

HYGIENE NORD GMBH
c/o BIOTECHNIKUM
WALTHER-RATHENAU-STRASSE 49 A
D - 17489 GREIFSWALD



HYGIENE NORD GMBH, c/o BIOTECHNIKUM, W.-RATHENAU-STR. 49 A, D-17489 GREIFSWALD

B. Braun Medical AG

Seesatz

6204 Sempach

Switzerland

CUSTOMER NUMBER DATE
324 July 30, 2008

REPORT A 08118-2

INSTRUMENT DISINFECTANT HELIX ULTRA

Purpose

The efficacy of **Helix ultra**, an instrument disinfectant produced by B. Braun Medical AG, Sempach, Switzerland, should to be evaluated in a quantitative suspension test against the test organism *A. niger* in accordance with the EN 13624 (2003).

Test description

Product name: **Helix ultra** (instrument disinfectant)

Batch number: 0705BH0013

Manufacturer: B. Braun Medical AG

Date of delivery: March 18, 2008

Storage conditions: Room temperature

Product dilution: Water

Date of order: June 04, 2008

Order number: A 08118

Sample number: P 080671

Test time: June 23, 2008 – July 27, 2008

Basis: DIN EN 13624 (2003): Chemical disinfectants and antiseptics – quantitative suspension test for the evaluation of fungicidal activity of chemical disinfectants for instruments used in the medical area – test method and requirements (phase 2, step 1)

Test organisms: *Aspergillus niger* ATCC 16404

Test solution: 2 % / 1.5 % / 1.0 % / 0.5 % / 0.25 % / 0.1 % / 0.01 %

Odor: aromatic

Appearance: white powder

Composition per 100 g: 0.16 % peracetic acid (in the 1 % product solution)

pH:

4 %:	7.63	2 %:	7.99	1 %:	8.18
1.5 %:	8.13	0.75 %:	8.25	0.5 %:	8.35

Neutralizer: 3 % Tween 80 + 0.3 % Lecithin + 0.1 % Histidine + 0.5 % Na-Thiosulfat (neutralizer III)

Contact time: 2 min / 5 min / 15min / 30 min / 60 min

Interfering substance: 0.03 % albumin

Test temperature: 21 ± 1°C

Incubation temperature: 30 ± 1°C

Test Method

Quantitative suspension test

Testing is based on the European Standard EN 13624 (2003). Validation and control procedures are therefore carried out in accordance with that standard.

For the test, suspension of test bacteria in a solution of the interfering substance is added to a sample of the test product (diluted with hard water). The mixture is maintained at $20\pm1^{\circ}\text{C}$ for the required contact times. At the end of the contact time, an aliquot of 1 ml is taken; the microbiocidal activity in this portion is immediately neutralized. Two 1 ml samples of this suspension are spread on 1 agar-plate each. The number of surviving test organisms in the test mixture is calculated for each sample and the reduction is determined with respect to the corresponding test suspension N_0 (EN 13624).

The experimental conditions (control A), the non-toxicity of the neutralizer (control B) and the dilution-neutralization method (control C) are validated.

The test is performed using *Aspergillus niger* as test-organism. Results are presented in table 1.

Results

Quantitative suspension test

The product possesses a sufficient activity ($\log \text{RF} \geq 4$) against reference strain *A. niger* according to the EN 13624 at a product concentration of **1.5 % / 15 min**, **1.0 % in 30 min** and at **0.5 % in 60 min**.

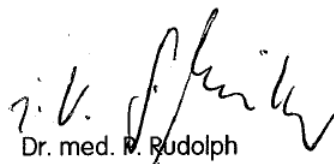
Results are successfully validated in accordance with the requirements of the EN 13624 (2003)

Greifswald, July 30, 2008



Dipl. Biol. T. Koburger

- Bereichsleiter Mikrobiologie -



Dr. med. R. Rudolph

- FA für Hygiene und Umweltmedizin -

Table 1: Results of the quantitative suspension test according to the EN 13624 (2003)

Date / performing:	June 23, 2008	Order number:	A 08118
Product:	Helix ultra	Sample number:	P 080671
Test organism:	<i>A. niger</i>	Lot number:	0705BH0013
Interfering substance:	0.03 % albumin	Neutralizer:	III
Incubation temperature:	30 ± 1 °C	Incubation time:	21 d
Test suspension (N):	2.40*10 ⁸ KbE/ml (7.38 log)	Test temperature	21± 1 °C
Test suspension (N₀):	2.40*10 ⁷ KbE/ml (6.38 log)		
Validation Suspension (N_v):	8.10*10 ² KbE/ml (2.91 log)		

contact time: 5 min						
concentration	dilution	cfu / plate 1	cfu / plate 2	V _{cl}	log ₁₀ Na	log ₁₀ R
2 %	1 ml (10 ⁰)	38	24	31	2.49	3.89
1 %	1 ml (10 ⁰)	> 330	> 330	> 660	> 3.82	< 2.56
0.5 %	1 ml (10 ⁰)	> 330	> 330	> 660	> 3.82	< 2.56

contact time: 15 min						
concentration	dilution	cfu / plate 1	cfu / plate 2	V _{cl}	log ₁₀ Na	log ₁₀ R
1 %	1 ml (10 ⁰)	34	24	29	2.46	3.92
0.5 %	1 ml (10 ⁰)	> 330	> 330	> 660	> 3.82	< 2.56
0.25 %	1 ml (10 ⁰)	> 330	> 330	> 660	> 3.82	< 2.56

contact time: 30 min						
concentration	dilution	cfu / plate 1	cfu / plate 2	V _{cl}	log ₁₀ Na	log ₁₀ R
1 %	1 ml (10 ⁰)	0	0	< 14	< 2.15	> 4.23
0.5 %	1 ml (10 ⁰)	> 330	> 330	> 660	> 3.82	< 2.56
0.1 %	1 ml (10 ⁰)	> 330	> 330	> 660	> 3.82	< 2.56

contact time: 60 min						
concentration	dilution	cfu / plate 1	cfu / plate 2	V _{cl}	log ₁₀ Na	log ₁₀ R
0.5 %	1 ml (10 ⁰)	0	0	< 14	< 2.15	> 4.23
0.25 %	1 ml (10 ⁰)	> 330	> 330	> 660	> 3.82	< 2.56
0.1 %	1 ml (10 ⁰)	> 330	> 330	> 660	> 3.82	< 2.56

Validation and Controls:

Validation - Suspension (N _{Vo})			Experimental condition control (A)			Neutralizer control (B)			Method validation (C): Product concentration: 2.0 %		
	cfu / plate 1 & 2	\bar{x}		cfu / plate 1 & 2	\bar{x}		cfu / plate 1 & 2	\bar{x}		cfu / plate 1 & 2	\bar{x}
V _{c1}	89	127.5	V _{c1}	66	62.5	V _{c1}	58	59.5	V _{c1}	65	62.5
V _{c2}	60		V _{c2}	59		V _{c2}	61		V _{c2}	60	
45 ≤ \bar{x} of N _{Vo} ≤ 180?			\bar{x} of A is ≥ 0.5* \bar{x} of N _{Vo} ?			\bar{x} of B is ≥ 0.5* \bar{x} of N _{Vo} ?			\bar{x} of C is ≥ 0.5* \bar{x} of N _{Vo} ?		
<input checked="" type="checkbox"/>	yes	<input type="checkbox"/> no	<input checked="" type="checkbox"/>	yes	<input type="checkbox"/> no	<input checked="" type="checkbox"/>	yes	<input type="checkbox"/> no	<input checked="" type="checkbox"/>	yes	<input type="checkbox"/> no

Table 1, continued: Results of the quantitative suspension test according to EN 13624 (2003)

Date / performing:	July 25, 2008	Order number:	A 08118
Product:	Helix ultra	Sample number:	P 080671
Test organism:	<i>A. niger</i>	Lot number:	0705BH0013
Interfering substance:	0.03 % albumin	Neutralizer:	III
Incubation temperature:	30 ± 1 °C	Incubation time:	21 d
Test suspension (N):	2.50*10 ⁸ KbE/ml (7.40 log)	Test temperature	21± 1 °C
Test suspension (N₀):	2.50*10 ⁷ KbE/ml (6.40 log)		

contact time: 15 min						
concentration	dilution	cfu / plate 1	cfu /plate 2	V _c	log ₁₀ Na	log ₁₀ R
2 %	1 ml (10 ⁰)	<u>0</u>	<u>0</u>	< 14	< 2.15	> 4.25
1.5 %	1 ml (10 ⁰)	<u>2</u>	<u>0</u>	< 14	< 2.15	> 4.25
1 %	1 ml (10 ⁰)	<u>112</u>	<u>181</u>	<u>146.5</u>	3.17	3.23

Table 1, continued: Results of the quantitative suspension test according to EN 13624 (2003)

Date / performing:	July 25, 2008	Order number:	A 08118
Product:	Helix ultra	Sample number:	P 080671
Test organism:	<i>A. niger</i>	Lot number:	0705BH0013
Interfering substance:	0.03 % albumin	Neutralizer:	III
Incubation temperature:	30 ± 1 °C	Incubation time:	21 d
Test suspension (N):	3.10*10 ⁸ KbE/ml (7.49 log)	Test temperature	21± 1 °C
Test suspension (N₀):	3.10*10 ⁷ KbE/ml (6.49 log)		

contact time: 15 min						
concentration	dilution	cfu / plate 1	cfu /plate 2	V _c	log ₁₀ Na	log ₁₀ R
2 %	1 ml (10 ⁰)	<u>0</u>	<u>0</u>	< 14	< 2.15	> 4.35
1.5 %	1 ml (10 ⁰)	<u>3</u>	<u>3</u>	< 14	< 2.15	> 4.35
1 %	1 ml (10 ⁰)	<u>109</u>	<u>155</u>	<u>132</u>	3.12	3.37

Legend

MW	=	average value
x	=	average value
RF	=	reduction factor
> 330	=	not countable
> 660	=	not countable
n.d.	=	not done
n.n.	=	not necessary
E	=	self-inhibition
Co 1	=	Control 1
Co 2	=	Control 2
Co 3	=	Control 3