditions (300ppm) and soiling agent (3g/l bovine albumin)
Klebsiella pneumoniae	1% dilution, 5 min contact time, hard water conditions (300ppm) and soiling agent (3g/l bovine albumin)
MRSA – (Methycillin Resistant Staph aureus)	0.1% dilution, 5 min contact time, hard water conditions (300ppm) and soiling agent (3g/l bovine albumin)
Intermediate -VRSA (Vancomycin Resistant Staphylococcus aureus)	0.05% dilution, 5 min contact time, hard water conditions (300ppm) and soiling agent (3g/l bovine albumin)

Fungicidal Activity according to EN 1650, 1998:

September 2005:

Candida albicans	0.25% dilution, 15 min contact time, hard water conditions (300ppm) and soiling agent (3g/l bovine albumin)
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October 2005:

Aspergillus niger	2% dilution, 15 min contact time, hard water conditions (300ppm) and soiling agent (3g/l bovine albumin)
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Everbrite

Test results, continued...

II - TESTS CARRIED OUT AT MGS LABORATORIES Limited, UK:

Bactericidal Activity according to EN 1276, 1997:

November 2005:

Clostridium difficile 4% dilution, 60 min contact time, hard water conditions (300ppm)
and soiling agent (3g/l bovine albumin)*

* Pass criteria = log 3 reduction only, as the organisms is a spore forming bacteria.

II - TESTS CARRIED OUT AT IMSL LABORATORY, UK:

EN 1276

Bactericidal results in presence of organic load (Albumin)

Test strains: Legionella pneumophila Serogroup 1

Results: **1.0 % 5 min.**

Certificate: IMSL Laboratory, UK, 3 May 2006

prEN 13623

Bactericidal results in presence of organic load (yeast) and buffered ferrous hard water

Test strains: Legionella pneumophila Serogroup 1

Results: **1.0 % 60 min.**

Certificate: IMSL Laboratory, UK, 3 May 2006

**Everbrite (NP1679) Efficacy Against
Intermediate Vancomycin Resistant Staphylococcus aureus (VRSA)
Using EN 1276 : 1997**

Introduction

Staphylococcus aureus is a bacteria commonly found on the skin and in the nose of healthy people. Occasionally Staph. aureus can get into the body and can cause infection. This can be minor pimples and boils or more serious blood infections or pneumonia. In the past Staph aureus infections have been treated with antibiotics, especially penicillin, then methicillin. With many years of treatment, some antibiotic resistant strains of Staphylococcus aureus have emerged and in particular the MRSA strains that are resistant to methicillin and other commonly used antibiotics. The alternative antibiotic that has been used and that is the most effective and reliable drug in these cases is Vancomycin.

More recently, in the last couple of years, some strains of Staph. aureus that are 4 times more resistant to vancomycin have been isolated. This means that usual doses of vancomycin treatment are not effective for treating these infections. Those strains have been called Intermediate Vancomycin Resistant Staph. aureus or also Vancomycin Intermediate Staph aureus (VISA).

However, to date, all infections due to VISA have been cured using very high doses of Vancomycin along with other antimicrobial drugs.

The ultimate strain, the Vancomycin Resistant Staph. aureus (VRSA) would be resistant to vancomycin, and vancomycin would not be effective at all for treating these infections.

Because Staph. aureus can survive dry conditions, they can remain alive for long periods on dust particles, clothing, furniture, or hospital equipment. Staph. aureus often spreads from patient to patient via the hands of hospital workers or via dust, clothing, furniture or medical equipment that has been in contact with infected patients.

The use of infections control practices (such as washing hands and disinfecting hard surfaces and equipment) can significantly reduce the spread of VRSA, as VRSA are NOT resistant to disinfectants and can be easily removed and killed by cleaning and disinfection procedures.

The Test

This test was carried out to assess the efficacy of Everbrite, a sanitiser/disinfectant, against Intermediate VRSA.

The EN1276:1997 efficacy tests were carried out on the disinfectant solutions made up from Everbrite. The concentrations tested were 0.25%, 0.10%, 0.05% & 0.01% v/v of the concentrated product. The contact time was 5 minutes at 20°C.

**Determination of Bactericidal Activity for
Intermediate VRSA mu50 PHLS (Prof. Keiichi Hiramatsu)**

a) ID Test Lab.....NCH European Technical Centre

b) ID Samples

Name Everbrite
Batch No..... NP 1679 167/87A
Manufacturer..... NCH
Date of Delivery..... 15/07/05
Storage..... Room Temperature
Active ingredients..... QAC

c) Test Method and its validation

Method..... EN1276:1997 Quantitative Suspension Test for the Evaluation of bactericidal activity of chemical disinfectants and antiseptics used in food, industrial, domestic and institutional areas - Test Method.

Neutraliser 6g/l Lecithin, 60g/l polysorbate 80, 2g/l L-Histidine, 10g/l Sodium Thiosulphate in Maximum Recovery Diluent.

d) Experimental Conditions

Period of analysis..... 1/08/05 - 05/08/05
Product Diluent Sterile hard water 300mg/kg CaCO₃
Test Concentrations..... 0.25%, 0.10%,0.05%, 0.01% v/v.
Appearance of Dilutions Colourless, clear product
Temperature..... 20°C
Contact time..... 5 mins ± 10s
Interfering substance... 3g/l bovine albumin
Incubation Temp..... 36°C ± 1°C
Bacterial Strain used... Intermediate VRSA mu50 PHLS (Prof. Keiichi Hiramatsu)

e) Test Results

See Tables 1 and 2

f) Conclusion

According to EN 1276:1997, Everbrite NP1679 167/87A when diluted to 0.05% v/v in hard water possesses bactericidal activity in 5 minutes at 20°C under dirty conditions (3g/l bovine albumin) for the strain Intermediate VRSA mu50 PHLS (Prof. Keiichi Hiramatsu).

5 min Contact Time

Everbrite 0.05% dilution produced a 5 log reduction in viable count of a strain of Intermediate-VRSA and PASSED the EN1276:1997

Results

Pass Rate for EN 1276:1997 is 5 log reduction or 99.999% kill.

Table 1 Verification of the methodology and validation of dilution-neutralisation method for the test concentration of 0.05% of the test product - Everbrite

Test organism	Viable Count (cfu/ml)				
	Bacterial Test suspension (N)	Bacterial Suspension (Nv)	Experimental conditions (A)	Neutraliser toxicity control (B)	Dilution-Neutralisation control (C)
Intermediate VRSA mu50	10 ⁻⁶ : 209 10 ⁻⁷ : 29 N: 2.5 x10 ⁸	2.4 x10 ³	2.1 x10 ²	2.0 x10 ²	2.2 x 10 ²

For the strains tested:

N = number of cfu/ml of the bacterial test suspension

Nv = number of cfu/ml of the bacterial suspension

A = number of cfu/ml of the experimental conditions validation

B = number of cfu/ml of the neutraliser toxicity validation

C = number of cfu/ml of the dilution-neutralisation validation

The Neutraliser is validated for the test concentration of 0.05% solution of Everbrite for the VRSA strain tested.

Table 2 Test Results

Test Organism	Viable counts (cfu/ml) for the test mixture			
	0.25% Everbrite 5 min	0.10% Everbrite 5 min	0.05% Everbrite 5 min	0.01% Everbrite 5 min
Intermediate-VRSA mu50	Vc 0, 0 Na <1.5 x 10 ²	Vc 0, 0 Na <1.5 x 10 ²	Vc 0, 0 Na <1.5 x 10 ²	Vc >300, >300 Na >3.0 x 10 ³
Reduction in viability at test concentration (R) i.e. Log reduction/kill	> 10 ⁵	> 10 ⁵	>10 ⁵	<10 ⁵
Test Result	Pass	Pass	Pass	Fail

Vc = Viable Count

Na = number of cfu/ml in test mixture

R = reduction in viability

CERTIFICATE OF ANALYSIS

EN 1276 : 1997 Chemical Disinfectants and Antiseptics –Quantitative suspension test for the evaluation of bactericidal activity of chemical disinfectant and antiseptics used in food, industrial, domestic, and institutional areas- Test Method and Requirements

SAMPLE: EVERBRITE NP1679 167/87A

Challenge Organism Intermediate Vancomycin Resistant *Staphylococcus aureus*
Intermediate-VRSA mu50 PHLS (Prof. Keiichi Hiramatsu)

Contact Time 5 minutes

Temp 20°C

Soil 3g/l Bovine Albumin

Diluent Hard Water 300mg/kg CaCO₃

PASS Level for EN 1276:1997 - 5 log ↓ in Challenge Organism Inoculum in 5 minutes

Object of Test: To achieve a 5 log reduction in Challenge Organism Inoculum

5 Minute Contact Time

Everbrite 0.05% Passed EN 1276:1997
Producing a 5 log ↓ Against
Intermediate-VRSA

Signed: Joanne Green
Microbiologist

15 August 2005

Everbrite Efficacy Against Klebsiella pneumoniae using EN 1276 : 1997

Introduction

This test was carried out to assess the efficacy of Everbrite, a sanitiser/disinfectant, against *Klebsiella pneumoniae*.

The EN1276:1997 efficacy tests were carried out on the disinfectant solutions made up from Everbrite. The concentrations tested were 2%, 1%, 0.5% & 0.1% v/v of the concentrated product. The contact time was 5 minutes at 20°C.

Everbrite

**Determination of Bactericidal Activity for
Klebsiella pneumoniae NCTC 9633**

a) **ID Test Lab**.....NCH European Technical Centre

g) ID Samples

Name Everbrite
Batch No..... NP 1679 167/87A
Manufacturer..... NCH
Date of Delivery..... 15/07/05
Storage..... Room Temperature
Active ingredients..... QAC

h) Test Method and its validation

Method..... EN1276:1997 Quantitative Suspension Test for the Evaluation of bactericidal activity of chemical disinfectants and antiseptics used in food, industrial, domestic and institutional areas - Test Method.

Neutraliser 6g/l Lecithin, 60g/l polysorbate 80, 2g/l L-Histidine, 10g/l Sodium Thiosulphate in Maximum Recovery Diluent.

i) Experimental Conditions

Period of analysis..... 08/08/05 - 12/08/05
Product Diluent Sterile hard water 300mg/kg CaCO₃
Test Concentrations..... 2%, 1%, 0.5%, 0.1% v/v.
Appearance of Dilutions Colourless, clear product
Temperature..... 20°C
Contact time..... 5 mins ± 10s
Interfering substance... 3g/l bovine albumin
Incubation Temp..... 36°C ± 1°C
Bacterial Strain used... Klebsiella pneumoniae NCTC 9633

e) See Tables 1 and 2

f) Conclusion

According to EN 1276:1997, Everbrite NP1679 167/87A when diluted to 1% v/v in hard water possesses bactericidal activity in 5 minutes at 20°C under dirty conditions (3g/l bovine albumin) for the strain Klebsiella pneumoniae NCTC 9633.

5 min Contact Time

Everbrite 1% dilution produced a 5 log reduction in viable count of a strain of Klebsiella pneumoniae NCTC 9633 and PASSED the EN1276:1997

Results

Pass Rate for EN 1276:1997 is 5 log reduction or 99.999% kill.

Table 1 Verification of the methodology and validation of dilution-neutralisation method for the test concentration of 1% of the test product – Everbrite

Test organism	Viable Count (cfu/ml)				
	Bacterial Test suspension (N)	Bacterial Suspension (Nv)	Experimental conditions (A)	Neutraliser toxicity control (B)	Dilution-Neutralisation control (C)
Kleb. pneumoniae	10 ⁻⁶ : 168 10 ⁻⁷ : 16 N: 1.6 x10 ⁸	1.5 x10 ³	1.5 x10 ²	1.5 x10 ²	1.6 x 10 ²

For the strains tested:

N = number of cfu/ml of the bacterial test suspension

Nv = number of cfu/ml of the bacterial suspension

A = number of cfu/ml of the experimental conditions validation

B = number of cfu/ml of the neutraliser toxicity validation

C = number of cfu/ml of the dilution-neutralisation validation

The Neutraliser is validated for the test concentration of 1% solution of Everbrite for the Klebsiella pneumoniae strain tested.

Table 2 Test Results

Test Organism	Viable counts (cfu/ml) for the test mixture			
	2% Everbrite 5 min	1% Everbrite 5 min	0.5% Everbrite 5 min	0.1% Everbrite 5 min
Klebsiella pneumoniae	Vc 0, 0 Na <1.5 x 10 ²	Vc 0, 0 Na <1.5 x 10 ²	Vc 35, 41 Na >3.0 x 10 ³	Vc >300, >300 Na >3.0 x 10 ³
Reduction in viability at test concentration (R) i.e. Log reduction/kill	> 10 ⁵	> 10 ⁵	<10 ⁵	<10 ⁵
Test Result	Pass	Pass	Fail	Fail

Vc = Viable Count

Na = number of cfu/ml in test mixture

R = reduction in viability

CERTIFICATE OF ANALYSIS

EN 1276 : 1997 Chemical Disinfectants and Antiseptics –Quantitative suspension test for the evaluation of bactericidal activity of chemical disinfectant and antiseptics used in food, industrial, domestic, and institutional areas- Test Method and Requirements

SAMPLE: EVERBRITE NP1679 167/87A

Challenge Organism *Klebsiella pneumoniae* **NCTC 9633**

Contact Time 5 minutes

Temp 20°C

Soil 3g/l Bovine Albumin

Diluent Hard Water 300mg/kg CaCO₃

PASS Level for EN 1276:1997 - 5 log ↓ in Challenge Organism Inoculum in 5 minutes

Object of Test: To achieve a 5 log reduction in Challenge Organism Inoculum

5 Minute Contact Time

**Everbrite 1% Passed EN 1276:1997
Producing a 5 log ↓ Against
Klebsiella pneumoniae NCTC 9633**

**Signed: Joanne Green
Microbiologist**

15 August 2005

Everbrite Efficacy Against Listeria monocytogenes using EN 1276 : 1997

Introduction

This test was carried out to assess the efficacy of Everbrite, a sanitiser/disinfectant, against *Listeria monocytogenes*.

The EN1276:1997 efficacy tests were carried out on the disinfectant solutions made up from Everbrite. The concentrations tested were 0.25%, 0.1%, 0.05% & 0.01% v/v of the concentrated product. The contact time was 5 minutes at 20°C.

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**Determination of Bactericidal Activity for
Listeria monocytogenes NCTC 11994**

a) **ID Test Lab**.....NCH European Technical Centre

j) ID Samples

Name Everbrite
Batch No..... NP 1679 167/87A
Manufacturer..... NCH
Date of Delivery..... 15/07/05
Storage..... Room Temperature
Active ingredients..... QAC

k) Test Method and its validation

Method..... EN1276:1997 Quantitative Suspension Test for the Evaluation of bactericidal activity of chemical disinfectants and antiseptics used in food, industrial, domestic and institutional areas - Test Method.

Neutraliser 6g/l Lecithin, 60g/l polysorbate 80, 2g/l L-Histidine, 10g/l Sodium Thiosulphate in Maximum Recovery Diluent.

l) Experimental Conditions

Period of analysis..... 01/08/05 - 05/08/05
Product Diluent Sterile hard water 300mg/kg CaCO₃
Test Concentrations..... 0.25%, 0.1%, 0.05%, 0.01% v/v.
Appearance of Dilutions Colourless, clear product
Temperature..... 20°C
Contact time..... 5 mins ± 10s
Interfering substance... 3g/l bovine albumin
Incubation Temp..... 36°C ± 1°C
Bacterial Strain used... Listeria monocytogenes NCTC 11994

e) See Tables 1 and 2

f) Conclusion

According to EN 1276:1997, Everbrite NP1679 167/87A when diluted to 0.1% v/v in hard water possesses bactericidal activity in 5 minutes at 20°C under dirty conditions (3g/l bovine albumin) for the strain Listeria monocytogenes NCTC 11994.

5 min Contact Time

Everbrite 0.1% dilution produced a 5 log reduction in viable count of a strain of Listeria monocytogenes NCTC 11994 and PASSED the EN1276:1997

Everbrite

Results

Pass Rate for EN 1276:1997 is 5 log reduction or 99.999% kill.

Table 1 Verification of the methodology and validation of dilution-neutralisation method for the test concentration of 0.1% of the test product – Everbrite

Test organism	Viable Count (cfu/ml)				
	Bacterial Test suspension (N)	Bacterial Suspension (Nv)	Experimental conditions (A)	Neutraliser toxicity control (B)	Dilution-Neutralisation control (C)
Listeria monocytogenes NCTC 11994	10 ⁻⁶ : 290 10 ⁻⁷ : 24 N: 2.7 x10 ⁸	2.6 x10 ³	2.8 x10 ²	3.0 x10 ²	2.7 x 10 ²

For the strains tested:

N = number of cfu/ml of the bacterial test suspension

Nv = number of cfu/ml of the bacterial suspension

A = number of cfu/ml of the experimental conditions validation

B = number of cfu/ml of the neutraliser toxicity validation

C = number of cfu/ml of the dilution-neutralisation validation

The Neutraliser is validated for the test concentration of 0.1% solution of Everbrite for the Listeria monocytogenes NCTC 11994 strain tested.

Table 2 Test Results

Test Organism	Viable counts (cfu/ml) for the test mixture			
	0.25% Everbrite 5 min	0.1% Everbrite 5 min	0.05% Everbrite 5 min	0.01% Everbrite 5 min
Listeria monocytogenes	Vc 0, 0 Na <1.5 x 10 ²	Vc 0, 0 Na <1.5 x 10 ²	Vc 36, 28 Na >3.0 x 10 ³	Vc >300, >300 Na >3.0 x 10 ³
Reduction in viability at test concentration (R) i.e. Log reduction/kill	> 10 ⁵	> 10 ⁵	<10 ⁵	<10 ⁵
Test Result	Pass	Pass	Fail	Fail

Vc = Viable Count

Na = number of cfu/ml in test mixture

R = reduction in viability

CERTIFICATE OF ANALYSIS

EN 1276 : 1997 Chemical Disinfectants and Antiseptics –Quantitative suspension test for the evaluation of bactericidal activity of chemical disinfectant and antiseptics used in food, industrial, domestic, and institutional areas- Test Method and Requirements

SAMPLE: EVERBRITE NP1679 167/87A

Challenge Organism **Listeria monocytogenes NCTC 11994**

Contact Time 5 minutes

Temp 20°C

Soil 3g/l Bovine Albumin

Diluent Hard Water 300mg/kg CaCO₃

PASS Level for EN 1276:1997 - 5 log ↓ in Challenge Organism Inoculum in 5 minutes

Object of Test: To achieve a 5 log reduction in Challenge Organism Inoculum

5 Minute Contact Time

**Everbrite 0.1% Passed EN 1276:1997
Producing a 5 log ↓ Against
*Listeria monocytogenes NCTC 11994***

Signed: **Joanne Green**
Microbiologist

15 August 2005

Everbrite Efficacy Against Methicillin Resistant Staphylococcus aureus (MRSA) Using EN 1276 : 1997

Introduction

Staphylococcus aureus is a common cause of skin and wound infections and food poisoning in hospitals. MRSA is an antibiotic resistant strain of Staphylococcus aureus. MRSA is the most reported organism associated with infection, causing 30% of infections in European intensive care units. Some strains are resistant to antibiotics and it has been reported that 60% of isolates found in hospitals are resistant to the antibiotic Methicillin. The increase in use of antibiotic treatment has been associated with the increased prevalence of MRSA.

The Test

This test was carried out to assess the efficacy of Everbrite, a sanitiser/disinfectant, against MRSA.

The EN1276:1997 efficacy tests were carried out on the disinfectant solutions made up from Everbrite. The concentrations tested were 0.25%, 0.10%, 0.05% & 0.01% v/v of the concentrated product. The contact time was 5 minutes at 20°C.

Everbrite

Determination of Bactericidal Activity for MRSA NCTC 12493

a) **ID Test Lab**.....NCH European Technical Centre

m) ID Samples

Name Everbrite
Batch No..... NP 1679 167/87A
Manufacturer..... NCH
Date of Delivery..... 15/07/05
Storage..... Room Temperature
Active ingredients..... QAC

n) Test Method and its validation

Method..... EN1276:1997 Quantitative Suspension Test for the Evaluation of bactericidal activity of chemical disinfectants and antiseptics used in food, industrial, domestic and institutional areas - Test Method.

Neutraliser 6g/l Lecithin, 60g/l polysorbate 80, 2g/l L-Histidine, 10g/l Sodium Thiosulphate in Maximum Recovery Diluent.

o) Experimental Conditions

Period of analysis..... 08/08/05 - 11/08/05
Product Diluent Sterile hard water 300mg/kg CaCO₃
Test Concentrations..... 0.25%, 0.10%,0.05%, 0.01% v/v.
Appearance of Dilutions Colourless, clear product
Temperature..... 20°C
Contact time..... 5 mins ± 10s
Interfering substance... 3g/l bovine albumin
Incubation Temp..... 36°C ± 1°C
Bacterial Strain used... Methicillin Resistant Staphylococcus aureus MRSA NCTC 12493

e) See Tables 1 and 2

f) Conclusion

According to EN 1276:1997, Everbrite NP1679 167/87A when diluted to 0.10% v/v in hard water possesses bactericidal activity in 5 minutes at 20°C under dirty conditions (3g/l bovine albumin) for the strain MRSA NCTC 12493.

5 min Contact Time

Everbrite 0.10% dilution produced a 5 log reduction in viable count of a strain of MRSA NCTC 12493 and PASSED the EN1276:1997

Results

Pass Rate for EN 1276:1997 is 5 log reduction or 99.999% kill.

Table 1 Verification of the methodology and validation of dilution-neutralisation method for the test concentration of 0.1% of the test product – Everbrite

Test organism	Viable Count (cfu/ml)				
	Bacterial Test suspension (N)	Bacterial Suspension (Nv)	Experimental conditions (A)	Neutraliser toxicity control (B)	Dilution-Neutralisation control (C)
MRSA NCTC 12493	10 ⁻⁶ : 146 10 ⁻⁷ : 17 N: 1.6 x10 ⁸	1.5 x10 ³	1.5 x10 ²	1.6 x10 ²	1.6 x 10 ²

For the strains tested:

N = number of cfu/ml of the bacterial test suspension

Nv = number of cfu/ml of the bacterial suspension

A = number of cfu/ml of the experimental conditions validation

B = number of cfu/ml of the neutraliser toxicity validation

C = number of cfu/ml of the dilution-neutralisation validation

The Neutraliser is validated for the test concentration of 0.1% solution of Everbrite for the MRSA strain tested.

Table 2 Test Results

Test Organism	Viable counts (cfu/ml) for the test mixture			
	0.25% Everbrite 5 min	0.10% Everbrite 5 min	0.05% Everbrite 5 min	0.01% Everbrite 5 min
MRSA	Vc 0, 0 Na <1.5 x 10 ²	Vc 2, 4 Na <1.5 x 10 ²	Vc 36, 45 Na >3.0 x 10 ³	Vc >300, >300 Na >3.0 x 10 ³
Reduction in viability at test concentration (R) i.e. Log reduction/kill	> 10 ⁵	> 10 ⁵	<10 ⁵	<10 ⁵
Test Result	Pass	Pass	Fail	Fail

Vc = Viable Count

Na = number of cfu/ml in test mixture

R = reduction in viability

CERTIFICATE OF ANALYSIS

EN 1276 : 1997 Chemical Disinfectants and Antiseptics –Quantitative suspension test for the evaluation of bactericidal activity of chemical disinfectant and antiseptics used in food, industrial, domestic, and institutional areas- Test Method and Requirements

SAMPLE: EVERBRITE NP1679 167/87A

Challenge Organism **Methicillin Resistant Staphylococcus aureus MRSA**
NCTC 12493

Contact Time 5 minutes

Temp 20°C

Soil 3g/l Bovine Albumin

Diluent Hard Water 300mg/kg CaCO₃

PASS Level for EN 1276:1997 - 5 log ↓ in Challenge Organism Inoculum in 5 minutes

Object of Test: To achieve a 5 log reduction in Challenge Organism Inoculum

5 Minute Contact Time

Everbrite 0.1% Passed EN 1276:1997
Producing a 5 log ↓ Against
MRSA NCTC 12493

Signed: **Joanne Green**
Microbiologist

15 August 2005

Everbrite Efficacy Against Candida albicans using EN1650: 1998

Introduction

This test was carried out to determine the efficacy of Everbrite, a sanitising concentrate. The EN 1650 Fungicidal suspension test was used and the efficacy was tested against 1 fungal strain, *Candida albicans*. A soiling agent and hard water was used in the test.

NP 1679 (Everbrite)

**Fungicidal activity in general use conditions
Candida albicans**

a) ID Test Lab.....NCH European Technical Centre

p) ID Sample

Name Everbrite
Batch No..... NP 1679 167/87A
Manufacturer..... NCH
Date of Delivery..... 15/07/05
Storage..... Room Temperature
Active ingredients... QAC

q) Test Method and its validation

Method..... EN1650:1998 Fungicidal Activity (phase 2 step 1)
Neutraliser 60ml/l Tween 80, 6g/l Lecithin, 2g/l L-Histidine, 10g/l Sodium
Thiosulphate in Diluent.

r) Experimental Conditions

Period of analysis..... 12/09/05 to 19/09/05
Product diluent in test.. Sterile hard water 300 mg/kg CaCO₃
Test Concentrations. ...1, 0.5, 0.25, 0.1%.
Test Temperature..... 20°C
Contact time..... 15 minutes
Interfering substance...3g/l of bovine albumin ('dirty conditions')
Incubation Temp..... 36°C ± 1°C
Identification of fungal strain used Candida albicans NCTC 3179

s) Test Results

See Tables 1 and 2

t) Conclusion

According to EN 1650:1998 the batch, NP 1679 167/87A, of the product Everbrite,- possesses fungicidal activity in 15 minutes and at 20°C under 'dirty' conditions (3g/l bovine albumin) for the Candida albicans NCTC 3179

15 min Contact Time

Everbrite (0.25%) produced a 4 log reduction in viable count of Candida albicans and PASSED the EN1650:1998

RESULTS

Pass Rate for EN 1650 is 4 log or 99.99% reduction

Table 1 Verification of the methodology and validation of dilution-neutralisation method for the test concentration of the test product –Everbrite

Test organism	Viable Count (cfu/ml)				
	Fungal Test suspension (N)	Fungal Suspension (Nv)	Experimental Conditions (A)	Neutraliser toxicity Control (B)	Dilution neutralisation control (C)
C albicans	1.9×10^7	1.8×10^3	1.8×10^2	2.0×10^2	1.7×10^2

For the strains tested:

N = number of cfu/ml of the fungal test suspension

Nv = number of cfu/ml of the fungal suspension

A = number of cfu/ml of the experimental conditions validation

B = number of cfu/ml of the neutraliser toxicity validation

C = number of cfu/ml of the dilution-neutralisation validation

The inactivation is validated for the test concentration of Everbrite for the strain tested.

Table 2 Test Results

Vc = Viable Count

Na = Number of cfu/ml in the test mixture

R = reduction in viability

5 minutes

Test Organism	Viable counts (cfu/ml) for the test mixture			
	1% Everbrite 15 min	0.5% Everbrite 15 min	0.25% Everbrite 15 min	0.1% Everbrite 15 min
Candida albicans NCTC 3179	Vc 0, 0 Na $<1.5 \times 10^2$	Vc 5, 0 Na $<1.5 \times 10^2$	Vc 0, 0 Na $<1.5 \times 10^2$	Vc 35, 55 Na $>1.5 \times 10^3$
Reduction in viability at test concentration (R) i.e. Log reduction/ kill	$> 10^4$	$> 10^4$	$>10^4$	$<10^4$
Test Result	Pass	Pass	Pass	Fail

CERTIFICATE OF ANALYSIS

EN 1650 : 1998 Chemical Disinfectants and Antiseptics – Quantitative suspension test for the fungicidal activity of chemical disinfectants and antiseptics used in the food, industrial, domestic, and institutional areas - Test method and requirements

SAMPLE: Everbrite - NP 1679 167/87A

Challenge Organism **Candida albicans NCTC 3179**

Contact Time 15 minutes

Temp 20°C

Hard water Diluent 300 mg/kg CaCO₃

'Dirty' conditions 3 g/l Bovine albumin

PASS Level for EN 1650:1998 - 4 log ↓ in Challenge Organism Inoculum in 15 mins

Object of Test: To achieve a 4 log reduction in Challenge Organism Inoculum

15 Minute Contact Time

**Everbrite (0.25%) Passed EN 1650:1998
Producing a 4 log ↓ Against
Candida albicans NCTC 3179**

**Signed: Joanne Green
Microbiologist**

19 September 2005

Everbrite Efficacy Against Aspergillus niger using EN1650: 1998

Introduction

This test was carried out to determine the efficacy of Everbrite, a sanitising concentrate. The EN 1650 Fungicidal suspension test was used and the efficacy was tested against 1 fungal strain, *Aspergillus niger*. A soiling agent and hard water was used in the test.

NP 1679 (Everbrite)

**Fungicidal activity in general use conditions
Aspergillus niger**

a) ID Test Lab.....NCH European Technical Centre

u) ID Sample

Name Everbrite
Batch No..... NP 1679 167/87A
Manufacturer..... NCH
Date of Delivery..... 15/07/05
Storage..... Room Temperature
Active ingredients... QAC

v) Test Method and its validation

Method..... EN1650:1998 Fungicidal Activity (phase 2 step 1)
Neutraliser 60ml/l Tween 80, 6g/l Lecithin, 2g/l L-Histidine, 10g/l Sodium
Thiosulphate in Diluent.

w) Experimental Conditions

Period of analysis..... 05/10/05 to 17/10/05
Product diluent in test.. Sterile hard water 300 mg/kg CaCO₃
Test Concentrations. ...4, 3, 2, 1%.
Test Temperature..... 20°C
Contact time..... 15 min & 1 hour
Interfering substance...3g/l of bovine albumin ('dirty conditions')
Incubation Temp..... 30°C ± 1°C
Identification of fungal strain used Aspergillus niger NCTC 2275

x) Test Results

See Tables 1 and 2

y) Conclusion

According to EN 1650:1998 the batch, NP 1679 167/87A, of the product Everbrite,- possesses fungicidal activity in 15 minutes & 1 hour and at 20°C under 'dirty' conditions (3g/l bovine albumin) for the Aspergillus niger NCTC 2275

15 min Contact Time

Everbrite (2%) produced a 4 log reduction in viable count of
Aspergillus niger and PASSED the EN1650:1998

1 hour Contact Time

Everbrite (2%) produced a 4 log reduction in viable count of
Aspergillus niger and PASSED the EN1650:1998

NP 1679 (Everbrite)

RESULTS

Pass Rate for EN 1650 is 4 log or 99.99% reduction

Table 1 Verification of the methodology and validation of dilution-neutralisation method for the test concentration of the test product –Everbrite

Test organism	Viable Count (cfu/ml)				
	Fungal Test suspension (N)	Fungal Suspension (Nv)	Experimental Conditions (A)	Neutraliser toxicity Control (B)	Dilution neutralisation control (C)
Asp niger	1.6×10^7	1.6×10^3	1.6×10^2	1.5×10^2	1.5×10^2

For the strains tested:

N = number of cfu/ml of the fungal test suspension

Nv = number of cfu/ml of the fungal suspension

A = number of cfu/ml of the experimental conditions validation

B = number of cfu/ml of the neutraliser toxicity validation

C = number of cfu/ml of the dilution-neutralisation validation

The inactivation is validated for the test concentration of Everbrite for the strain tested.

Table 2 Test Results

Vc = Viable Count

Na = Number of cfu/ml in the test mixture

R = reduction in viability

15 minutes

Test Organism	Viable counts				(cfu/ml) for the test mixture			
	4% Everbrite 15 min		3% Everbrite 15 min		2% Everbrite 15 min		1% Everbrite 15 min	
Asp niger NCTC 2275	Vc 0, 0 Na $<1.5 \times 10^2$		Vc 2, 0 Na $<1.5 \times 10^2$		Vc 1, 3 Na $<1.5 \times 10^2$		Vc >300 , >300 Na $>1.5 \times 10^3$	
Reduction in viability at test concentration (R) i.e. Log reduction/ kill	$> 10^4$		$> 10^4$		$>10^4$		$<10^4$	
Test Result	Pass		Pass		Pass		Fail	

Test Organism	Viable counts				(cfu/ml) for the test mixture			
	4% Everbrite 1 hour		3% Everbrite 1 hour		2% Everbrite 1 hour		1% Everbrite 1 hour	
Asp niger NCTC 2275	Vc 0, 0 Na $<1.5 \times 10^2$		Vc 0, 0 Na $<1.5 \times 10^2$		Vc 1, 0 Na $<1.5 \times 10^2$		Vc >300 , >300 Na $>1.5 \times 10^3$	
Reduction in viability at test concentration (R) i.e. Log reduction/ kill	$> 10^4$		$> 10^4$		$>10^4$		$<10^4$	
Test Result	Pass		Pass		Pass		Fail	

CERTIFICATE OF ANALYSIS

EN 1650 : 1998 Chemical Disinfectants and Antiseptics – Quantitative suspension test for the fungicidal activity of chemical disinfectants and antiseptics used in the food, industrial, domestic, and institutional areas - Test method and requirements

SAMPLE: Everbrite - NP 1679 167/87A

Challenge Organism **Aspergillus niger NCTC 2275**

Contact Time 15 minutes

Temp 20°C

Hard water Diluent 300 mg/kg CaCO₃

'Dirty' conditions 3 g/l Bovine albumin

PASS Level for EN 1650:1998 - 4 log ↓ in Challenge Organism Inoculum in 15 mins

Object of Test: To achieve a 4 log reduction in Challenge Organism Inoculum

15 Minute Contact Time

**Everbrite (2%) Passed EN 1650:1998
Producing a 4 log ↓ Against
Aspergillus niger NCTC 2275**

**Signed: Joanne Green
Microbiologist**

17 October 2005

CERTIFICATE OF ANALYSIS

EN 14476: 2005 Chemical disinfectants and antiseptics - Virucidal quantitative suspension test for chemical disinfectants and antiseptics used in human medicine - Test method and requirements (phase 2, step 1)

NOTE: This European Norm specifies a test method and the minimum requirements for virucidal activity of chemical disinfectants or antiseptic products for instruments, surfaces or hands that form a homogeneous physically stable preparation when diluted with hard water – or in the case of ready-to-use products – with water. This EN test is applicable to a broad spectrum of viruses (Annex B) and 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 may occur in the workplace and in the home. It may also include services such as laundries and kitchens supplying products directly for the patients.

PRODUCT NAME: EVERBRITE

Challenge Organism Avian influenza virus (H3N8/H5N1)
Influenza virus A /duck/Ukraine/1/63 (H3N8) was incorporated as surrogate of Avian influenza virus H5N1, due to bio safety reasons

Contact Time 5 and 10 minutes

Temp 20°C ±1°C

Soil 0.03% bovine serum albumin (clean conditions) / 0.3% bovine serum albumin and 0.3% sheep erythrocytes (dirty conditions)

Diluent Hard Water 300mg/kg CaCO₃

PASS Level for EN 14476:2005 - 5 log ↓ in Challenge Organism Inoculum

Objective of Test: To achieve a 4 log reduction in Challenge Organism Inoculum

5 and 10 minute Contact Time

**Everbrite 1% (1:100) Passed EN 14476:2005
with 5 minutes contact time (clean conditions)
and 10 minutes contact time (dirty conditions)**

Producing a 4 log ↓ Against

Influenza virua A H3N8, surrogate for Avian influenza H5N1