

A: Identification of the test laboratory	Cheshire Scientific Limited 7 Brunel Road Bromborough Wirral Merseyside CH62 3NY
B: Identification of the sample	159392 - 504850
Name of the product	Clean & Protect – Multipurpose Cleaner and Sanitizer
Batch Number	No Batch Number Given – N/A
Manufacturer	Natrusan
Date of delivery	27.11.2018
Storage Conditions	Room Temperature and darkness
Product Diluent recommended by the manufacturer for use	Tested Neat
Active substance (s) and its (their) concentrations(s)	Not specified
C: Test method and its validation	
Method Based on	BS EN 13697:2015 – Dilution Neutralization.
Neutraliser	Lecithin 3g/l, polysorbate 80 30g/l, sodium thiosulphate 5g/l, L-histadine 1g/l, saponin 30g/l in 1 litre of water.
D: Experimental conditions	
Period of analysis	07.01.2019 - 17.01.2019
Product diluent used during test	Sterile Deionised Water
Product test concentrations	Tested Neat, 10% and 1% concentrations.
Appearance product dilutions	Clear and colourless.
Interfering substances	Bovine Albumen 0.3g/100ml
Test temperature	21.0 Deg C
Temperature of incubation	37 +/- 1 Deg C
Identification of bacterial strains used	Pseudomonas aeruginosa NCTC10662 + Pseudomonas denitrificans NCTC9496.
Tested by	A Griffiths
Report Authorised by	A.Griffiths
Report dated	18.01.2019
Test Results – See page 4	
Conclusion	
The product did give a >log 4 reduction for Pseudomonas aeruginosa + Pseudomonas denitrificans for Neat, 10% and 1% concentrations. This is a Pass for this reference strains used.	

In accordance with EN 13697:2015, the product Clean & Protect made by Natrusan, when tested at neat concentration, 10% concentration, and 1% concentration, does possess antibacterial activity on surfaces in 5 minutes at 20°C under dirty conditions (3g/l bovine albumin) for referenced strains *Pseudomonas aeruginosa* + *Pseudomonas denitrificans*.

Test method principle

The lab was asked if the product supplied killed biofilms. A biofilm comprises any syntrophic consortium of microorganisms in which cells stick to each other and often also to a surface. *Pseudomonas aeruginosa* represents a commonly used biofilm model organism since it is involved in different types of biofilm-associated infections. So to test if the supplied product killed biofilms, a combination of two *Pseudomonas* species were used – *Pseudomonas aeruginosa*, and *Pseudomonas denitrificans* – using the BS EN 13697:2015 method which is suitable for testing surface cleaning products.

A suspension of the combined bacteria in a solution of interfering substance is applied onto a stainless steel non-porous surface. After the bacteria has been allowed to dry, the product under test is applied to the bacterial suspension on the disc. This is maintained at a specified temperature for 5 minutes. The surface is then transferred to a previously validated neutralization medium so the action of the disinfectant is immediately neutralized. The number of surviving organisms which can be recovered from the surface is determined quantitatively.

The number of bacteria on a surface treated with water in place of disinfectant is also determined, and the reduction in viable counts attributed to the product is calculated by difference.

BS EN13697:2015 says to test the product in question at three different concentrations. The concentrations used were Neat, 10%, and 1%.

To pass the BS EN 13697 standard there would have to be at least a 4 log₁₀ reduction in the bacteria used. The standard states to use four different bacteria – *Escherichia coli* ATCC 10536, *Pseudomonas aeruginosa* ATCC 15442, *Enterococcus hirae* ATCC 10541, and *Staphylococcus aureus* ATCC 6538. For this test, the lab is using the BS EN 13697 pass grade as a guide to see if the product has worked or not.

Verification of Methodology

For each test, check that:

a). The mean counts from duplicate plates used for calculation of N, Nc, Nd, NC, and NT are between 14 and 330.

b). $6.57 \leq N \leq 7.10$ Logs for bacteria.

Pseudomonas aeruginosa + Pseudomonas denitrificans : 7.26

c). $Nc \geq 6.27$ Logs for bacteria.

Pseudomonas aeruginosa + Pseudomonas denitrificans 6.30

d). $NC > 0.5 Nc$.

Pseudomonas aeruginosa + Pseudomonas denitrificans: NC: 5.80 Nc: $6.30 \times 0.5 = 3.15$

e). $NT > 0.5 Nc$

Pseudomonas aeruginosa + Pseudomonas denitrificans: NT: 6.33 Nc: $6.30 \times 0.5 = 3.15$

f). Nts is less than 100 cfu/ml for active concentrations. If not, the recovery of micro-organisms has not been sufficient. For no active concentrations, Nts may not be countable.

Pseudomonas aeruginosa + Pseudomonas denitrificans:

Neat Disinfectant: Nts=0

Water Control: Nts=234

10% Disinfectant: Nts=0

1% Disinfectant: Nts=0

NT = \log_{10} number of cfu per test surface of the neutralization test.

NC = \log_{10} number of cfu per test surface of the neutralization control.

Nc = \log_{10} number of cfu per test surface of the water control.

Nd = \log_{10} number of cfu per test surface of the disinfectant test

Nts = number of colony forming units remaining on the test surface.

Table 1: Test Results

Test Organism	Bacterial Test Suspension: N	Validation Test		Water Control : Nc	Test Procedure 5 mins contact time Neat Concentration	Test Procedure 5 mins contact time 10% Concentration	Test Procedure 5 mins contact time 1% Concentration
		Neutraliser Validation NT	Neutraliser Control NC				
Pseudomonas aeruginosa NCTC10662 + Pseudomonas denitrificans NCTC9496	10 ⁻⁶ : 304,364 10 ⁻⁷ : 37,37 N: 7.26	10 ⁻³ : >300,>300 10 ⁻⁴ : 139,146 10 ⁻⁵ : 19,17 NT: 6.33	10 ⁻³ : >300,>300 10 ⁻⁴ : 52,74 10 ⁻⁵ : 8,11 NC: 5.80	10 ⁻³ : >300,>300 10 ⁻⁴ : 192,211 10 ⁻⁵ : 21,27 10 ⁻⁶ : 1,3 Nc : 6.30 Nts: 234	Neat: 0,0 10 ⁻¹ : 0,0 10 ⁻² : 0,0 Nd: 0 Nts : 0 R: 6.30	Neat: 4,6 10 ⁻¹ : 0,0 10 ⁻² : 0,0 Nd: 0.70 Nts : 0 R: 5.60	Neat: 7,7 10 ⁻¹ : 0,1 10 ⁻² : 0,0 Nd: 0.85 Nts : 0 R: 5.45

Microbicidal Effect (R value) = Nc - Nd