

Campden Technology Limited
Station Road
Chipping Campden
Gloucestershire
GL55 6LD, UK



Tel: +44 (0)1386 842000
Fax: +44 (0)1386 842100
www.campden.co.uk

Investigation into the effectiveness of Sanitizer Plus when tested in accordance with BS EN 13704:2002 - 'Chemical disinfectants - Quantitative suspension test for the evaluation of sporicidal activity of chemical disinfectants used in food, industrial, domestic and institutional areas'

CONFIDENTIAL TO:

Mr Mal Stevens
RJN Chemicals Limited
Unit 8, The Ridgeway
Thorney Lane, Iwer
Bucks SL0 9HX

PROJECT: MB/REP/123670/1

Lawrence Staniforth

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Report Approved By:

Report Checked By:

Name:

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Registered Office: Station Road, Chipping Campden, Gloucestershire. GL55 6LD
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SUMMARY

Sanitizer Plus was tested at 2% possesses sporicidal activity against the obligatory *Bacillus subtilis* and the additional challenge organism *Clostridium difficile* within 60 minutes at 20°C under clean conditions (0.03% bovine albumen final concentration in the test) when tested using the method BS EN 13704:2002.

INTRODUCTION

RJN Chemicals Limited requested that Campden Technology Limited (Campden BRI) assess the efficacy of Sanitizer Plus for sporicidal activity using the method BS EN 13704:2002 - 'Chemical disinfectants - Quantitative suspension test for the evaluation of sporicidal activity of chemical disinfectants used in food, industrial, domestic and institutional areas' and the additional challenge organism *Clostridium difficile*

METHOD

For the sporicidal test, the principles of BS EN 13704:2002 - 'Chemical disinfectants - Quantitative suspension test for the evaluation of sporicidal activity of chemical disinfectants used in food, industrial, domestic and institutional areas' were followed.

NOTES

The additional challenge organism, *Clostridium difficile* was tested at the standard 60 minutes.

Sporicidal activity in general use conditions

a) IDENTIFICATION OF THE TEST LABORATORY	Campden BRI
b) IDENTIFICATION OF THE SAMPLE	FH/123670/1
Name of the product	Sanitizer Plus
Batch number	Not supplied
Manufacturer	RJN Chemicals Limited
Condition on receipt	OK
Date of delivery	05.05.11
Storage conditions	dark, ambient (Laboratory sample cupboard)
Product diluent recommended by the manufacturer for use	N/A
Active substance(s) and its (their) concentrations(optional)	N/A
c) EXPERIMENTAL CONDITIONS	
Period of analysis	July 2011
Product diluent used during the test	Water of Standard Hardness (WSH)
Product test concentrations	20%, 10%, 1%
Appearance of diluted product	White crystalline powder
Contact time	Sporicidal test – 60 minutes
Test temperature	Sporicidal test - 20°C
Interfering substance	Bovine Serum Albumin @ 0.03% ("CLEAN") in Sterile Distilled Water (SDW)
Stability of mixture (interfering substance and product diluted in hard water)	Stable
Temperature of incubation	30°C ± 1°C
Identification of the spore strains used	<i>Bacillus subtilis</i> <i>Clostridium difficile</i>
Preparation of the spore suspension	<i>Bacillus subtilis</i> NCIMB 8054 (Campden ref. FH/77/a) Spores cropped on: 11/01/10 Heat treated on day of testing
	Additional sporicidal test strain: <i>C. difficile</i> NCIMB 10666 (Campden FH147) Spores cropped on 6.4.11 Heat treated on day of testing

d) OPERATING PROCEDURE
Methods used
Deviations from methods

Sporicidal test – BS EN 13704:2002
***Clostridium difficile*, anaerobic conditions,**
Tryptone Soya agar + 5% defibrinated sheeps
blood incubated at 30°C ± 1°C for 5 days
Dilution neutralisation

Neutralisation method

e) TEST RESULTS

See attached table

f) CONCLUSION

When tested *according* to the method BS
EN 13704:2002, the supplied Sanitizer Plus
(FH/123670/1), at 2% possesses sporicidal
activity against *Bacillus subtilis* and
***Clostridium difficile* spores in 60 minutes at**
20°C under clean conditions.

N.B. KEY TO CODES ON RESULT SHEET

Vc:	viable count
R:	reduction in viability
Na:	the number of cfu/ml in the test mixture
Nv:	the number of cfu/ml of the spore suspension for validation controls
N:	the number of cfu/ml of the spore test suspension
A:	the number of cfu/ml of the experimental conditions control
B:	the number of cfu/ml of the neutraliser toxicity validation or of the filtration validation
C:	the number of cfu/ml of the dilution neutralisation test validation, or of the membrane filtration test validation

TABLE 1 - RESULTS AND VALIDATIONS
(Dilution Neutralisation)

Test criteria	Validation Test				Test suspension	Test procedure at concentration % (v/v)				Result
	Vc: 84, 89 Nv: 8.6 x 10 ²	Vc: 43, 46 A: 44.5	Vc: 42, 44 B: 43	Vc: 32, 31 C: 31.5		cfu/ml in test pot	0.01	1-	2	
<i>Bacillus Subtilis</i> NC/MB 8054 Clean 60 minutes	Vc: 84, 89 Nv: 8.6 x 10 ²	Vc: 43, 46 A: 44.5	Vc: 42, 44 B: 43	Vc: 32, 31 C: 31.5	Vc: -4 150, 156 -5: 14, 16 N: 1.53 x 10 ⁶	1.53 x 10 ⁵	Vc: >300, >300 Na: >3.0 x 10 ³ R: <1.0 x 10 ³	Vc: 240, 192 Na: 2.16 x 10 ³ R: <1.0 x 10 ³	Vc: 0, 0 Na: < 1.5 x 10 ² R: >1.0 x 10 ³	Pass at 2% Test Valid
<i>Clostridium difficile</i> NC/MB 10666 Clean 60 minutes	Vc: 64, 52 Nv: 5.8 x 10 ²	Vc: 64, 62 A: 63	Vc: 58, 66 B: 62	Vc: 60, 56 C: 58	Vc: -4 230, 242 -5: 22, 20 N: 2.36 x 10 ⁶	2.3 x 10 ⁵	Vc: >300, >300 Na: >3.0 x 10 ³ R: <1.3 x 10 ²	Vc: >300, >300 Na: >3.0 x 10 ³ R: < 1.3 x 10 ²	Vc: 0, 0 Na: < 1.5 x 10 ² R: >2.5 x 10 ³	Pass at 2% Test Valid

