Determination of the Bactericidal Activity of Green Up when tested Against MRSA, *Salmonella typhimurium* & *Listeria monocytogenes* using the European Standard Test method BS EN 1276:1997.

~

Prepared by
Dr Paul Humphreys
May 07

Determination of the Bactericidal Activity of Green Up when tested Against MRSA, *Salmonella typhimurium* & *Listeria monocytogenes* using the European Standard Test method BS EN 1276:1997.

25 May 2007

Author: P. Humphreys	**Signature:**	**Date:**
Checked by: C. Tillson	**Signature:**	**Date:**
Authorised by: P. Humphreys	**Signature:**	**Date:**

Report No:	**HMS/PC/4/07**
ISSUE:	**Date:**
Version 1	**24/05/07**

Tests Carried Out By:

University of Huddersfield	School of Applied Sciences Queensgate Huddersfield HD1 3DH

Microbiological Tests

Test Method	British/European Standard BS EN 1276:1997. Membrane filtration Technique
Test Procedures	Full details of all the test and control procedures used are given in the Test Method
Disinfectant	Green Up Batch number: N/A Date of delivery: March 2007 Storage conditions: $20^{o}C–25^{o}C$ Active substances: Refer to Manufacturer

Interfering Substance (Organic Challenge)

1. Simulated clean conditions:
   0.3 g l^{-1} bovine albumin (final concentration)

2. Simulated dirty conditions:
   3.0 g l^{-1} bovine albumin (final concentration)

Temperature	Ambient (23-25ºC)
Contact Time Tested	5 (± 10 s) minute.
Test Organisms	*Salmonella typhimurium* ATCC 14028, *Listeria monocytogenes* ATCC 7644, Methicillin resistant *Staphylococcus aureus* (MRSA, ATCC 43300)
Culture Medium	Tryptone Soya Agar, LabM.
Incubation	Plates were incubated at 35 or 37ºC for 24-48hrs.
Diluent	MRD, Lab M
Neutraliser	N/A membrane filtration approach employed

General Method
A standard suspension of test organisms containing 1.5 – 5.0 x 10^8 cells ml^{-1} of bacteria was prepared. 1 ml of interfering substance was pipetted into a Universal bottle, followed by 1 ml of test organism suspension. The mixture was mixed and left for 2 minutes. After 2 minutes 8 ml of product was added and mixed. After a contact time of 5 minutes, a 0.1 ml sample of the reaction mixture was filtered through a 0.45µm sterile filter. After filtration the filter was placed on the surface of a TSA agar plate and incubated. Colony forming units were counted after 24 hours incubation and the fraction of surviving organisms calculated.

Requirements of this standard
The product, when tested as stipulated under simulated clean conditions (0.3 g/l bovine albumin) or dirty conditions (3 g/l bovine albumin) under the required test conditions (23-25ºC, 5 minute contact, for the selected reference strain), shall demonstrate at least a 5 $\log_{10}$ reduction in viable counts.

Results
Results from the test are summarised in Table 1 a full set of results can be found in Appendix 1. In order to pass the test a 5 $\log_{10}$ reduction is required.

Product	**Referenced Organism**	**Pass or Fail?**	
		Clean	**Dirty**
Green Up	*Salmonella typhimurium* ATCC 14028	Pass	Pass
	Listeria monocytogenes ATCC 7644	Pass	Pass
	Staphylococcus aureus (MRSA, ATCC 43300)	Pass	Pass

Table 1. Summary of Product Test Results

Interpretation of the Results
When tested against *Salmonella typhimurium* (ATCC 14028), *Listeria monocytogenes* (ATCC 7644, and Methicillin resistant *Staphylococcus aureus* (MRSA, ATCC 43300) with a 5 minute contact time the product produced greater than a 5 $\log_{10}$ reduction in viable counts.

Conclusion
According to EN 1276:1997 Green UP possesses bactericidal activity in 5 minutes at ambient temperature (23-25^{o}C) under clean conditions (0.3g/l bovine albumin) and dirty conditions (3g/l bovine albumin) for the three bacteria under tests, *Salmonella typhimurium* (ATCC 14028), *Listeria monocytogenes* (ATCC 7644) and Methicillin resistant *Staphylococcus aureus* (MRSA, ATCC 43300).

Signed:

Dr Paul Humphreys
School of Applied Sciences
University of Huddersfield

Appendix 1. Bactericidal Activity of Green Up

Test Organism	VALIDATIONS										Bacterial Test Suspension			Test Procedure Results	
	Bacterial Suspension	Experimental Conditions Validation				Neutraliser Toxicity Control		Dilution Neutralisation Control						Clean	Dirty
		Clean		Dirty				Clean		Dirty					
MRSA		Vc	268 225	249	250	Vc	260 248	Vc	236 234	241 249	10-6	179	175	Vc < 15 15	< 15 15
											10-7	15	12	Na < 1.5E+02	< 1.5E+02
	Nv 1.8E+03	A	2.5E+02	2.5E+02		B	2.5E+02	C	2.4E+02	2.5E+02	N	1.8E+08		R > 2.E+05	> 2.E+05
Verification of Methodology					**Passed**	**Log10 Reductions/cfu/ml**									
N is between 1.5E+8 cfu/ml and 5E+8 cfu/ml, N =				1.8E+08		Clean	5.373								
Nv is between 6E+2 cfu/ml and 3E+3 cfu/ml, Nv =				1.8E+03		Dirty	5.373								
CLEAN A ≥ 0.05 x Nv when 0.05 x Nv =				8.9E+01	Yes										
DIRTY A ≥ 0.05 x Nv when 0.05 x Nv =				8.9E+01	Yes										
B ≥ 0.05 x Nv when 0.05 x Nv =				8.9E+01	Yes										
CLEAN C ≥ 0.5 x B when 0.5 x B =				1.3E+02	Yes										
DIRTY C ≥ 0.5 x B when 0.5 x B =				1.3E+02	Yes										

Table 2. Biocidal Testing Against MRSA using the Test Method Outlined in BS EN 1276:1997.

Test Organism	VALIDATIONS										Bacterial Test Suspension			Test Procedure Results	
	Bacterial Suspension	Experimental Conditions Validation				Neutraliser Toxicity Control		Dilution Neutralisation Control						Clean	Dirty
		Clean		Dirty				Clean		Dirty					
Salmonella typhimurium		Vc	214 125	129	164	Vc	169 166	Vc	132 178	104 179	10-6	185	187	Vc < 15 15	< 15 15
											10-7	25	15	Na < 1.5E+02	< 1.5E+02
	Nv 1.9E+03	A	1.7E+02	1.5E+02		B	1.7E+02	C	1.6E+02	1.4E+02	N	1.9E+08		R > 2.E+05	> 2.E+05
Verification of Methodology					**Passed**	**Log10 Reductions/cfu/ml**									
N is between 1.5E+8 cfu/ml and 5E+8 cfu/ml, N =				1.9E+08		Clean	5.394								
Nv is between 6E+2 cfu/ml and 3E+3 cfu/ml, Nv =				1.9E+03		Dirty	5.394								
CLEAN A ≥ 0.05 x Nv when 0.05 x Nv =				9.3E+01	Yes										
DIRTY A ≥ 0.05 x Nv when 0.05 x Nv =				9.3E+01	Yes										
B ≥ 0.05 x Nv when 0.05 x Nv =				9.3E+01	Yes										
CLEAN C ≥ 0.5 x B when 0.5 x B =				8.4E+01	Yes										
DIRTY C ≥ 0.5 x B when 0.5 x B =				8.4E+01	Yes										

Table 3. Biocidal Testing Against *S. typhimurium* using the Test Method Outlined in BS EN 1276:1997.

Test Organism	VALIDATIONS										Bacterial Test Suspension			Test Procedure Results				
	Bacterial Suspension		Experimental Conditions Validation				Neutraliser Toxicity Control			Dilution Neutralisation Control								
			Clean			Dirty				Clean			Dirty				Clean			Dirty	
Listeria monocytogenes			Vc	232	155	161	178	Vc	259	294	Vc	164	221	147	141	10-6	296	284	Vc	15	15	15	15
																10-7	32	29	Na	<	1.5E+02	<	1.5E+02
	Nv	2.9E+03	A	1.9E+02		1.7E+02		B	2.8E+02		C	1.9E+02		1.4E+02		N	2.9E+08		R	>	4.E+05	>	4.E+05

Verification of Methodology		Passed
N is between 1.5E+8 cfu/ml and 5E+8 cfu/ml, N =	2.9E+08	
Nv is between 6E+2 cfu/ml and 3E+3 cfu/ml, Nv =	2.9E+03	
CLEAN A ≥ 0.05 x Nv when 0.05 x Nv =	1.5E+02	Yes
DIRTY A ≥ 0.05 x Nv when 0.05 x Nv =	1.5E+02	Yes
B ≥ 0.05 x Nv when 0.05 x Nv =	1.5E+02	Yes
CLEAN C ≥ 0.5 x B when 0.5 x B =	1.4E+02	Yes
DIRTY C ≥ 0.5 x B when 0.5 x B =	1.4E+02	Yes

Log10 Reductions/cfu/ml	
Clean	5.589
Dirty	5.589

Table 4. Biocidal Testing Against *L. monocytogenes* using the Test Method Outlined in BS EN 1276:1997.