Analysis Report

REPORT NUMBER:
926474_REV 1
Replaces report 926474

DANISH
TECHNOLOGICAL
INSTITUTE

Teknologiparken
Kongsvang Allé 29
DK-8000 Aarhus C
+45 72 20 20 00
Info@teknologisk.dk
www.teknologisk.dk

Assignor:	Biotech Force Danmark ApS Gl. Donsvej 6 DK-6000 Kolding
Item:	Analysis of two products, Hånddesinfektion 0.15% ddac and Hånddesinfektion 0.15% ddac Gel, intended for hygienic handrub according to EN 13727:2012+A2:2015.
Sampling:	The assignor
Period:	Samples received: 7 May 2020 Test performed: 7 – 18 May 2020
Storage:	The test material will be destroyed after 3 months, unless otherwise agreed in writing.
Remark:	The account of the method(s) used only concerns the analysed sample(s).
Terms:	This test was conducted in accordance with international requirements (ISO/IEC 17025:2017) and in accordance with the General Terms and Conditions of Danish Technological Institute. The test results solely apply to the tested item(s) or to the sub-sample(s) selected for analysis. This analysis report may be quoted in extract only if Danish Technological Institute has granted its written consent.
Date/place:	27 May 2020 Danish Technological Institute, Aarhus Laboratory for Chemistry and Microbiology
Signature:	Digitally signed by: Helle Stendahl Andersen Date: 2020.05.27 14:32:59 +02'00' Helle Stendahl Andersen Business Manager

Table of Contents

Introduction

The efficacy of the two products, Hånddesinfektion 0.15% ddac and Hånddesinfektion 0.15% ddac Gel, both intended for hygienic handrub were tested according to DS/EN 13727:2012+A2:2015.

When tested in accordance with the test method under simulated clean conditions and under the required test conditions, the product must demonstrate ≥ log 5 reductions in viable counts for the four bacteria test strains in order to fulfill the requirements of DS/EN 13727:2012+A2:2015 for a bactericidal effect.

For each test organism, the test suspension was mixed with the interfering substance and the product at the given concentration. Following a given contact time, a subsample was neutralized using the membrane filtration methods.

A subsample was transferred to a membrane filter and filtered. The membrane filter was rinsed with a neutralizing liquid to stop the effect of the active substance. The membrane filter was transferred to an agar plate, incubated and evaluated for the number of colony-forming-units (cfu).

With a contact time of 10 min or shorter, the product must be neutralized in 10 sec. As this was not possible, when using the neutralization-dilution method, the membrane filtration method was used instead.

Validation tests were performed to check the effect of the experimental conditions, the effect of the filtration and the neutralizing liquid.

Experimental conditions

Test organisms:	*Pseudomonas aeruginosa* ATCC 15442, DSM 939 *Escherichia coli* K12 NCTC 10538, DSM 11250 *Staphylococcus aureus* ATCC 6538; DSM 799 *Enterococcus hirae* ATCC 10541, DSM 10541
Contact times:	(10 ± 1) sec.
Test temperature:	Room temperature (20 - 25°C)
Incubation of bacteria:	(37 ± 1) °C for (48 ±2) hours
Growth media for bacteria:	Tryptone soya agar (TSA)
Interfering substances:	0.3 g/L bovine serum albumin (simulated clean conditions)
Rinsing liquid:	Polysorbate 80: 5 g/L NaCl: 9 g/L Tryptone: 1 g/L Dissolved in 0.25 M phosphate buffer, pH 7.2
Diluent for the product:	Demineralized water

Products

Name: Hånddesinfektion 0.15% ddac
Hånddesinfektion 0.15% ddac Gel

Batch No.: not given

Active substance: didecyldimethylammoniumchlorid (DDAC)

Manufacturer: -

The following products and concentrations were tested.

Product, concentration	Appearance for Hånddesinfektion 0.15% ddac	pH for Hånddesinfektion 0.15% ddac	Appearance for Hånddesinfektion 0.15% ddac Gel	pH for Hånddesinfektion 0.15% ddac Gel
1,5%	Clear liquid	7.0	Clear	6.9
0,15%	-	6.9	-	6.9
0,015%	-	6.8	-	6.9

Table 1: The products were diluted to 80% during the test, so e.g. a 1.875 % concentrated batch of the product was used to achieve the 1.5 % concentration in the test.

Results

The product must achieve a ≥5 log reduction to fulfill the requirements of bactericidal activity according to DS/EN 13727:2012+A2:2015.

Hånddesinfektion 0.15% ddac

Contact time: 10 sec.	**Hånddesinfektion 0.15% ddac**		
Test organism	**1.5%**	**0.15%**	**0.015%**
P. aeruginosa	≥5.47	≥5.47	≥5.47
E. coli	≥5.43	≥5.43	≥5.43
S. aureus	≥5.52	≥5.52	≥5.52
E. hirae	≥5.36	≥5.36	≥5.36

Table 2: Results of the bactericidal activity given as a log reduction when the product is tested using a contact time of 10 sec.

See enclosure 1-4 for detailed results for a contact time of 10 sec.

Conclusion for Hånddesinfektion 0.15% ddac

The products fulfil the requirements according to DS/EN 13727:2012+A2:2015 for all three product concentrations, 1.5%, 0.15% and 0.015% with a contact time of 10 sec.

Hånddesinfektion 0.15% ddac Gel

Contact time: 10 sec.	**Hånddesinfektion 0.15% ddac Gel**		
Test organism	**1.5%**	**0.15%**	**0.015%**
P. aeruginosa	≥5.47	≥5.47	≥5.23
E. coli	≥5.43	≥5.43	≥5.43
S. aureus	≥5.52	≥5.52	≥5.52
E. hirae	≥5.36	≥5.36	≥5.36

Table 3: Results of the bactericidal activity given as a log reduction when the product is tested using a contact time of 10 sec.

See enclosure 5-8 for detailed results for a contact time of 10 sec.

Conclusion for Hånddesinfektion 0.15% ddac Gel

The products fulfil the requirements according to DS/EN 13727:2012+A2:2015 for all three product concentrations, 1.5%, 0.15% and 0.015% with a contact time of 10 sec.

Analysis method

The samples were analysed according to:

Reference method:	DS/EN 13727:2012 + A2:2015. Quantitative suspension test for the evaluation of bactericidal activity in the medical area - Test method and requirements (phase 2, step 1), modified version.

Revision 1

The product names were corrected.

.

Enclosure 1: Hånddesinfektion 0.15% ddac

Test organism: Pseudomonas aeruginosa, ATCC 15442, DSM 939
Contact time: 10 sec.

Controls and validation						
	log(FF)	**Vc1**	**Vc2**	**Value**	**Control Check**	
N	7	43	40	4.2E+08	$1.5 \cdot 10^8 \leq N \leq 5 \cdot 10^8$	OK
Nv	1	95	84	895	300≤Nv≤1600	OK
Control A	0	45	54	50	A≥0.5Nv0	OK
Control B	0	46	52	49	B≥0.5Nv0	OK
Control C	0	48	50	49	C≥0.5Nv0	OK

Results						
	log(FF)	**Vc1**	**Vc2**	**Value**	**WMC-check**	**LogR**
N	7	43	40	4.15E+08	not relevant	-
Na (Produkt CS1, 1.5%)	0	<14	<14	<1.40E+02	not relevant	≥5.47
Na (Produkt CS1, 0.15%)	0	<14	<14	<1.40E+02	not relevant	≥5.47
Na (Produkt CS1, 0.015%)	0	<14	<14	<1.40E+02	not relevant	≥5.47

Log(FF) is the log-value of the dilution factor. N is the number of cfu per ml in the test suspension. N0 is the number of cfu per ml in the test mixture at the beginning of the contact time. N0 = N/10. Nv is the number of cfu per ml in the validation suspension. Nv0 = Nv/10 is the number of cfu in the validation mixtures A, B and C at the beginning of the contact time. A, B and C is the number of cfu per ml following the tests for the experimental conditions (A), the toxicity of the neutralizer (B) and the efficacy of the neutralizer (C). Na is the number of cfu in the test mixture at the end of the contact time. R is the ratio between the starting concentration of cfu and the concentration of cfu following the exposure time. Only cfu counts between 14 and 330 was used for calculations. <14 cfu/plate was used for calculations in case of the undiluted samples showing below 14 cfu/plate. WMC is the weighted mean count.

Enclosure 2: Hånddesinfektion 0.15% ddac

Test organism: Escherichia coli K12, DSM 11250
Contact time: 10 sec.

Controls and validation						
	log(FF)	**Vc1**	**Vc2**	**Value**	**Control Check**	
N	7	43	32	3.8E+08	$1.5 \cdot 10^8 \leq N \leq 5 \cdot 10^8$	OK
Nv	1	64	70	670	300≤Nv≤1600	OK
Control A	0	36	39	38	A≥0.5Nv0	OK
Control B	0	49	49	49	B≥0.5Nv0	OK
Control C	0	34	40	37	C≥0.5Nv0	OK

Results						
	log(FF)	**Vc1**	**Vc2**	**Value**	**WMC-check**	**LogR**
N	7	43	32	3.75E+08	not relevant	-
Na (Produkt CS1, 1.5%)	0	<14	<14	<1.40E+02	not relevant	≥5.43
Na (Produkt CS1, 0.15%)	0	<14	<14	<1.40E+02	not relevant	≥5.43
Na (Produkt CS1, 0.015%)	0	<14	<14	<1.40E+02	not relevant	≥5.43

Log(FF) is the log-value of the dilution factor. N is the number of cfu per ml in the test suspension. N0 is the number of cfu per ml in the test mixture at the beginning of the contact time. N0 = N/10. Nv is the number of cfu per ml in the validation suspension. Nv0 = Nv/10 is the number of cfu in the validation mixtures A, B and C at the beginning of the contact time. A, B and C is the number of cfu per ml following the tests for the experimental conditions (A), the toxicity of the neutralizer (B) and the efficacy of the neutralizer (C). Na is the number of cfu in the test mixture at the end of the contact time. R is the ratio between the starting concentration of cfu and the concentration of cfu following the exposure time. Only cfu counts between 14 and 330 was used for calculations. <14 cfu/plate was used for calculations in case of the undiluted samples showing below 14 cfu/plate. WMC is the weighted mean count.

Enclosure 3: Hånddesinfektion 0.15% ddac

Test organism: Staphylococcus aureus, ATCC 6538, DSM 799
Contact time: 10 sec.

Controls and validation						
	log(FF)	**Vc1**	**Vc2**	**Value**	**Control Check**	
N	7	44	48	4.6E+08	$1.5 \cdot 10^8 \leq N \leq 5 \cdot 10^8$	OK
Nv	1	84	88	860	300≤Nv≤1600	OK
Control A	0	66	70	68	A≥0.5Nv0	OK
Control B	0	62	61	62	B≥0.5Nv0	OK
Control C	0	59	55	57	C≥0.5Nv0	OK

Results						
	log(FF)	**Vc1**	**Vc2**	**Value**	**WMC-check**	**LogR**
N	7	44	48	4.60E+08	not relevant	-
Na (Produkt CS1, 1.5%)	0	<14	<14	<1.40E+02	not relevant	≥5.52
Na (Produkt CS1, 0.15%)	0	<14	<14	<1.40E+02	not relevant	≥5.52
Na (Produkt CS1, 0.015%)	0	<14	<14	<1.40E+02	not relevant	≥5.52

Log(FF) is the log-value of the dilution factor. N is the number of cfu per ml in the test suspension. N0 is the number of cfu per ml in the test mixture at the beginning of the contact time. N0 = N/10. Nv is the number of cfu per ml in the validation suspension. Nv0 = Nv/10 is the number of cfu in the validation mixtures A, B and C at the beginning of the contact time. A, B and C is the number of cfu per ml following the tests for the experimental conditions (A), the toxicity of the neutralizer (B) and the efficacy of the neutralizer (C). Na is the number of cfu in the test mixture at the end of the contact time. R is the ratio between the starting concentration of cfu and the concentration of cfu following the exposure time. Only cfu counts between 14 and 330 was used for calculations. <14 cfu/plate was used for calculations in case of the undiluted samples showing below 14 cfu/plate. WMC is the weighted mean count.

Enclosure 4: Håndesinfektion 0.15% ddac

Test organism: Enterococcus hirae, ATCC 10541, DSM 3320
Contact time: 10 sec.

Controls and validation						
	log(FF)	**Vc1**	**Vc2**	**Value**	**Control Check**	
N	6 7	319 34	314 32	3.2E+08	$1.5 \cdot 10^8 \leq N \leq 5 \cdot 10^8$	OK
Nv	1	68	73	705	300≤Nv≤1600	OK
Control A	0	66	71	69	A≥0.5Nv0	OK
Control B	0	58	65	62	B≥0.5Nv0	OK
Control C	0	55	54	55	C≥0.5Nv0	OK

Results						
	log(FF)	**Vc1**	**Vc2**	**Value**	**WMC-check**	**LogR**
N	6 7	319 34	314 32	3.18E+08	OK	-
Na (Produkt CS1, 1.5%)	0	<14	<14	<1.40E+02	not relevant	≥5.36
Na (Produkt CS1, 0.15%)	0	<14	<14	<1.40E+02	not relevant	≥5.36
Na (Produkt CS1, 0.015%)	0	<14	<14	<1.40E+02	not relevant	≥5.36

Log(FF) is the log-value of the dilution factor. N is the number of cfu per ml in the test suspension. N0 is the number of cfu per ml in the test mixture at the beginning of the contact time. N0 = N/10. Nv is the number of cfu per ml in the validation suspension. Nv0 = Nv/10 is the number of cfu in the validation mixtures A, B and C at the beginning of the contact time. A, B and C is the number of cfu per ml following the tests for the experimental conditions (A), the toxicity of the neutralizer (B) and the efficacy of the neutralizer (C). Na is the number of cfu in the test mixture at the end of the contact time. R is the ratio between the starting concentration of cfu and the concentration of cfu following the exposure time. Only cfu counts between 14 and 330 was used for calculations. <14 cfu/plate was used for calculations in case of the undiluted samples showing below 14 cfu/plate. WMC is the weighted mean count.

Enclosure 5: Håndesinfektion 0.15% ddac Gel

Test organism: Pseudomonas aeruginosa, ATCC 15442, DSM 939
Contact time: 10 sec.

Controls and validation						
	log(FF)	**Vc1**	**Vc2**	**Value**	**Control Check**	
N	7	43	40	4,2E+08	$1.5 \cdot 10^8 \leq N \leq 5 \cdot 10^8$	OK
Nv	1	95	84	895	300≤Nv≤1600	OK
Control A	0	45	54	50	A≥0.5Nv0	OK
Control B	0	46	52	49	B≥0.5Nv0	OK
Control C	0	48	50	49	C≥0.5Nv0	OK

Results						
	log(FF)	**Vc1**	**Vc2**	**Value**	**WMC-check**	**LogR**
N	7	43	40	4,15E+08	not relevant	-
Na (Produkt CS1 Gel, 1.5%)	0	<14	<14	1,40E+02	not relevant	≥5.47
Na (Produkt CS1 Gel, 0.15%)	0	<14	<14	1,40E+02	not relevant	≥5,47
Na (Produkt CS1 Gel, 0.015%)	0	35	<14	2,45E+02	not relevant	≥5.23

Log(FF) is the log-value of the dilution factor. N is the number of cfu per ml in the test suspension. N0 is the number of cfu per ml in the test mixture at the beginning of the contact time. N0 = N/10. Nv is the number of cfu per ml in the validation suspension. Nv0 = Nv/10 is the number of cfu in the validation mixtures A, B and C at the beginning of the contact time. A, B and C is the number of cfu per ml following the tests for the experimental conditions (A), the toxicity of the neutralizer (B) and the efficacy of the neutralizer (C). Na is the number of cfu in the test mixture at the end of the contact time. R is the ratio between the starting concentration of cfu and the concentration of cfu following the exposure time. Only cfu counts between 14 and 330 was used for calculations. <14 cfu/plate was used for calculations in case of the undiluted samples showing below 14 cfu/plate. WMC is the weighted mean count.

Enclosure 6: Håndesinfektion 0.15% ddac Gel

Test organism: Escherichia coli K12, DSM 11250
Contact time: 10 sec.

Controls and validation						
	log(FF)	**Vc1**	**Vc2**	**Value**	**Control Check**	
N	7	43	32	3.8E+08	$1.5 \cdot 10^8 \leq N \leq 5 \cdot 10^8$	OK
Nv	1	64	70	670	300≤Nv≤1600	OK
Control A	0	36	39	38	A≥0.5Nv0	OK
Control B	0	49	49	49	B≥0.5Nv0	OK
Control C	0	36	37	37	C≥0.5Nv0	OK

Results						
	log(FF)	**Vc1**	**Vc2**	**Value**	**WMC-check**	**LogR**
N	7	43	32	3.75E+08	not relevant	-
Na (Produkt CS1, 1,5%)	0	<14	<14	<1.40E+02	not relevant	≥5.43
Na (Produkt CS1, 0,15%)	0	<14	<14	<1.40E+02	not relevant	≥5.43
Na (Produkt CS1, 0,015%)	0	<14	<14	<1.40E+02	not relevant	≥5.43

Log(FF) is the log-value of the dilution factor. N is the number of cfu per ml in the test suspension. N0 is the number of cfu per ml in the test mixture at the beginning of the contact time. N0 = N/10. Nv is the number of cfu per ml in the validation suspension. Nv0 = Nv/10 is the number of cfu in the validation mixtures A, B and C at the beginning of the contact time. A, B and C is the number of cfu per ml following the tests for the experimental conditions (A), the toxicity of the neutralizer (B) and the efficacy of the neutralizer (C). Na is the number of cfu in the test mixture at the end of the contact time. R is the ratio between the starting concentration of cfu and the concentration of cfu following the exposure time. Only cfu counts between 14 and 330 was used for calculations. <14 cfu/plate was used for calculations in case of the undiluted samples showing below 14 cfu/plate. WMC is the weighted mean count.

Enclosure 7: Håndesinfektion 0.15% ddac Gel

Test organism: Staphylococcus aureus, ATCC 6538, DSM 799
Contact time: 10 sec.

Controls and validation						
	log(FF)	**Vc1**	**Vc2**	**Value**	**Control Check**	
N	7	44	48	4.6E+08	$1.5 \cdot 10^8 \leq N \leq 5 \cdot 10^8$	OK
Nv	1	84	88	860	300≤Nv≤1600	OK
Control A	0	66	70	68	A≥0.5Nv0	OK
Control B	0	62	61	62	B≥0.5Nv0	OK
Control C	0	60	69	65	C≥0.5Nv0	OK

Results						
	log(FF)	**Vc1**	**Vc2**	**Value**	**WMC-check**	**LogR**
N	7	44	48	4.60E+08	not relevant	-
Na (Produkt CS1 Gel, 1,5%)	0	<14	<14	<1.40E+02	not relevant	≥5.52
Na (Produkt CS1 Gel, 0,15%)	0	<14	<14	<1.40E+02	not relevant	≥5.52
Na (Produkt CS1 Gel, 0,015%)	0	<14	<14	<1.40E+02	not relevant	≥5.52

Log(FF) is the log-value of the dilution factor. N is the number of cfu per ml in the test suspension. N0 is the number of cfu per ml in the test mixture at the beginning of the contact time. N0 = N/10. Nv is the number of cfu per ml in the validation suspension. Nv0 = Nv/10 is the number of cfu in the validation mixtures A, B and C at the beginning of the contact time. A, B and C is the number of cfu per ml following the tests for the experimental conditions (A), the toxicity of the neutralizer (B) and the efficacy of the neutralizer (C). Na is the number of cfu in the test mixture at the end of the contact time. R is the ratio between the starting concentration of cfu and the concentration of cfu following the exposure time. Only cfu counts between 14 and 330 was used for calculations. <14 cfu/plate was used for calculations in case of the undiluted samples showing below 14 cfu/plate. WMC is the weighted mean count.

Enclosure 8: Håndesinfektion 0.15% ddac Gel

Test organism: Enterococcus hirae, ATCC 10541, DSM 3320
Contact time: 10 sec.

Controls and validation						
	log(FF)	**Vc1**	**Vc2**	**Value**	**Control Check**	
N	6 7	319 34	314 32	3.2E+08	$1.5 \cdot 10^8 \leq N \leq 5 \cdot 10^8$	OK
Nv	1	68	73	705	300≤Nv≤1600	OK
Control A	0	66	71	69	A≥0.5Nv0	OK
Control B	0	58	65	62	B≥0.5Nv0	OK
Control C	0	55	56	56	C≥0.5Nv0	OK

Results						
	log(FF)	**Vc1**	**Vc2**	**Value**	**WMC-check**	**LogR**
N	6 7	319 34	314 32	3.18E+08	OK	-
Na (Produkt CS1, 1.5%)	0	<14	<14	<1.40E+02	not relevant	≥5.36
Na (Produkt CS1, 0.15%)	0	<14	<14	<1.40E+02	not relevant	≥5.36
Na (Produkt CS1, 0.015%)	0	<14	<14	<1.40E+02	not relevant	≥5.36

Log(FF) is the log-value of the dilution factor. N is the number of cfu per ml in the test suspension. N0 is the number of cfu per ml in the test mixture at the beginning of the contact time. N0 = N/10. Nv is the number of cfu per ml in the validation suspension. Nv0 = Nv/10 is the number of cfu in the validation mixtures A, B and C at the beginning of the contact time. A, B and C is the number of cfu per ml following the tests for the experimental conditions (A), the toxicity of the neutralizer (B) and the efficacy of the neutralizer (C). Na is the number of cfu in the test mixture at the end of the contact time. R is the ratio between the starting concentration of cfu and the concentration of cfu following the exposure time. Only cfu counts between 14 and 330 was used for calculations. <14 cfu/plate was used for calculations in case of the undiluted samples showing below 14 cfu/plate. WMC is the weighted mean count.