



**Henkel AG & Co. KGaA**  
Microbiology

## Test Report

12-16819

On the  
sporicidal activity of

**Sekusept aktiv PC**

TS  
1.2.7.

**According to EN 13704**

Chemical disinfectants and antiseptics –  
Quantitative suspension test for the evaluation of sporicidal (EN 13704) activity of chemical  
disinfectants used in food, industrial, domestic and institutional areas  
Test method and requirements (phase 2, step 1);  
German version EN 13704:2002

This test report comprises 5 pages and may be reproduced only in complete form.  
It bases on a study which was performed in May 2008 under the internal number 08.00772

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**1. Laboratory**

Henkel AG & Co. KGaA  
HSA Corporate Scientific Services  
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**2. Identity of the test substance**

2.1. Product name	Sekusept aktiv PC
2.1.1 Batch	MD11554-62-1
2.1.2 Formula Code	FC 908706
2.1.3 Filling date	April 2008
2.1.4 Manufacturing date	February 2008
2.1.5 Expiry date	unknown
2.1.6 Manufacturer	Ecolab Deutschland GmbH, Düsseldorf
2.1.7 Date of sample entry	16 April 2008
2.1.8 Storage conditions in the laboratory	RT
2.1.9 Appearance	white granules with blue speckles
2.1.10 Active substances	PAA

**3. Test method**

- 3.1 Quantitative Suspension test according to EN 13704  
Chemical disinfectants and antiseptics –  
Quantitative suspension test for the evaluation of sporicidal (EN 13704) activity of  
chemical disinfectants used in food, industrial, domestic and institutional areas –  
Test method and requirements (phase 2, step 1);  
German version EN 13704:2002

## 3.2

The chosen neutralisation method was in each case the membrane filtration method according to EN 13704 with following dilution series by pour plate method to demonstrate killing rates less than  $10^2$ .

**Neutralizer** based on Tween, Lecithin, Histidin, Na-thiosulphate (3%, 0.3%, 0.1%, 0.5%)

**4. Experimental conditions**

- 4.1 Test period:  
May 2008
- 4.2 Diluent:  
standard hard water according DIN EN 13704
- 4.3 Test concentration:  
1000ppm PAA  
the test solution was prepared and adjusted by the client. It contained 1284ppm PAA (1.25 x test concentration) at pH 8.06

- 4.4 Appearance of the test solution  
colourless, clear, liquid
- 4.4 Test organism  
*Bacillus subtilis* (spores) DSM 347  
(test strain was given by the client)  
spore suspension prepared in February 2007
- 4.5 Test suspension  
 $1.5 \times 10^6 < N < 5 \times 10^6$  spores/ml
- 4.6 Contact times  
15 - 30 minutes  
contact times were given by the client
- 4.7 Test temperature  
20°C
- 4.8 Interfering substance  
0.3g/l bovine albumin (simulated clean conditions following EN 13704)
- 4.9 Incubation temperature  
30°C

## Results

The chosen neutralisation method was in each case the membrane filtration method with following dilution series by pour plate method to demonstrate killing rates less than  $10^2$ .

Sporicidal activity according to EN 13704			TS 1.2.7.
R			
Room temperature		clean conditions	
Sample	Conc.	15 min.	30 min.
Sekusept aktiv PC	1000ppm PAA	$>2.53 \times 10^3$	$>2.53 \times 10^3$

## 6. Conclusion

The standard EN 13704 defines the method and the minimum requirements for the sporicidal activity of chemical disinfectants in food, industrial domestic and institutional areas. The products must be capable to reduce the viable counts of spores of *Bacillus subtilis* by a factor of  $10^3$  within 60 minutes at 20°C under the influence of interfering substance simulating clean conditions to fulfil the requirement of EN 13704 for sporicidal activity.

In this study the substances were tested under simulated clean conditions at 15 and 30 minutes at room temperature against *Bacillus subtilis* spores. Deviated from the norm the samples were tested with following dilution series by pour plate method to demonstrate killing rates less than  $10^2$ .

The tested formulation **Sekusept aktiv PC** demonstrated the required reduction of the viable counts of *Bacillus subtilis* spores of  $\geq 10^3$  spores with an application concentration which corresponded to 1000ppm PAA at 20°C within 15 minutes contact time.

Thus the product fulfilled the requirements of EN 13704 regarding the sporicidal efficacy.

Düsseldorf, 15 November 2012



Dr. R. Breves



U. Bäumer

## Test results

## DIN EN 13704: 2002

Test strain	Validation			Test suspension N (see 5.4.1.2)	Test with concentration in ppm
	Test suspension N <sub>v</sub> (see B.2)	Validation of selected experimental conditions A (see 4.1.2.a)	Validation of non-toxicity of neutralizer B (see 4.1.2.b)	Validation of dilution-neutralization-method C (see 4.1.2.c)	
<i>Bacillus subtilis</i> DSMZ 347	V <sub>C</sub> : 205/217 N <sub>v</sub> : 2.11x10 <sup>3</sup>	15'/clean cond. V <sub>C</sub> : 181/109 N <sub>v</sub> : 1.86x10 <sup>2</sup>	V <sub>C</sub> : 195/203 N <sub>v</sub> : 1.99x10 <sup>2</sup>	15'/clean cond. V <sub>C</sub> : 177/184 N <sub>v</sub> : 1.81x10 <sup>2</sup>	V <sub>C</sub> :  N <sub>a</sub> : R:
		30'/clean cond. V <sub>C</sub> : 193/186 N <sub>v</sub> : 1.9x10 <sup>2</sup>		30'/clean cond. V <sub>C</sub> : 192/205 N <sub>v</sub> : 1.99x10 <sup>2</sup>	
	/			10 <sup>-4</sup> : >300/>300 10 <sup>-5</sup> : 43/33 N: 3.8x10 <sup>6</sup>	V <sub>C</sub> :  N <sub>a</sub> : R:
					15' 10 <sup>0</sup> : 0/0 10 <sup>-1</sup> : 0/0 10 <sup>-3</sup> : 0/0 <1.5x10 <sup>2</sup> >2.53x10 <sup>3</sup>
					30' 10 <sup>0</sup> : 0/0 10 <sup>-1</sup> : 0/0 10 <sup>-3</sup> : 0/0 <1.5x10 <sup>2</sup> >2.53x10 <sup>3</sup>

**Validation of the dilution neutralisation method and membrane filtration method**

Neutralizer based on Tween, Lecithin, Histidin, Na-thiosulphate (3%, 0.3%, 0.1%, 0.5%)

- N the number of cfu (colony forming units; spores) per ml of the bacterial test suspension  
required by EN 13704:  $1.5 \times 10^6 < N < 5 \times 10^6$
- $N_V$  the number of cfu/ml of the bacterial suspension for verification tests  
required by EN 13704:  $6 \times 10^2 < N_V < 3 \times 10^3$
- A the number of cfu/ml for the validation of selected experimental conditions  
required by EN 13704:  $A \geq 0.05 \times N_V$
- B the number of cfu/ml of the neutralizer toxicity control  
required by EN 13704:  $B \geq 0.05 \times N_V$
- C the number of cfu/ml of the dilution-neutralization validation  
required by EN 13704:  $C \geq 0.5 \times B$

The elaborated test results are regarded as sufficiently valid. The non-toxicity of the chosen neutraliser solution and the sufficient neutralisation of the test substance by the neutralisation process were proven. It was proven as well that the selected experimental conditions had no adverse effect on the test organisms.