

**B. Braun Medical AG**  
**Seesatz**  
**6204 Sempach**  
**Schweiz / Switzerland**

DATE / DATUM  
March 16, 2010, 16.03.2010

**GUTACHTERLICHE STELLUNGNAHME / EXPERT'S REPORT**

**- SOFTASEPT®N (GEFÄRBT / COLORÉ) -**

Im Februar und März 2010 wurden durch die Hygiene Nord GmbH mit dem Prüfmuster **Softasept®N (gefärbt / coloré)**, einem Mittel zur Hautdesinfektion der Firma B. Braun Medical AG, Sempach, Schweiz, folgende Untersuchungen auf Basis der „Standardmethoden der DGHM zur Prüfung chemischer Desinfektionsverfahren“ (Stand: 01.09.2001) und des „Anforderungskatalogs für die Aufnahme von chemischen Desinfektionsverfahren in die Desinfektionsmittel-Liste der DGHM“ (Stand: 04.02.2002) durchgeführt:

*The efficacy of **Softasept®N (gefärbt / coloré)** as skin antiseptic (manufactured by B.Braun Medical AG, Sempach, Switzerland) was evaluated by the testing laboratory Hygiene Nord GmbH, Greifswald, Germany. The following tests were performed during February and March 2010 in accordance with the "Standard methods of the German Society of Hygiene and Microbiology (DGHM) for testing of chemical disinfection processes" (issue: 2001-09-01) and with respect to the "Catalogue of Requirements for Including Chemical Disinfection Processes in the DGHM List of Disinfectants (issue 02/2002):*

1. Bestimmung der **bakteriostatischen** und **levurostatischen Wirksamkeit** sowie geeigneter **Neutralisationsmittel**. Die Versuchsdurchführung und die Ergebnisse sind im Prüfbericht A 10030-1 der Hygiene Nord GmbH vom 16.03.2010 enthalten.

*Determination of a suitable **neutralizer** and analysis of the bacteriostatic and fungistatic efficacy. Results are presented in the Hygiene Nord GmbH test report A 10030-1 (March 16, 2010).*

2. Durch einen **qualitativen Suspensionstest** wurde die bakterizide und levurozide Wirksamkeit des Prüfmusters **Softasept®N (gefärbt / coloré)** untersucht. Die Versuchsdurchführung und die Ergebnisse sind im Prüfbericht A 10030-1 der Hygiene Nord GmbH vom 16.03.2010 enthalten.

*The bactericidal and yeasticidal efficacy of **Softasept®N (gefärbt / coloré)** was further analyzed in **qualitative suspension tests**. Results are presented in the Hygiene Nord GmbH test report A 10030-1 (March 16, 2010).*

3. Im **quantitativen Suspensionsversuch** wurde die bakterizide und levurozide Wirksamkeit des Prüfmusters **Softasept®N (gefärbt / coloré)** unter hoher organischer Belastung bestimmt (0,3 % Albumin + 0,3 % Schaferythrozyten) untersucht. Die Versuchsdurchführung und die Ergebnisse sind im Prüfbericht A 10030-1 der Hygiene Nord GmbH vom 16.03.2010 enthalten.

*The bactericidal and yeasticidal efficacy of **Softasept®N (gefärbt / coloré)** was evaluated by **quantitative suspension tests** under dirty conditions (0.3 % albumin + 0.3 % sheep erythrocytes). Results are presented in the Hygiene Nord GmbH test report A 10030-1 (March 16, 2010).*

4. Im **praxisnahen Versuch mit Probanden** wurde die Wirksamkeit von **Softasept®N (gefärbt / coloré)** auf talgdrüsenarmer (Oberarm) und talgdrüsenreicher Haut (Stirn) untersucht, letzteres bei einer statt 10 min auf 2,5 min verkürzten Einwirkzeiten. Die Versuchsdurchführung und die Ergebnisse sind im Prüfbericht A 10030-2 der Hygiene Nord GmbH vom 16.03.2010 enthalten.

*The efficacy of **Softasept®N (gefärbt / coloré)** was evaluated under conditions simulating **practical conditions** using volunteers in accordance with the "Standard methods of the German Society of Hygiene and Microbiology for testing of chemical disinfection processes" (issue: 2001-09-01). Testing was conducted on skin exhibiting a low or high density of sebaceous glands – upper arm or forehead, respectively. In deviation from the standard protocol, the contact time in the test on the forehead was reduced from 10 min to only 2.5 min. Results are presented in the Hygiene Nord GmbH test report A 10030-2, dated March 16, 2010).*

## ZUSAMMENFASSUNG / SUMMARY

Nach Bewertung der Ergebnisse kann festgestellt werden, dass das Prüfprodukt **Softasept®N (gefärbt / coloré)** dem „Anforderungskatalog für die Aufnahme von chemischen Desinfektionsverfahren in die Desinfektionsmittel-Liste der DGHM“ (Stand: 20.02.2002) genügt, da folgende Wirkungen beobachtet wurden:

*Upon evaluation of the test results it can be concluded that **Softasept®N (gefärbt / coloré)** complies with the requirements of the "Catalogue of Requirements for Including Chemical Disinfection Processes in the DGHM List of Disinfectants (issue 02/2002):*

- Bakterizide und levurozide Wirksamkeit in den *in vitro* - Tests: Im quantitativen Suspensionsversuch war das Präparat innerhalb von 15 s ab einer Konzentration von 75 % gegenüber den Prüforganismen *E. coli*, *P. mirabilis*, *P. aeruginosa*, *E. hirae*, *S. aureus* und *C. albicans* wirksam.

Im quantitativen Suspensionstest unter hoher Belastung wurden die Prüfspezies *P. aeruginosa*, *E. coli*, *E. hirae*, *S. aureus* und *C. albicans* bei der Konzentration-Zeit-Relation 80 % / 15 s in einem ausreichenden Maße inaktiviert ( $\log RF \geq 5$  bzw.  $\log RF \geq 4$ ).

*Bactericidal and yeasticidal activity in the in vitro tests: In the qualitative suspension test the product showed a sufficient activity against the test organisms E. coli, P. mirabilis, P. aeruginosa, E. hirae, S. aureus and C. albicans within a contact time of 15 s at a product concentration of 75 %.*

*In the quantitative suspension test under dirty conditions the product possesses bactericidal ( $\log RF \geq 5$ ) and yeasticidal ( $\log RF \geq 4$ ) activity against the test organisms P. aeruginosa, E. coli, E. hirae, S. aureus and C. albicans within the contact time of 15 s at a product concentrations of 80 %.*

- Wirksamkeit unter praxisnahen Bedingungen: Hautdesinfektion – praxisnaher Versuch mit Probanden an talgdrüsenarmer und talgdrüsenreicher Haut. Das Prüfprodukt **Softasept®N (gefärbt / coloré)** entsprach dabei den Anforderungen der DGHM innerhalb von 15 s, 30 s und 60 s (talgdrüsenarme Haut) bzw. 2.5 min und 30 min (talgdrüsenreiche Haut).

*The skin antiseptic **Softasept®N (gefärbt / coloré)** conforms to DGHM requirements (2001 / 2002) when skin exhibiting a low density of sebaceous glands is kept moist with the product for 15 s, 30 s and 60 s, and skin exhibiting a high density of sebaceous glands is kept moist with the product for 2.5 min and 30 min. A greater or not significantly smaller reduction of the transient skin flora was obtained with **Softasept®N (gefärbt / coloré)** within those contact times.*

Für die Aufnahme in die Desinfektionsmittelliste des VAH kann daher folgende Anwendungsempfehlung für **Softasept®N (gefärbt / coloré)** als **Mittel zur Hautdesinfektion** gegeben werden:

*It can therefore be recommended to include **Softasept®N (gefärbt / coloré)** in the VAH List of Disinfectants as a product for **instrument disinfection** as follows:*

**Softasept®N (gefärbt / coloré):**

Hautdesinfektion – Feuchthalten von talgdrüsenarmer Haut / Skin disinfection – skin with low density of sebaceous glands (keeping skin moist with the product)

- vor Punktion und Injektion / **100 % /  $\geq 15$  s**
- prior to punctures and injections

- vor Punktion von Gelenken, Körperhöhlen, **100 % /  $\geq 1$  min**  
Hohlorganen, sowie vor operativen Eingriffen /
- prior to punctures of joints, cavities and hollow  
organs; before surgical interventions

Hautdesinfektion - Feuchthalten von talgdrüsenreicher Haut / Skin disinfection - skin with high density of sebaceous glands (keeping skin moist with the product)

- vor allen Eingriffen / **100 % /  $\geq 2.5$  min**
- before all interventions

Greifswald, 16.03.2010 / March 16, 2010

  
Dipl. Biol. T. Koburger

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

**B.Braun Medical AG**

**Seesatz**

**CH-6204 Sempach**

**Schweiz / Switzerland**

CUSTOMER NUMBER  
324

DATE  
March 16, 2010

**TEST REPORT A 10030-1**  
**SOFTASEPT®N (GEFÄRBT / COLORÉ)**

## Purpose

The bactericidal and yeasticidal activity of the product **Softasept®N (gefärbt / coloré)**, manufactured by B. Braun Medical AG, Sempach, Switzerland, should to be evaluated by *in vitro* tests in accordance with the "Standard methods of the German Society of Hygiene and Microbiology (DGHM) for testing of chemical disinfection processes" and the "Catalogue of requirements for including chemical disinfectant processes in the DGHM list of disinfectants" (issue: 2002-02-04).

## Test description

Product name: **Softasept® N (gefärbt / coloré)**  
 Batch number: 9241M19  
 Manufacturer: B.Braun Medical AG  
 Date of delivery: February 15, 2010  
 Storage conditions: Room temperature  
 Product dilution: hard water  
 Date of order: February 09, 2010  
 Order number: A 10030  
 Sample number: P 100303  
 Test time: March 03, 2010 – March 17, 2010

Basis: Standard methods of the German Society of Hygiene and Microbiology (DGHM) for testing of chemical disinfection processes (issue: 2001-09-01)  
 Catalogue of requirements for including chemical disinfectant processes in the DGHM list of disinfectants (issue: 2002-02-04)

Test organisms:

<i>Escherichia coli</i> K12	NCTC 10538
<i>Pseudomonas aeruginosa</i>	ATCC 15442
<i>Enterococcus hirae</i>	ATCC 10541
<i>Staphylococcus aureus</i>	ATCC 6538
<i>Proteus mirabilis</i>	ATCC 14153
<i>Candida albicans</i>	ATCC 10231

Test solution: 100 % / 80 % / 50 % / 25 % / 12.5 %

Odor: alcoholic

Appearance: clear, red liquid

Active ingredients in 100 g: 74.1 g Ethanol  
 10 g Propan-2-ol

Active ingredients in 1 ml : 654.3 mg Ethanol (96 %)  
 83 mg Propan-2-ol

pH:

100 %:	2.73	50 %:	2.81
80 %:	3.12	25 %:	3.41

Neutralizer: 3.0 % Tween 80 + 3.0 % Saponin + 0.1 % L-Histidine + 0.1 % Cysteine (Neutralizer II)

Contact time: 15 s / 30 s / 1 min

Interfering substance: 0.3 % sheep erythrocytes + 0.3 % albumin (dirty conditions)

Test temperature: 21 ± 1 °C

Incubation temperature: 36 ± 1 °C (*C. albicans*: 30 ± 1 °C)

## Test Method

### Selection of a suitable neutralizer / MIC

The test was performed in accordance with the "Standard methods of the German Society of Hygiene and Microbiology (DGHM) for testing of chemical disinfection processes (issue: 2001-09-01)". Accordingly, the following test organisms were used:

-	<i>S. aureus</i>	ATCC 6538	<i>P. aeruginosa</i>	ATCC 15442
-	<i>E. coli</i> K12	NCTC 10538	<i>E. hirae</i>	ATCC 10541
-	<i>P. mirabilis</i>	ATCC 14153	<i>C. albicans</i>	ATCC 10231

The test is performed using the following growth medium:

- Tryptone Soya Broth (TSB)

The product is diluted with hard water; tests are performed at room temperature ( $21 \pm 1$  °C). The test organisms are incubated at  $36 \pm 1$  °C (*C. albicans*:  $30 \pm 1$  °C). Detailed results are presented in table 1.

### Qualitative suspension test

The test was performed in accordance with the "Standard methods of the German Society of Hygiene and Microbiology (DGHM) for testing of chemical disinfection processes (issue: 2001-09-01)". The bactericidal and yeasticidal activity of the test product **Softasept®N (gefärbt / coloré)** was evaluated using the following test organisms:

-	<i>S. aureus</i>	ATCC 6538	<i>P. aeruginosa</i>	ATCC 15442
-	<i>E. coli</i> K12	NCTC 10538	<i>E. hirae</i>	ATCC 10541
-	<i>P. mirabilis</i>	ATCC 14153	<i>C. albicans</i>	ATCC 10231

The product is diluted with hard water; tests are performed at room temperature ( $21 \pm 1$  °C). The test organisms are incubated at  $36 \pm 1$  °C (*C. albicans*:  $30 \pm 1$  °C). Detailed results are presented in table 2.

### Quantitative suspension test under dirty conditions

A test suspension of bacteria including an interfering substance (0.3% albumin + 0,3 % sheep erythrocytes – dirty conditions) is added to a sample of the product **Softasept®N (gefärbt / coloré)** (diluted with hard water, if necessary). The mixture is maintained at  $21 \pm 1$  °C for the required contact time. At the end of the contact time, an aliquot is taken; the microbiocidal activity in this portion is immediately neutralized. The number of surviving test organisms in each sample is determined by plating aliquots of the neutralized test suspensions and its dilutions. The reduction is calculated in relation to a sample containing water instead of the test product (water control, WSH control).

The experimental conditions, the non-toxicity of the neutralizer and the dilution-neutralization method are validated according to the DGHM standard methods:

- Co 1 = Water control (WSH)
- Co 2 = Method validation (Dilution-neutralization method)
- Co 3 = Non-toxicity of the neutralizer

The test was performed using *Pseudomonas aeruginosa*, *Staphylococcus aureus*, *Enterococcus hirae*, *E. coli* and *C. albicans* as test-organisms. Results are presented in tables 3.1 – 3.4

## Results

### Selection of a suitable neutralizer / Minimal inhibitory concentration (MIC)

In accordance with the test results presented in table 1, the combination 3.0 % Tween 80 + 3.0 % Saponin + 0.1 % L-Histidine + 0.1 % Cysteine (Neutralizer II) was selected as a suitable neutralizer. A product concentration of **50 %** was determined as the minimal inhibitory concentration at the methodically determined contact time of 24 h.

### Qualitative suspension test

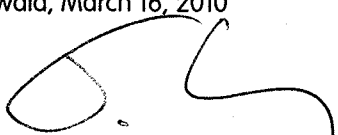
The test results presented in table 2 indicate a microbiocidal activity of the test product **Softasept®N (gefärbt / coloré)** at the concentration contact time relation **75 % / 15 s** for the test organisms tested. The activity of the test product against the test organism *P. aeruginosa* is not greater than that against *P. mirabilis*. The activity against *E. coli* is on par with that of *P. aeruginosa*. In agreement with the DGHM catalogue of requirements for including chemical disinfectant processes in the DGHM list of disinfectants (issue: 2002-02-04) *E. coli* was therefore included in the quantitative suspension test (see below).


### Quantitative suspension test

According to the "Catalogue of requirements for including chemical disinfectant processes in the DGHM list of disinfectants (issue: 2002-02-04)", the batch 9241M19 of the product **Softasept®N (gefärbt / coloré)**, applied at a product concentration of **80 %**, possesses bactericidal ( $\log R \geq 5$ ) and yeastocidal activity ( $\log R \geq 4$ ) in **15 s** at 20 °C under **dirty conditions** (0.3 % albumin + 0.3 % sheep erythrocytes) for reference strains *P. aeruginosa*, *S. aureus*, *E. hirae* and *C. albicans*.

Results are validated in accordance with DGHM requirements.

Greifswald, March 16, 2010

  
 Dipl. Biol. T. Koburger  
 - Geschäftsführer -

  
 Dr. med. P. Rudolph  
 FA für Hygiene und Umweltmedizin -

**Table 1: Identification of a suitable neutralizer (according to DGHM standard methods, 2001)**

**Date / performing:** March 03, 2010 **Order number:** A 10030  
**Substance:** Softasept®N (gefärbt / coloré) **Sample number:** P 091821  
**Test organism:** see table 1 **Batch number:** 9241M19  
**Incubation temperature:** 36 °C (30 °C *C. albicans*) **Test suspension:** see table 1  
**Incubation time:** 24 h

concentration (%)	<i>S. aureus</i> 3.00 *10 <sup>9</sup> cfu /ml (9.48 log)	<i>E. hirae</i> 1.70*10 <sup>9</sup> cfu/ml (9.23 log)	<i>E. coli</i> 1.50*10 <sup>9</sup> cfu/ml (9.18 log)	<i>P. mirabilis</i> 3.00*10 <sup>9</sup> cfu/ml (9.48 log)	<i>P. aeruginosa</i> 5.00*10 <sup>9</sup> cfu/ml (9.70 log)	<i>C. albicans</i> 3.30*10 <sup>9</sup> cfu/ml (8.452 log)
50	O I II III - - - -	O I II III - - - -	O I II III - - - -	O I II III - - - -	O I II III - - - -	O I II III - - - -
25	- - (+) -	(+) (+) + +	- - - -	- - - -	- - (+) (+)	+ + + +
12.5	+ + + +	+ + + +	+ + + +	+ + + +	- + + +	+ + + +
control*	+ + + +	+ + + +	+ + + +	+ + + +	+ + + +	+ + + +

control\* = WSH

- I = 3 % Tween 80 + 0.3 % Lecithin + 0.1 % Cysteine
- II = 3 % Tween 80 + 3 % Saponin + 0.1 % Histidine + 0.1 % Cysteine
- III = 3 % Tween 80 + 0.3 % Lecithin + 0.1 % Histidine + 0.5 % Na-Thiosulfate

- = no growth
- + = growth
- (+) = minimal growth



**Table 2: Results of the qualitative suspension test (according to DGHM standard methods, 2001)**

<b>Date / performing:</b>	March 03, 2010	<b>Order number:</b>	A 10030
<b>Product:</b>	<b>Softasept®N</b> (gefärbt / coloré)	<b>Sample number:</b>	P 091821
<b>Test organism:</b>	see table 2	<b>Batch number:</b>	9241M19
<b>Incubation temperature:</b>	36 °C (30 °C <i>C. albicans</i> )	<b>Test suspension:</b>	see table 2
<b>Incubation time:</b>	24 h (48 h <i>C. albicans</i> )	<b>Neutralizer:</b>	II

concentration	<i>S. aureus</i> 3.00*10 <sup>9</sup> cfu/ml (9.48 log)			<i>E. hirae</i> 1.70*10 <sup>9</sup> cfu/ml (9.23 log)			<i>E. coli</i> 1.50*10 <sup>9</sup> cfu/ml (9.18 log)			<i>P. mirabilis</i> 3.00*10 <sup>9</sup> cfu/ml (9.48 log)			<i>P. aeruginosa</i> 5.00*10 <sup>9</sup> cfu/ml (9.70 log)			<i>C. albicans</i> 3.30*10 <sup>9</sup> cfu/ml (8.52 log)		
%	15 s	30 s	60 s	15 s	30 s	60 s	15 s	30 s	60 s	15 s	30 s	60 s	15 s	30 s	60 s	15 s	30 s	60 s
75	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
50	-	-	-	(+)	(+)	(+)	(+)	(+)	(+)	(+)	-	-	(+)	(+)	(+)	-	-	-
25	+	+	+	+	+	+	+	+	+	+	+	+	+	(+)	(+)	+	+	+
12.5	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
control*	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+

\* = WSH (water of standardized hardness)  
 - = no growth  
 + = growth  
 (+) = minimal growth

**Table 3.1 Results of the quantitative suspension test (DGHM 2001)**

**Date / performing:** March 16, 2010  
**Substance:** Softasept®N (gefärbt / coloré)  
**Test organism:** *S. aureus*  
**Interfering substance:** 0.3 % sheep erythrocytes + 0.3 % albumin  
**Incubation temperature:** 36 ± 1 °C  
**Incubation time:** 24 h  
**Test suspension:** 2.70\*10<sup>9</sup> cfu/ml (9.43 log)  
**Method:** Dilution-neutralization-method

**Order number:** A 10030  
**Employee in charge:** U. Joachim  
**Neutralizer:** II  
**Reaction temperature:** 21 ± 1 °C  
**Batch number:** 9241M19

concentration	dilution	time: 15 s			time: 30 s			time: 60 s		
		cfu / plate	log <sub>10</sub> (x)	RF	cfu / plate	log <sub>10</sub> (x)	RF	cfu / plate	log <sub>10</sub> (x)	RF
80 %	1 ml 10 <sup>0</sup>	<u>4</u>	0.60	<b>6.78</b>	<u>0</u>	0.00	≥ <b>7.40</b>	<u>22</u>	1.34	<b>6.05</b>
	0.1 ml 10 <sup>0</sup>	<u>1</u>	1.00	<b>7.39</b>	<u>0</u>			<u>1</u>		
	0.1 ml 10 <sup>-1</sup>	<u>0</u>			<u>0</u>					
	0.1 ml 10 <sup>-2</sup>	<u>0</u>			<u>0</u>			<u>0</u>		
50 %	1 ml 10 <sup>0</sup>	> 300			<u>48</u>	1.68	<b>5.71</b>	<u>0</u>	0.00	≥ <b>7.39</b>
	0.1 ml 10 <sup>0</sup>	> 300			<u>5</u>			<u>0</u>		
	0.1 ml 10 <sup>-1</sup>	<u>57</u>	3.76	<b>3.64</b>	<u>0</u>			<u>0</u>		
	0.1 ml 10 <sup>-2</sup>	<u>7</u>			<u>0</u>			<u>0</u>		
25 %	1 ml 10 <sup>0</sup>	> 300			> 300			> 300		
	0.1 ml 10 <sup>0</sup>	> 300			> 300			> 300		
	0.1 ml 10 <sup>-1</sup>	> 300			> 300			> 300		
	0.1 ml 10 <sup>-2</sup>	> 300	> 5.48	<b>1.91</b>	> 300	> 5.48	<b>1.92</b>	> 300	> 5.48	<b>1.91</b>
WSH (co 1)	0.1 ml 10 <sup>-2</sup>	> 300			> 300			<u>300</u>		
	0.1 ml 10 <sup>-3</sup>	> 300			> 300			<u>300</u>		
	0.1 ml 10 <sup>-4</sup>	<u>220</u>	<b>7.39</b>		<u>238</u>	<b>7.40</b>		<u>252</u>	<b>7.39</b>	
	0.1 ml 10 <sup>-5</sup>	<u>27</u>			<u>26</u>			<u>24</u>		

**Controls and Validation:**

		number of colonies [cfu/plate]	cfu / ml (with regard to the validation suspension)	log <sub>10</sub> (x)
<b>control 2</b> (co 2)	0.1 ml 10 <sup>0</sup>	<u>196</u>	2.13E+04	4.33
	0.1 ml 10 <sup>-1</sup>	<u>23</u>		
<b>control 3</b> (co 3)	0.1 ml 10 <sup>0</sup>	<u>218</u>	1.94E+04	5.29
	0.1 ml 10 <sup>-1</sup>	<u>17</u>		
Validation suspension:			2.70E+05	4.43
		80 % - 60 s	controls o.k.?	<b>Yes</b>

**Table 3.2 Results of the quantitative suspension test (DGHM 2001)**

**Date / performing:** March 16, 2010  
**Substance:** Softasept®N (gefärbt / coloré)  
**Test organism:** *P. aeruginosa*  
**Interfering substance:** 0.3 % sheep erythrocytes + 0.3 % albumin  
**Incubation temperature:** 36 ± 1 °C  
**Incubation time:** 24 h  
**Test suspension:** 1.50\*10<sup>9</sup> cfu/ml (9.18 log)  
**Method:** Dilution-neutralization-method

**Order number:** A 10030  
**Employee in charge:** U. Joachim  
**Neutralizer:** II  
**Reaction temperature:** 21 ± 1 °C  
**Batch number:** 9241M19

concentration	dilution	time: 15 s			time: 30 s			time: 60 s		
		cfu / plate	log <sub>10</sub> (x)	RF	cfu / plate	log <sub>10</sub> (x)	RF	cfu / plate	log <sub>10</sub> (x)	RF
80 %	1 ml 10 <sup>0</sup>	0	0.00	≥ 7.19	0	0.00	≥ 7.17	0	0.00	≥ 7.23
	0.1 ml 10 <sup>0</sup>	0			0			0		
	0.1 ml 10 <sup>-1</sup>	0			0			0		
	0.1 ml 10 <sup>-2</sup>	0			0			0		
50 %	1 ml 10 <sup>0</sup>	0	0.00	≥ 7.19	0	0.00	≥ 7.17	2	0,30	6,93
	0.1 ml 10 <sup>0</sup>	0			0			0		
	0.1 ml 10 <sup>-1</sup>	0			0			0		
	0.1 ml 10 <sup>-2</sup>	0			0			0		
25 %	1 ml 10 <sup>0</sup>	> 300			> 300			8	0,90	6,33
	0.1 ml 10 <sup>0</sup>	> 300			> 300			0		
	0.1 ml 10 <sup>-1</sup>	> 300			> 300			0		
	0.1 ml 10 <sup>-2</sup>	≥ 300	> 5.48	≤ 1.71	40	4.60	2.57	0		
WSH (co 1)	0.1 ml 10 <sup>-2</sup>	> 300			> 300			> 300		
	0.1 ml 10 <sup>-3</sup>	> 300			> 300			> 300		
	0.1 ml 10 <sup>-4</sup>	148	7.19		157	7.17		152	7.23	
	0.1 ml 10 <sup>-5</sup>	16			14			19		

**Controls and Validation:**

		number of colonies [cfu/plate]	cfu /ml (with regard to the validation suspension)	log <sub>10</sub> (x)
control 2 (co 2)	0.1 ml 10 <sup>0</sup>	151	1.46E+04	5.16
	0.1 ml 10 <sup>-1</sup>	14		
control 3 (co 3)	0.1 ml 10 <sup>0</sup>	130	1.35E+04	5.13
	0.1 ml 10 <sup>-1</sup>	14		
Validation suspension:			1.50E+05	5.18
		80 % - 60 s	controls o.k.?	Yes

**Table 3.3 Results of the quantitative suspension test (DGHM 2001)**

**Date / performing:** March 16, 2010  
**Substance:** Softasept®N (gefärbt / coloré)  
**Test organism:** *E. hirae*  
**Interfering substance:** 0.3 % sheep erythrocytes + 0.3 % albumin  
**Incubation temperature:** 36 ± 1 °C  
**Incubation time:** 24 h  
**Test suspension:** 1.50\*10<sup>9</sup> cfu/ml (9.18 log)  
**Method:** Dilution-neutralization-method

**Order number:** A 10030  
**Employee in charge:** U. Joachim  
**Neutralizer:** II  
**Reaction temperature:** 21 ± 1 °C  
**Batch number:** 9241M19

concentration	dilution	time: 15 s			time: 30 s			time: 60 s		
		cfu / plate	log <sub>10</sub> (x)	RF	cfu / plate	log <sub>10</sub> (x)	RF	cfu / plate	log <sub>10</sub> (x)	RF
80 %	1 ml 10 <sup>0</sup>	0	0.00	≥ 7.17	0	0.00	≥ 7.23	0	0.00	≥ 7.25
	0.1 ml 10 <sup>0</sup>	0			0			0		
	0.1 ml 10 <sup>-1</sup>	0			0			0		
	0.1 ml 10 <sup>-2</sup>	0			0			0		
50 %	1 ml 10 <sup>0</sup>	0	0.00	≥ 7.17	0	0.00	≥ 7.23	0	0.00	≥ 7.25
	0.1 ml 10 <sup>0</sup>	0			0			0		
	0.1 ml 10 <sup>-1</sup>	0			0			0		
	0.1 ml 10 <sup>-2</sup>	0			0			0		
25 %	1 ml 10 <sup>0</sup>	> 300			> 300			> 300		
	0.1 ml 10 <sup>0</sup>	> 300			> 300			> 300		
	0.1 ml 10 <sup>-1</sup>	> 300			> 300			> 300		
	0.1 ml 10 <sup>-2</sup>	> 300	> 5.48	≤ 1.70	> 300	> 5.48	≤ 1.75	> 300	> 5.48	≤ 1.77
WSH (co 1)	0.1 ml 10 <sup>-2</sup>	> 300			> 300			> 300		
	0.1 ml 10 <sup>-3</sup>	> 300			> 300			> 300		
	0.1 ml 10 <sup>-4</sup>	158	7.17		169	7.23		152	7.25	
	0.1 ml 10 <sup>-5</sup>	14			17			19		

**Controls and Validation:**

		number of colonies [cfu/plate]	cfu / ml (with regard to the validation suspension)	log <sub>10</sub> (x)
control 2 (co 2)	0.1 ml 10 <sup>0</sup>	154	1.47E+04	4.12
	0.1 ml 10 <sup>-1</sup>	14		
control 3 (co 3)	0.1 ml 10 <sup>0</sup>	162	1.71E+04	4.26
	0.1 ml 10 <sup>-1</sup>	18		
Validation suspension:			1.50E+04	4.23
		80 % - 60 s	controls o.k.?	Yes

**Table 3.4 Results of the quantitative suspension test (DGHM 2001)**

**Date / performing:** March 16, 2010  
**Substance:** Softasept®N (gefärbt / coloré)  
**Test organism:** *E. coli*  
**Interfering substance:** 0.3 % sheep erythrocytes + 0.3 % albumin  
**Incubation temperature:** 36 ± 1 °C  
**Incubation time:** 24 h  
**Test suspension** 1.60\*10<sup>9</sup> cfu/ml (9.20 log)  
**Method:** Dilution-neutralization-method

**Order number:** A 10030  
**Employee in charge:** U. Joachim  
**Neutralizer:** II  
**Reaction temperature:** 21 ± 1 °C  
**Batch number:** 9241M19

concentration	dilution	time: 15 s			time: 30 s			time: 60 s		
		cfu / plate	log <sub>10</sub> (x)	RF	cfu / plate	log <sub>10</sub> (x)	RF	cfu / plate	log <sub>10</sub> (x)	RF
80 %	1 ml 10 <sup>0</sup>	0	0.00	≥ 7.51	0	0.00	≥ 7.54	0	0.00	≥ 7.58
	0.1 ml 10 <sup>0</sup>	0			0			0		
	0.1 ml 10 <sup>-1</sup>	0			0			0		
	0.1 ml 10 <sup>-2</sup>	0			0			0		
50 %	1 ml 10 <sup>0</sup>	0	0.00	≥ 7.51	0	0.00	≥ 7.54	0	0.00	≥ 7.58
	0.1 ml 10 <sup>0</sup>	0			0			0		
	0.1 ml 10 <sup>-1</sup>	0			0			0		
	0.1 ml 10 <sup>-2</sup>	0			0			0		
25 %	1 ml 10 <sup>0</sup>	> 300			> 300			> 300		
	0.1 ml 10 <sup>0</sup>	> 300			> 300			> 300		
	0.1 ml 10 <sup>-1</sup>	> 300			> 300			> 300		
	0.1 ml 10 <sup>-2</sup>	> 300	> 5.48	≤ 2.03	> 300	> 5.48	≤ 2.07	208	5.32	2.26
WSH (co 1)	0.1 ml 10 <sup>-2</sup>	> 300			> 300			> 300		
	0.1 ml 10 <sup>-3</sup>	> 300			> 300			> 300		
	0.1 ml 10 <sup>-4</sup>	> 300			> 300			> 300		
	0.1 ml 10 <sup>-5</sup>	32	7.51		32	7.54		38	7.58	

Controls and Validation:

		number of colonies [cfu/plate]	cfu / ml (with regard to the validation suspension)	log <sub>10</sub> (x)
control 2 (co 2)	0.1 ml 10 <sup>0</sup>	114		
	0.1 ml 10 <sup>-1</sup>	13	1.22E+04	4.09
control 3 (co 3)	0.1 ml 10 <sup>0</sup>	109		
	0.1 ml 10 <sup>-1</sup>	12	1.15E+04	4.06
Validation suspension:			1.60E+04	4.20
		80 % - 60 s	controls o.k.?	Yes

**Table 3.5 Results of the quantitative suspension test (DGHM 2001)**

**Date / performing:** March 16, 2010  
**Substance:** Softasept®N (gefärbt / coloré)  
**Test organism:** *C. albicans*  
**Interfering substance:** 0.3 % sheep erythrocytes + 0.3 % albumin  
**Incubation temperature:** 30 ± 1 °C  
**Incubation time:** 48 h  
**Test suspension** 2.20\*10<sup>8</sup> cfu/ml (8.34 log)  
**Method:** Dilution-neutralization-method

**Order number:** A 10030  
**Employee in charge:** U. Joachim  
**Neutralizer:** II  
**Reaction temperature:** 21 ± 1 °C  
**Batch number:** 9241M19

concentration	dilution	time: 15 s			time: 30 s			time: 60 s		
		cfu / plate	log <sub>10</sub> (x)	RF	cfu / plate	log <sub>10</sub> (x)	RF	cfu / plate	log <sub>10</sub> (x)	RF
80 %	1 ml 10 <sup>0</sup>	0	0.00	≥ 6.23	0	0.00	≥ 6.20	0	0.00	≥ 6.28
	0.1 ml 10 <sup>0</sup>	0			0			0		
	0.1 ml 10 <sup>-1</sup>	0			0			0		
	0.1 ml 10 <sup>-2</sup>	0			0			0		
50 %	1 ml 10 <sup>0</sup>	0	0.00	≥ 6.23	0	0.00	≥ 6.20	0	0.00	≥ 6.28
	0.1 ml 10 <sup>0</sup>	0			0			0		
	0.1 ml 10 <sup>-1</sup>	0			0			0		
	0.1 ml 10 <sup>-2</sup>	0			0			0		
25 %	1 ml 10 <sup>0</sup>	> 300			> 300			> 300		
	0.1 ml 10 <sup>0</sup>	> 300			> 300			> 300		
	0.1 ml 10 <sup>-1</sup>	> 300			> 300			> 300		
	0.1 ml 10 <sup>-2</sup>	> 300	> 5.48	≤ 0.75	> 300	> 5.48	≤ 0.72	> 300	> 5.48	≤ 0.80
WSH (co 1)	0.1 ml 10 <sup>-2</sup>	> 300			> 300			> 300		
	0.1 ml 10 <sup>-3</sup>	181	6.23		179	6.20		188	6.28	
	0.1 ml 10 <sup>-4</sup>	16			14			19		
	0.1 ml 10 <sup>-5</sup>	n.d.			n.d.			n.d.		

Controls and Validation:

		number of colonies [cfu/plate]	cfu / ml (with regard to the validation suspension)	log <sub>10</sub> (x)
control 2 (co 2)	0.1 ml 10 <sup>0</sup>	300		
	0.1 ml 10 <sup>-1</sup>	81	8.10E+04	4.91
control 3 (co 3)	0.1 ml 10 <sup>0</sup>	300		
	0.1 ml 10 <sup>-1</sup>	92	9.20E+04	4.96
Validation suspension:			1.60E+04	4.20
		80 % - 60 s	controls o.k.?	Yes

**Legend:**

MW	=	average value
x	=	average value
RF	=	reduction factor
> 300	=	not countable
n.d.	=	not done
E	=	self-inhibition
Co 1	=	Control 01
Co 2	=	Control 02
Co 3	=	Control 03

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

**B.Braun Medical AG**

**Seesatz**

**CH-6204 Sempach**

**Schweiz / Switzerland**

CUSTOMER NUMBER  
324

DATE  
March 16, 2010

TEST REPORT A 10030-2  
**SOFTASEPT® N (GEFÄRBT / COLORÉ)**  
- SKIN DISINFEKTION -

## Purpose

The efficacy of **Softasept® N** (B. Braun Medical AG, Sempach, Switzerland) as a skin antiseptic should be evaluated in a test simulating practical conditions in accordance with the Standard Methods of the German Society for of Hygiene and Microbiology (DGHM, 2001).



## Test description

Manufacturer: B.Braun Medical AG

Product name: **Softasept®N (gefärbt / coloré)**

Sample number: P 100303

Batch number: 9241M19

Best before: 05/2014

Date of order: February 09, 2010

Date of delivery: February 15, 2010

Test date: February 25, 2010 – March 15, 2010

Basis: Standard methods of the German Society of Hygiene and Microbiology (DGHM) for testing of chemical disinfection processes (issue: 2001-09-01)  
Catalogue of requirements for including chemical disinfectant processes in the DGHM list of disinfectants (issue: 2002-02-04)

Test organisms: not applicable

Test solution: 100 %

Contact times: upper arm: 15 s, 30 s, 1 min  
forehead: 2,5 min, 30 min

Active ingredients in 100 ml: 74.1 g Ethanol  
10 g Propan-2-ol

Active ingredients in 1 ml : 654.3 mg Ethanol (96 %)  
83 mg Propan-2-ol

pH: 100 %: 2.73                      50 %: 2.81  
80 %: 3.12                      25 %: 3.41

Odour: alcoholic

Appearance: clear, red liquid

Neutralizer: 3.0 % Tween 80 + 3.0 % Saponin + 0.1 % L-Histidine + 0.1 % Cysteine  
(Neutralizer II)

Test temperature:  $21 \pm 1$  °C

Incubation temperature:  $36 \pm 1$  °C

## Test method

The evaluation of the efficacy of the test product **Softasept®N (gefärbt / coloré)** was performed in accordance with the "Standard methods of the German Society of Hygiene and Microbiology (DGHM) for testing of chemical disinfection processes" (issue: 2001-09-01) and the "Catalogue of Requirements for Including Chemical Disinfection Processes in the DGHM List of Disinfectants" (issue 2002-02-04). Testing was therefore based on the DGHM standard method 13:

- Skin disinfection – practical test with test subjects on skin exhibiting a high density of sebaceous glands (forehead) and a low density of sebaceous glands (upper arm)

The test evaluates the efficacy of a disinfectant against the resident skin flora, pre- and postvalues are assessed by the quantitative swab method on test areas measuring 5 cm<sup>2</sup>. Data of at least 18 out of 20 subjects are required for a conclusive statement regarding the efficacy of a skin antiseptic.

### **Skin disinfection – skin exhibiting a low density of sebaceous glands (upper arm)**

The tests evaluating the efficacy of the reference product (70 % propan-2-ol) on skin with low density of sebaceous glands were performed on the right upper arm, for testing the efficacy of **Softasept®N (gefärbt / coloré)** the left upper arm was used.

The contact times were set to 15 s, 30 s and 1 min. The products were applied only once at the beginning of those contact times on the previously selected test areas measuring 5 cm<sup>2</sup>.

The bacterial counts of the test areas before and after the disinfection were analyzed by the quantitative swab method as described by the DGHM Standard Methods. Prevalues were determined on a separate test area that neither product had been applied to. Results are summarized in table 1.1 and presented in detail in tables 2.1 to 2.2.

### **Skin disinfection – skin exhibiting a high density of sebaceous glands forehead**

The tests evaluating the efficacies of the reference- and the test product on skin exhibiting a high density of sebaceous glands were performed on the test subject's forehead.

The contact time of the test product were set to **2.5 min** and 30 min, whereas the reference product was tested at the standard contact times of 10 min and 30 min. In each case, the test areas were kept moist for the shorter contact time – 2.5 min (3 applications) and 10 min (5 applications), respectively. Samples (postvalues) were taken after 2.5 min or 10 min, respectively, and after 30 min as described above.

Prevalues were determined in each case on a separate test area that neither product had been applied to. The reduction factors (RF) were calculated by subtracting the log postvalue from respective log prevalue. Results are summarized in table 1.2 and presented in detail in tables 3.1 to 3.2.

The tests were performed at room temperature (21 ± 1 °C).

## Results

The efficacy of **Softasept®N (gefärbt / coloré)** as a skin antiseptic was analyzed in a test simulating practical conditions in accordance with DGHM requirements (2001/2002) on skin exhibiting high or low density of sebaceous glands. Results are valid, as the following requirements according to the DGHM standard methods were fulfilled: Data of 20 test subjects were acceptable for further analysis. Pre- and postvalues are presented in tables 2.1 to 3.2. The log reduction is calculated by subtracting the log prevalues from the log postvalues for each contact time. Results are summarized in tables 1.1 (upper arm) and 1.2 (forehead) and presented in detail in tables 2.1 to 3.2.

**Table 1.1: Summary of the mean reduction factors obtained with the reference product propan-2-ol and the test product Softasept®N (gefärbt / coloré) on skin exhibiting a low density of sebaceous glands (upper arm) at contact times of 15 s, 30 s and 1 min.**

contact time	Mean log RF	
	Reference product	Test product
15 s	<b>0.96</b>	<b>0.87</b>
30 s	<b>0.83</b>	<b>0.86</b>
1 min	<b>0.78</b>	<b>0.80</b>

**Table 1.2: Summary of the mean reduction factors obtained with the reference product propan-2-ol and the test product Softasept®N (gefärbt / coloré) on skin exhibiting a high density of sebaceous glands (forehead) at contact times of 2.5 min and 30 min.**

contact time	Mean log RF	
	Reference product	Test product
2.5 min	<b>0.92</b>	<b>1.07</b>
30 min	<b>1.37</b>	<b>1.36</b>

At the contact times of **15 s**, the mean reduction factors obtained with test product **Softasept®N (gefärbt / coloré)** on **skin exhibiting a low density of sebaceous glands** (upper arm) is smaller than that of the reference product Propan-2-ol. In Wilcoxon Signed Ranks Test (1-tailed), this difference is not considered significant at the agreed significance level of  $p = 0.1$  (15 s:  $p = 0.320$ ; see Table 4.1). The mean reduction factors of **Softasept®N (gefärbt / coloré)** at contact times of **30 s** and **1 min** are greater than that of the reference. The differences are also not considered significant (30 s:  $p = 0.446$ ; 1 min:  $p = 0.453$ ; see Table 4.2 and 4.3), significance testing is not necessary in this case, though, anyway.

On **skin exhibiting a high density of sebaceous glands** (forehead), the mean reduction factor obtained with test product **Softasept®N (gefärbt / coloré)** is greater at the contact time of **2.5 min** when compared to the results obtained with the reference product at the standard contact time of 10 min. The difference is just not considered significant In Wilcoxon Signed Ranks Test at the agreed significance level of  $p = 0.1$  ( $P_{2.5 \text{ min}}$  vs.  $R_{10 \text{ min}}$ :  $p = 0.121$ ; Table 5.1).

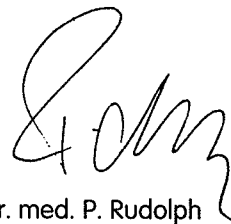
At the contact time **30 min**, the reduction obtained with **Softasept®N (gefärbt / coloré)** on **skin exhibiting a high density of sebaceous glands** is slightly, yet not significantly lower than that obtained with the reference product Propan-2-ol ( $P_{30 \text{ min}}$  vs.  $P_{30 \text{ min}}$ ;  $p = 0.446$ ; Table 5.2)

Therefore, the skin antiseptic **Softasept®N (gefärbt / coloré)** conforms to DGHM requirements (2001 / 2002) when skin exhibiting a low density of sebaceous glands (upper arm) and when skin exhibiting a high density of sebaceous glands is kept moist with this product for **15 s** or **2.5 min.** (forehead) , respectively

Greifswald, March 16, 2010



Dipl. Biol. T. Koburger  
- Geschäftsführer -



Dr. med. P. Rudolph  
- FA für Hygiene und Umweltmedizin -

**Tabelle 2.1: Skin disinfection – Skin with low density of sebaceous glands (upper arm), test results and reduction factors – reference product (70 Vol % Propan-2-ol)**

**Date:**  
**Substance:**  
**Neutralizer:**

March 11, 2010  
70 % Propan-2-ol  
II

**Order-Number:**  
**Sample-Number:**  
**Batch:**

A 10030-1  
not applicable  
081290026

Subject	Prevalue	Postvalue 15 s	Postvalue 30 s	Postvalue 1 min	log VW	log 15 s	log 30 s	log 1 min	RF 15 s	RF 30 s	RF 1 min
1	6.70E+03	2.82E+02	7.60E+02	3.48E+02	3.83	2.45	2.88	2.54	1.38	0.95	1.28
2	2.26E+02	2.80E+01	1.60E+01	6.20E+01	2.35	1.45	1.20	1.79	0.91	1.15	0.56
3	1.82E+02	8.80E+01	4.00E+00	6.00E+00	2.26	1.94	0.60	0.78	0.32	1.66	1.48
4	5.90E+02	3.60E+01	1.16E+02	5.00E+01	2.77	1.56	2.06	1.70	1.21	0.71	1.07
5	5.70E+02	2.00E+00	1.80E+01	2.80E+01	2.76	0.30	1.26	1.45	2.45	1.50	1.31
6	9.40E+01	1.40E+01	1.00E+00	8.20E+01	1.97	1.15	0.00	1.91	0.83	1.97	0.06
7	3.40E+01	2.00E+01	4.00E+00	8.00E+00	1.53	1.30	0.60	0.90	0.23	0.93	0.63
8	1.36E+02	4.00E+00	3.80E+01	1.00E+01	2.13	0.60	1.58	1.00	1.53	0.55	1.13
9	1.14E+02	6.00E+00	8.00E+00	6.00E+01	2.06	0.78	0.90	1.78	1.28	1.15	0.28
10	2.60E+01	2.60E+01	4.00E+00	6.00E+00	1.41	1.41	0.60	0.78	0.00	0.81	0.64
11	3.60E+01	4.00E+00	4.00E+00	4.00E+00	1.56	0.60	0.60	0.60	0.95	0.95	0.95
12	2.16E+02	4.60E+01	6.47E+03	9.20E+01	2.33	1.66	3.81	1.96	0.67	-1.48	0.37
13	2.14E+02	4.00E+00	6.60E+01	3.20E+01	2.33	0.60	1.82	1.51	1.73	0.51	0.83
14	4.94E+02	1.98E+02	1.60E+01	2.76E+02	2.69	2.30	1.20	2.44	0.40	1.49	0.25
15	8.00E+00	1.00E+00	4.00E+00	8.00E+00	0.90	0.00	0.60	0.90	0.90	0.30	0.00
16	7.20E+01	1.60E+01	6.90E+01	4.00E+00	1.86	1.20	1.84	0.60	0.65	0.02	1.26
17	2.08E+02	8.00E+00	6.00E+00	4.00E+00	2.32	0.90	0.78	0.60	1.41	1.54	1.72
18	3.40E+01	6.00E+00	2.00E+01	2.00E+00	1.53	0.78	1.30	0.30	0.75	0.23	1.23
19	3.00E+01	1.40E+01	6.00E+00	3.00E+01	1.48	1.15	0.78	1.48	0.33	0.70	0.00
20	1.60E+01	1.00E+00	2.00E+00	4.00E+00	1.20	0.00	0.30	0.60	1.20	0.90	0.60
Arithm. Mean											
Standard deviation (absolute)											
Standard deviation (relative)											
					2.06	1.11	1.24	1.28	0.96	0.83	0.78
					0.65	0.66	0.89	0.64	0.57	0.72	0.51
					31.67	59.98	71.86	50.19	59.93	87.37	64.71

**Tabelle 2.2: Skin disinfection – Skin with low density of sebaceous glands (upper arm), test results and reduction factors – test product (Softasept®N)**

**Date:** March 11, 2010 **Order-Number:** A 10030-1  
**Substance:** Softasept®N (gefärbt / coloré) **Sample-Number:** P 100303  
**Neutralizer:** II **Batch:** 9241M19

Subject	Prevalue	Postvalue 15 s	Postvalue 30 s	Postvalue 1 min	log VW	log 15 s	log 30 s	log 1 min	RF 15 s	RF 30 s	RF 1 min
1	3.76E+04	8.16E+03	3.38E+03	8.24E+03	4.58	3.91	3.53	3.92	0.66	1.05	0.66
2	3.00E+03	1.20E+01	1.40E+01	3.60E+01	3.48	1.08	1.15	1.56	2.40	2.33	1.92
3	2.96E+02	6.00E+00	1.00E+02	6.00E+00	2.47	0.78	2.00	0.78	1.69	0.47	1.69
4	1.58E+03	2.82E+02	1.82E+02	1.86E+02	3.20	2.45	2.26	2.27	0.75	0.94	0.93
5	5.10E+02	1.50E+02	1.84E+02	4.22E+02	2.71	2.18	2.26	2.63	0.53	0.44	0.08
6	4.80E+01	1.00E+00	2.00E+00	6.00E+00	1.68	0.00	0.00	0.78	1.68	1.68	0.90
7	1.80E+02	7.60E+01	4.00E+00	1.00E+01	2.26	1.88	0.60	1.00	0.37	1.65	1.26
8	2.18E+02	1.20E+01	5.20E+01	3.80E+01	2.34	1.08	1.72	1.58	1.26	0.62	0.76
9	8.60E+01	1.20E+01	8.00E+00	2.80E+01	1.93	1.08	0.90	1.45	0.86	1.03	0.49
10	1.12E+02	1.60E+01	1.20E+01	3.20E+01	2.05	1.20	1.08	1.51	0.85	0.97	0.54
11	1.00E+01	1.00E+01	1.60E+01	6.00E+00	1.00	1.00	1.20	0.78	0.00	-0.20	0.22
12	4.36E+02	3.20E+01	3.60E+01	8.00E+01	2.64	1.51	1.56	1.90	1.13	1.08	0.74
13	3.82E+02	1.40E+01	1.00E+01	4.40E+01	2.58	1.15	1.00	1.64	1.44	1.58	0.94
14	1.62E+03	2.66E+02	4.02E+02	2.76E+02	3.21	2.42	2.60	2.44	0.78	0.61	0.77
15	8.00E+00	4.00E+00	8.00E+00	1.00E+00	0.90	0.60	0.90	0.00	0.30	0.00	0.90
16	2.60E+01	2.00E+00	1.80E+01	6.00E+00	1.41	0.30	1.26	0.78	1.11	0.16	0.64
17	4.36E+02	5.60E+01	1.00E+01	6.80E+01	2.64	1.75	1.00	1.83	0.89	1.64	0.81
18	6.80E+01	9.80E+01	6.00E+00	6.00E+00	1.83	1.99	0.78	0.78	-0.16	1.05	1.05
19	2.60E+01	4.00E+00	2.00E+01	8.00E+00	1.41	0.60	1.30	0.90	0.81	0.11	0.51
20	8.00E+00	6.00E+00	8.00E+00	6.00E+00	0.90	0.78	0.90	0.78	0.12	0.00	0.12
Arithm. Mean											
Standard deviation (absolute)					2.26	1.39	1.40	1.46	0.87	0.86	0.80
Standard deviation (relative)					0.91	0.88	0.78	0.86	0.61	0.67	0.45
					40.33	63.60	55.86	58.82	69.53	77.34	56.04

**Table 3.1: Skin disinfection – Skin with high density of sebaceous glands (forehead), test results and reduction factors - reference product (70 Vol % Propan-2-ol)**

**Date:** February 25, 2010  
**Product:** 70 % Propan-2-ol  
**Neutralizer:** II

**Order number:** A 10030-1  
**Sample number:** not applicable  
**Batch:** 081290026

Subject	Prevalue	Postvalue 10 min	Postvalue 30 min	log prevalue	log postvalue 10 min	log postvalue 30 min	RF 10 min	RF 30 min
1	2.16E+04	2.06E+03	1.62E+03	4.33	3.31	3.21	1.02	1.12
2	4.80E+04	1.62E+04	1.38E+03	4.68	4.21	3.14	0.47	1.54
3	7.90E+03	1.52E+03	1.38E+03	3.90	3.18	3.14	0.72	0.76
4	1.50E+03	3.80E+01	6.00E+00	3.18	1.58	0.78	1.60	2.40
5	5.90E+03	2.88E+03	1.72E+02	3.77	3.46	2.24	0.31	1.54
6	4.00E+02	1.92E+02	7.40E+01	2.60	2.28	1.87	0.32	0.73
7	2.22E+04	1.62E+04	8.60E+02	4.35	4.21	2.93	0.14	1.41
8	1.03E+04	2.50E+02	2.82E+02	4.01	2.40	2.45	1.61	1.56
9	1.87E+04	2.62E+04	1.70E+04	4.27	4.42	4.23	-0.15	0.04
10	8.30E+03	5.60E+02	1.18E+03	3.92	2.75	3.07	1.17	0.85
11	3.50E+03	7.40E+02	8.80E+02	3.54	2.87	2.94	0.67	0.60
12	6.90E+03	5.40E+01	1.00E+01	3.84	1.73	1.00	2.11	2.84
13	2.06E+02	2.40E+01	2.00E+00	2.31	1.38	0.30	0.93	2.01
14	8.00E+01	2.22E+02	4.00E+00	1.90	2.35	0.60	-0.44	1.30
15	2.24E+04	2.72E+02	1.04E+02	4.35	2.43	2.02	1.92	2.33
16	5.00E+01	2.00E+00	3.06E+03	1.70	0.30	3.49	1.40	-1.79
17	4.56E+02	5.20E+01	2.00E+00	2.66	1.72	0.30	0.94	2.36
18	1.02E+05	3.58E+04	5.80E+01	5.01	4.55	1.76	0.45	3.25
19	3.60E+03	2.48E+02	7.80E+02	3.56	2.39	2.89	1.16	0.66
20	1.70E+03	1.40E+01	2.80E+01	3.23	1.15	1.45	2.08	1.78

	log prevalue	log postvalue 10 min	log postvalue 30 min	RF 10 min	RF 30 min
<b>Arithm. Mean</b>	3.56	2.63	2.19	<b>0.92</b>	<b>1.37</b>
<b>Standard deviation (absolute)</b>	0.90	1.13	1.12	0.70	1.08
<b>Standard deviation (relative)</b>	25.17	43.04	51.12	76.37	78.99

**Table 3.2: Skin disinfection – Skin with high density of sebaceous glands (forehead), test results and reduction factors – test product Softasept®N (gefärbt / coloré)**

**Date :** February 25, 2010 **Order number:** A 10030-1  
**Product:** Softasept®N (gefärbt / coloré) **Sample number:** P 100303  
**Neutralizer :** II **Batch:** 9241M19

Subject	Prevalue	Postvalue 2.5 min	Postvalue 30 min	log prevalue	log postvalue 2.5 min	log postvalue 30 min	RF 10 min	RF 30 min
1	2.16E+04	1.16E+02	5.08E+02	4.33	2.06	2.71	2.27	1.63
2	4.80E+04	1.04E+04	1.44E+03	4.68	4.02	3.16	0.66	1.52
3	7.90E+03	5.28E+03	4.20E+02	3.90	3.72	2.62	0.17	1.27
4	1.50E+03	1.00E+01	3.80E+01	3.18	1.00	1.58	2.18	1.60
5	5.90E+03	6.00E+02	1.02E+03	3.77	2.78	3.01	0.99	0.76
6	4.00E+02	4.00E+01	4.60E+01	2.60	1.60	1.66	1.00	0.94
7	2.22E+04	3.34E+03	3.66E+02	4.35	3.52	2.56	0.82	1.78
8	1.03E+04	1.68E+02	1.84E+02	4.01	2.23	2.26	1.79	1.75
9	1.87E+04	1.06E+04	2.60E+03	4.27	4.03	3.41	0.25	0.86
10	8.30E+03	1.38E+03	1.64E+03	3.92	3.14	3.21	0.78	0.70
11	3.50E+03	2.54E+03	5.46E+02	3.54	3.40	2.74	0.14	0.81
12	6.90E+03	1.40E+01	2.80E+01	3.84	1.15	1.45	2.69	2.39
13	2.06E+02	1.14E+02	1.00E+01	2.31	2.06	1.00	0.26	1.31
14	8.00E+01	1.40E+01	6.00E+00	1.90	1.15	0.78	0.76	1.12
15	2.24E+04	2.80E+01	1.68E+02	4.35	1.45	2.23	2.90	2.12
16	5.00E+01	7.60E+01	2.46E+02	1.70	1.88	2.39	-0.18	-0.69
17	4.56E+02	1.60E+01	3.20E+01	2.66	1.20	1.51	1.45	1.15
18	1.02E+05	2.38E+03	2.80E+01	5.01	3.38	1.45	1.63	3.56
19	3.60E+03	6.40E+02	3.80E+01	3.56	2.81	1.58	0.75	1.98
20	1.70E+03	1.66E+03	3.84E+02	3.23	3.22	2.58	0.01	0.65

	log prevalue	log postvalue 2.5 min	log postvalue 30 min	RF 2.5 min	RF 30 min
<b>Arithm. Mean</b>	3.56	2.49	2.19	<b>1.07</b>	<b>1.36</b>
<b>Standard deviation (absolute)</b>	0.90	1.01	0.75	0.89	0.83
<b>Standard deviation (relative)</b>	25.17	40.38	34.19	83.49	60.83



**Table 4.1: Statistical evaluation of test results using the Wilcoxon matched-pairs signed-ranks test – RF Softasept®N (gefärbt / coloré) < RF Propan-2-ol**

**Level of significance:  $p = 0.1$  (1-tailed)** (Calculation with SPSS 13.0)

**Test on skin exhibiting a low density of sebaceous glands**

Reference (R): Propan-2-ol Contact time 15 s RF (mean) = 0.96  
 Test product (P): Softasept®N Contact time 15 s RF (mean) = 0.87

Ranks				Test Statistics <sup>b</sup>	
		N	Mean Rank	Sum of Ranks	
R15s - P15s	Negative Ranks	9 <sup>a</sup>	10,22	92,00	Z
	Positive Ranks	11 <sup>b</sup>	10,73	118,00	Asymp. Sig. (2-tailed)
	Ties	0 <sup>c</sup>			Exact Sig. (2-tailed)
	Total	20			Exact Sig. (1-tailed)
					Point Probability
a. R15s < P15s					
b. R15s > P15s					
c. R15s = P15s					
					a. Based on negative ranks.
					b. Wilcoxon Signed Ranks Test

For the calculated smaller sum of ranks (sum of negative ranks = **92**), the p-value was determined with  **$p = 0.320$**  (1-tailed). As this value does not fall below the required level of significance of  $p = 0.1$ , P is **not significantly** less effective than R.

**Table 4.2: Statistical evaluation of test results using the Wilcoxon matched-pairs signed-ranks test – RF Softasept®N (gefärbt / coloré) > RF Propan-2-ol**

**Level of significance:  $p = 0.1$  (1-tailed)** (Calculation with SPSS 13.0)

**Test on skin exhibiting a low density of sebaceous glands**

Reference (R): Propan-2-ol Contact time 30 s RF (mean) = 0.83  
 Test product (P): Softasept®N (gefärbt / coloré) Contact time 30 s RF (mean) = 0.86

Ranks				Test Statistics <sup>b</sup>	
		N	Mean Rank	Sum of Ranks	
R30s - P30s	Negative Ranks	11 <sup>a</sup>	9,18	101,00	Z
	Positive Ranks	9 <sup>b</sup>	12,11	109,00	Asymp. Sig. (2-tailed)
	Ties	0 <sup>c</sup>			Exact Sig. (2-tailed)
	Total	20			Exact Sig. (1-tailed)
					Point Probability
a. R30s < P30s					
b. R30s > P30s					
c. R30s = P30s					
					a. Based on negative ranks.
					b. Wilcoxon Signed Ranks Test

For the calculated smaller sum of ranks (sum of negative ranks = **101**), the p-value was determined with  **$p = 0.446$**  (1-tailed). As this value does not fall below the required level of significance of  $p = 0.1$ , P is **not significantly** more effective than R.

**Table 4.3: Statistical evaluation of test results using the Wilcoxon matched-pairs signed-ranks test – RF Softasept®N > RF Propan-2-ol**

**Level of significance:  $p = 0.1$  (1-tailed)** (Calculation with SPSS 13.0)

**Test on skin exhibiting a low density of sebaceous glands**

**Reference (R):** Propan-2-ol **Contact time** 1 min **RF (mean) = 0.78**  
**Test product (P):** Softasept®N (gefärbt / coloré) **Contact time** 1 min **RF (mean) = 0.80**

Ranks				Test Statistics <sup>b</sup>	
		N	Mean Rank	Sum of Ranks	
R60s - P60s	Negative Ranks	10 <sup>a</sup>	10,85	108,50	Z
	Positive Ranks	10 <sup>b</sup>	10,15	101,50	Asymp. Sig. (2-tailed)
	Ties	0 <sup>c</sup>			Exact Sig. (2-tailed)
	Total	20			Exact Sig. (1-tailed)
a. R60s < P60s b. R60s > P60s c. R60s = P60s					Point Probability
					a. Based on positive ranks.
					b. Wilcoxon Signed Ranks Test

For the calculated smaller sum of ranks (sum of positive ranks = **101.50**), the p-value was determined with  **$p = 0.453$**  (1-tailed). As this value does not fall below the required level of significance of  $p = 0.1$ , P is **not significantly** more effective than R.

**Table 5.1: Statistical evaluation of test results using the Wilcoxon matched-pairs signed-ranks test – RF Softasept®N (gefärbt / coloré) > RF Propan-2-ol**

**Level of significance:  $p = 0.1$  (1-tailed)** (Calculation with SPSS 13.0)

**Test on skin exhibiting a high density of sebaceous glands**

**Reference (R):** Propan-2-ol **Contact time 10 min** **RF (mean) = 0.92**  
**Test product (P):** Softasept®N (gefärbt / coloré) **Contact time 2,5 min** **RF (mean) = 1.07**

Ranks				Test Statistics <sup>b</sup>	
		N	Mean Rank	Sum of Ranks	
R - P	Negative Ranks	13 <sup>a</sup>	10,54	137,00	Z
	Positive Ranks	7 <sup>b</sup>	10,43	73,00	-1,195 <sup>a</sup>
	Ties	0 <sup>c</sup>			Asymp. Sig. (2-tailed)
	Total	20			,232
					Exact Sig. (2-tailed)
					,242
					Exact Sig. (1-tailed)
					,121
					Point Probability
					,004

a.  $R < P$   
b.  $R > P$   
c.  $R = P$

a. Based on positive ranks.  
b. Wilcoxon Signed Ranks Test

For the calculated smaller sum of ranks (sum of positive ranks = **73**), the p-value was determined with  **$p = 0.121$**  (1-tailed). As this value does not fall below the required level of significance of  $p = 0.1$ , P is **not significantly** more effective than R.

**Table 5.2: Statistical evaluation of test results using the Wilcoxon matched-pairs signed-ranks test – RF Softasept®N (gefärbt / coloré) < RF Propan-2-ol**

**Level of significance:  $p = 0.1$  (1-tailed)** (Calculation with SPSS 13.0)

**Test on skin exhibiting a high density of sebaceous glands**

**Reference (R):** Propan-2-ol **Contact time 30 min** **RF (mean) = 1.37**  
**Test product (P):** Softasept®N (gefärbt / coloré) **Contact time 30 min** **RF (mean) = 1.36**

Ranks				Test Statistics <sup>b</sup>	
		N	Mean Rank	Sum of Ranks	
R - P	Negative Ranks	10 <sup>a</sup>	10,90	109,00	Z
	Positive Ranks	10 <sup>b</sup>	10,10	101,00	-,149 <sup>a</sup>
	Ties	0 <sup>c</sup>			Asymp. Sig. (2-tailed)
	Total	20			,881
					Exact Sig. (2-tailed)
					,891
					Exact Sig. (1-tailed)
					,445
					Point Probability
					,007

a.  $R < P$   
b.  $R > P$   
c.  $R = P$

a. Based on positive ranks.  
b. Wilcoxon Signed Ranks Test

For the calculated smaller sum of ranks (sum of positive ranks = **101**), the p-value was determined with  **$p = 0.445$**  (1-tailed). As this value does not fall below the required level of significance of  $p = 0.1$ , P is **not significantly** less effective than R.

**Legend:**

MW	=	mean
x	=	mean
RF	=	Reduction factor
> 300	=	not countable
n.d.	=	not determined
WSH	=	hard water (water of standardized hardness)
DGHM	=	Deutsche Gesellschaft für Hygiene und Mikrobiologie
cfu	=	colony forming unit