Nordic Tersus
Laboratory

Test report No. hd0219

EVALUATION OF FUNGICIDAL OR YEASTICIDAL ACTIVITY IN THE MEDICAL AREA (EN 13624)

Name of the product:	CHEMISEPT WIPES MD
Batch number:	79040818
Order number:	18023
Manufacturer:	Chemi-Pharm Ltd.
Client, representative:	Chemi-Pharm Ltd., Põllu 132, Tallinn, 10917, ESTONIA
Date of delivery:	05.12.2018
Test material conditions:	No specific features, sample in the manufacturers tare
Storage conditions:	In room temperature, dark
Active substance – conc.:	Ethyl alcohol 72% wt
Appearance of the product:	Transparent liquid
Test concentration:	Ready to use
Contact time:	60 s and 120 s
Interfering substance:	3.0 g/l sheep blood erythrocytes = clean conditions
Rinsing liquid:	Tryptone 1 g/l + NaCl 9 g/l
Neutralizer:	-
Test organisms:	*Aspergillus brasiliensis* ATCC 16404
Testing method:	**EVS-EN 13624:2013** **Quantitative suspension test for the evaluation of fungicidal or yeasticidal activity in the medical area.**
Testing date:	02.01.2019 – 04.01.2019
Results:	look appendix 1-2

Date of test report: 07.01.2019

Nordic Tersus Laboratory OÜ
Harjumaa, Tallinn, Serva tn 44a, 11618
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Appendix 1

TEST RESULTS (yeasticidal suspension test)

EVS-EN 13624:2013; Phase 2, step 1;
Membrane filtration method;
Rinsing liquid: Tryptone 1 g/l + NaCl 9 g/l;
Test organism: *Aspergillus brasiliensis* ATCC 16404;
Test temperature: +20° C; Incubation temperature: +30° C
Interfering substance: 3.0g/l sheep blood erythrocytes = clean conditions;
Nordic Tersus Laboratory LLC.; Date of test: 02.01.2019 – 04.01.2019
Responsible person: Allar Laaneleht

Validation and controls

Clean conditions

Validation suspension N_{vo}			Experimental conditions control (A)			Filtration control (B)			Method validation (C)		
V_{C1}	48	$\bar{x}$ = 45	V_{C1}	33	$\bar{x}$ = 34	V_{C1}	37	$\bar{x}$ = 33.5	V_{C1}	39	$\bar{x}$ = 40.5
V_{C2}	42		V_{C2}	35		V_{C2}	30		V_{C2}	42	
30 ≤ $\bar{x}$ N_{vo} ≤160?yes X; no □			$\bar{x}$ A is ≥ 0.5 $\bar{x}$ N_{vo}?yes X; no □			$\bar{x}$ B is ≥ 0.5 $\bar{x}$ N_{vo}?yesX; no□			$\bar{x}$ C is ≥ 0.5 $\bar{x}$ N_{vo}? yesX; no□		

Test suspension and test

Testsuspension:	N	V_{C1}	V_{C2}	$\bar{x}_{wm} = 1.67 \times 10^8$; log$N$ = 8.22
N and N_0	10^{-6}	154	181	$N_0 = N/100$; logN_0 = 6.22
	10^{-7}	16	16	6.17≤ logN_0≤6.70; yesX; no □

Experimental results

Concentration of the product	Dilution step	V_{C1}	V_{C2}	Na (=$\bar{x}$*10)	log Na	logR	Contact time	Conditions
Ready to use	-	<14	<14	<140	<2.15	>4.07	60 s	clean
Ready to use	-	<14	<14	<140	<2.15	>4.07	120 s	clean

Explanations:

V_C = count per ml (one plate or more)

$\bar{x}$ = average of V_{C1} and V_{C2} (1. + 2. duplicate)

N = cfu/ml microbes in testsuspension

N_0 = cfu/ml at the start of the contact time (t=0)

N_{vo} = cfu/ml in the validation suspension (t=0)

Na = surviving microbes after the test

R = reduction factor (R= N_0/ Na; LogR=LogN_0 - Log Na)

Appendix 2

Interpretation:

The ready to use product for hand disinfection **CHEMISEPT WIPES MD** (batch no. 79040818) was tested according to the test method EVS-EN 13624:2013. The test was performed at 20 °C ± 1 °C, clean conditions during contact times of 60 s and 120 s for fungus. The membrane filtration method was used for testing the produts effectiveness against the reference strains: *Aspergillus brasiliensis* ATCC 16404. Under clean conditions the tested product was effective against reference strain within contact times tested.

Conclusion:

The surviving count of the reference strain showed at least 4 lg reduction meaning that **the ready to use product CHEMISEPT WIPES MD has a fungicidal effect in case of hand disinfection under clean conditions within 60 s.**

Nordic Tersus Laboratory

Test report No. hd0419

EVALUATION OF FUNGICIDAL OR YEASTICIDAL ACTIVITY IN THE MEDICAL AREA (EN 13624)

Name of the product:	CHEMISEPT WIPES MD
Batch number:	79040818
Order number:	18023
Manufacturer:	Chemi-Pharm Ltd.
Client, representative:	Chemi-Pharm Ltd., Põllu 132, Tallinn, 10917, ESTONIA
Date of delivery:	05.12.2018
Test material conditions:	No specific features, sample in the manufacturers tare
Storage conditions:	In room temperature, dark
Active substance – conc.:	Ethyl alcohol 72% wt
Appearance of the product:	Transparent liquid
Test concentration:	Ready to use
Contact time:	15 s
Interfering substance:	15 g/l bovine albumin + 15 ml/l sheep blood erythrocytes = dirty conditions
Rinsing liquid:	Tryptone 1 g/l + NaCl 9 g/l
Neutralizer:	-
Test organisms:	*Candida albicans* ATCC 10231
Testing method:	EVS-EN 13624:2013 Quantitative suspension test for the evaluation of fungicidal or yeasticidal activity in the medical area.
Testing date:	02.01.2019 – 04.01.2019
Results:	look appendix 1-2

Date of test report: 07.01.2019

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Appendix 1

TEST RESULTS (yeasticidal suspension test)

EVS-EN 13624:2013; Phase 2, step 1;
Membrane filtration method;
Rinsing liquid: Tryptone 1 g/l + NaCl 9 g/l;
Test organism: *Candida albicans* ATCC 10231;
Test temperature: +20° C; Incubation temperature: +30° C
Interfering substance: 15 g/l bovine albumin + 15 ml/l sheep blood erythrocytes = dirty conditions;
Nordic Tersus Laboratory LLC.; Date of test: 02.01.2019 – 04.01.2019
Responsible person: Allar Laaneleht

Validation and controls

Dirty conditions

Validation suspension N_{vo}			Experimental conditions control (A)			Filtration control (B)			Method validation (C)		
V_{C1}	62	$\bar{x}$ = 58.5	V_{C1}	48	$\bar{x}$ = 44	V_{C1}	39	$\bar{x}$ = 42.5	V_{C1}	38	$\bar{x}$ = 38
V_{C2}	55		V_{C2}	40		V_{C2}	46		V_{C2}	38	
30 ≤ $\bar{x}$ N_{vo} ≤160?yes X; no □			$\bar{x}$ A is ≥ 0.5 $\bar{x}$ N_{vo}?yes X; no □			$\bar{x}$ B is ≥ 0.5 $\bar{x}$ N_{vo}?yesX; no□			$\bar{x}$ C is ≥ 0.5 $\bar{x}$ N_{vo}? yesX; no□		

Test suspension and test

Testsuspension:	N	V_{C1}	V_{C2}	$\bar{x}_{win}$ = 1.60 x 10^8; log$N$ = 8.20
	10^{-6}	167	153	N_0 = N/100; logN_0 = 6.20
N and N_0	10^{-7}	16	15	6.17≤ logN_0≤6.70; yesX; no □

Experimental results

Concentration of the product	Dilution step	V_{C1}	V_{C2}	Na (=$\bar{x}$*10)	log Na	logR	Contact time	Conditions
Ready to use	-	<14	<14	<140	<2.15	>4.05	15 s	dirty

Explanations:

V_C = count per ml (one plate or more)

$\bar{x}$ = average of V_{C1} and V_{C2} (1. + 2. duplicate)

N = cfu/ml microbes in testsuspension

N_0 = cfu/ml at the start of the contact time (t=0)

N_{vo} = cfu/ml in the validation suspension (t=0)

Na = surviving microbes after the test

R = reduction factor (R= N_0/ Na; LogR=LogN_0 - Log Na)

Appendix 2

Interpretation:

The ready to use product for hand disinfection **CHEMISEPT WIPES MD** (batch no. 79040818) was tested according to the test method EVS-EN 13624:2013. The test was performed at 20 °C ± 1 °C, under dirty conditions during contact time of 15 s. The membrane filtration method was used for testing the produts effectiveness against the reference strains: *Candida albicans* ATCC 10231. Under dirty conditions the tested product was effective against the reference strain within contact time tested.

Conclusion:

The surviving count of the reference strain showed at least 4 lg reduction meaning that **the ready to use product CHEMISEPT WIPES MD has a yeasticidal effect under dirty conditions in case of hand disinfection within 15s.**

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Nordic Tersus
Laboratory

Test report No. hd0119

EVALUATION OF BACTERICIDAL ACTIVITIES OF A DISINFECTANTS AND ANTISEPTICS USED IN THE MEDICAL AREA (EN 13727)

Name of the product:	CHEMISEPT WIPES MD
Batch number:	79040818
Order number:	18023
Manufacturer:	Chemi-Pharm Ltd
Client, representative:	Chemi-Pharm Ltd., Põllu 132, Tallinn, 10917, ESTONIA
Date of delivery:	05.12.2018
Test material conditions:	No specific features, sample in the manufacturers tare
Storage conditions:	In room temperature, dark
Active substance – conc.:	Ethyl alcohol – 72 % wt;
Appearance of the product:	Transparent liquid
Test concentration:	Ready to use
Contact time:	15 s
Interfering substance:	15 g/l bovine albumin + 15 ml/l sheep blood erythrocytes = dirty conditions
Neutralizer:	-
Rinsing liquid:	Tryptone 1 g/l + NaCl, 9 g/l
Test organisms:	*Pseudomonas aeruginosa* ATCC 15442 *Staphylococcus aureus* ATCC 6538 *Enterococcus hirae* ATCC 10541 *Escherichia coli* K12, NTCT 10538
Testing method:	**EVS-EN 13727:2012+A2:2015** **Chemical disinfectants and antiseptics - Quantitative suspension test for the evaluation of bactericidal activity in the medical area - Test method and requirements (phase 2, step 1)**
Testing date:	03.01.2019 – 04.01.2019
Results:	look appendix 1-5

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Appendix 1

TEST RESULTS (bactericidal suspension test)

EVS-EN 13727:2012+A2:2015; Phase 2, step 1;
Membrane filtration method;
Rinsing liquid: Tryptone 1 g/l + NaCl 9 g/l;
Test organism: *Staphylococcus aureus* ATCC 6538;
Test temperature: +20° C; Incubation temperature: +37 °C
Interfering substance: 15 g/l bovine albumin + 15 ml/l sheep blood erythrocytes = dirty conditions;
Nordic Tersus Laboratory LLC.;
Date of test: 03.01.2019 – 04.01.2019
Responsible person: Allar Laaneleht

Validation and controls

Dirty conditions

Validation suspension N_{vo}			Experimental conditions control (A)			Filtration control (B)			Method validation (C)		
V_{C1}	62	$\bar{x}$ = 56	V_{C1}	47	$\bar{x}$ = 44.5	V_{C1}	58	$\bar{x}$ = 51	V_{C1}	55	$\bar{x}$ = 53
V_{C2}	50		V_{C2}	42		V_{C2}	44		V_{C2}	51	
30 ≤ $\bar{x}$ N_{vo} ≤160? yes X; no□			$\bar{x}$ A is ≥ 0,5 $\bar{x}$ N_{vo}? yes X; no□			$\bar{x}$ B is ≥ 0,5 $\bar{x}$ N_{vo}? yesX; no□			$\bar{x}$ C is ≥ 0,5 $\bar{x}$ N_{vo}? yes X; no□		

Test suspension and test

Testsuspension:	N	V_{C1}	V_{C2}	$\bar{x}_{wm}$ = 2.15 x 10^9; log$N$ = 9.33
N and N_0	10^{-7}	228	203	N_0 = N/100; logN_0 =7.33
	10^{-8}	24	19	7,17≤ logN_0≤7,70; yesX; no □

Experimental results

Concentration of the product %	Dilution step	V_{C1}	V_{C2}	Na (=$\bar{x}$*10)	log Na	logR	Contact time	Conditions
Ready to use	-	<14	<14	<140	<2.15	>5.18	15 sec	dirty

Explanations:
V_C = count per ml (one plate or more)
$\bar{x}$ = average of V_{C1} and V_{C2} (1. + 2. duplicate)
N = cfu/ml microbes in testsuspension
N_0 = cfu/ml at the start of the contact time (t=0)
N_{vo} = cfu/ml in the validation suspension (t=0)
Na = surviving microbes after the test
R = reduction factor (R= N_0/ Na; LogR=LogN_0 - Log Na)

Appendix 2

TEST RESULTS (bactericidal suspension test)

EVS-EN 13727:2012+A2:2015; Phase 2, step 1;
Membrane filtration method;
Rinsing liquid: Tryptone 1 g/l + NaCl 9 g/l;
Test organism: *Enterococcus hirae* ATCC 10541;
Test temperature: +20° C; Incubation temperature: +37 °C
Interfering substance: 15 g/l bovine albumin + 15 ml/l sheep blood erythrocytes = dirty conditions;
Nordic Tersus Laboratory LLC.;
Date of test: 03.01.2019 – 04.01.2019
Responsible person: Allar Laaneleht

Validation and controls

Dirty conditions

Validation suspension N_{vo}			Experimental conditions control (A)			Filtration control (B)			Method validation (C)		
V_{C1}	89	$\bar{x}$ = 93	V_{C1}	68	$\bar{x}$ = 70.5	V_{C1}	55	$\bar{x}$ = 58.5	V_{C1}	75	$\bar{x}$ = 79.5
V_{C2}	97		V_{C2}	73		V_{C2}	62		V_{C2}	84	
30 ≤ $\bar{x}$ N_{vo} ≤160? yes X; no□			$\bar{x}$ A is ≥ 0,5 $\bar{x}$ N_{vo}? yes X; no□			$\bar{x}$ B is ≥ 0,5 $\bar{x}$ N_{vo}? yesX; no□			$\bar{x}$ C is ≥ 0,5 $\bar{x}$ N_{vo}? yes X; no□		

Test suspension and test

Testsuspension:	N	V_{C1}	V_{C2}	$\bar{x}_{wm}$ = 2.55 x 10^9; log$N$ = 9.41
N and N_0	10^{-7}	266	247	N_0 = N/100; logN_0 =7.41
	10^{-8}	21	28	7,17≤ logN_0≤7,70; yesX; no □

Experimental results

Concentration of the product %	Dilution step	V_{C1}	V_{C2}	Na (=$\bar{x}$*10)	log Na	logR	Contact time	Conditions
Ready to use	-	<14	<14	<140	<2.15	>5.26	15 s	dirty

Explanations:
V_C = count per ml (one plate or more)
$\bar{x}$ = average of V_{C1} and V_{C2} (1. + 2. duplicate)
N = cfu/ml microbes in testsuspension
N_0 = cfu/ml at the start of the contact time (t=0)
N_{vo} = cfu/ml in the validation suspension (t=0)
Na = surviving microbes after the test
R = reduction factor (R= N_0/ Na; LogR=LogN_0 - Log Na)

Appendix 3

TEST RESULTS (bactericidal suspension test)

EVS-EN 13727:2012+A2:2015; Phase 2, step 1;
Membrane filtration method;
Rinsing liquid: tryptone 1 g/l + NaCl 9 g/l;
Test organism: *Pseudomonas aeruginosa* ATCC 15442
Test temperature: +20° C; Incubation temperature: +37 °C
Interfering substance: 15 g/l bovine albumin + 15 ml/l sheep blood erythrocytes = Dirty conditions;
Nordic Tersus Laboratory LLC.;
Date of test: 03.01.2019 – 04.01.2019
Responsible person: Allar Laaneleht

Validation and controls

Dirty conditions

Validation suspension N_{vo}			Experimental conditions control (A)			Filtration control (B)			Method validation (C)		
V_{C1}	74	$\bar{x}$ = 77	V_{C1}	51	$\bar{x}$ = 47.5	V_{C1}	49	$\bar{x}$ = 45.5	V_{C1}	68	$\bar{x}$ = 64.5
V_{C2}	80		V_{C2}	44		V_{C2}	42		V_{C2}	61	
30 ≤ $\bar{x}$ N_{vo} ≤160? yes X; no□			$\bar{x}$ A is ≥ 0,5 $\bar{x}$ N_{vo}? yes X; no□			$\bar{x}$ B is ≥ 0,5 $\bar{x}$ N_{vo}? yesX; non			$\bar{x}$ C is ≥ 0,5 $\bar{x}$ N_{vo}? yes X; no□		

Test suspension and test

Testsuspension:	N	V_{C1}	V_{C2}	$\bar{x}_{wm}$ 2.30 = x 10^9; log$N$ = 9.36
N and N_0	10^{-7}	238	224	N_0 = N/100; logN_0 =7.36
	10^{-8}	22	22	7,17≤ logN_0≤7,70; yesX; no □

Experimental results

Concentration of the product %	Dilution step	V_{C1}	V_{C2}	Na (=$\bar{x}$*10)	log Na	logR	Contact time	Conditions
Ready to use	-	<14	<14	<140	<2.15	>5.21	15 s	dirty

Explanations:
V_C = count per ml (one plate or more)
$\bar{x}$ = average of V_{C1} and V_{C2} (1. + 2. duplicate)
N = cfu/ml microbes in testsuspension
N_0 = cfu/ml at the start of the contact time (t=0)
N_{vo} = cfu/ml in the validation suspension (t=0)
Na = surviving microbes after the test
R = reduction factor (R= N_0/ Na; LogR=LogN_0 - Log Na)

Appendix 4

TEST RESULTS (bactericidal suspension test)

EVS-EN 13727:2012+A2:2015; Phase 2, step 1;
Membrane filtration method;
Rinsing liquid: tryptone 1 g/l + NaCl 9 g/l;
Test organism: *Escherichia coli* K12, NTCT 10538
Test temperature: +20° C; Incubation temperature: +37 °C
Interfering substance: 15 g/l bovine albumin + 15 ml/l sheep blood erythrocytes = Dirty conditions;
Nordic Tersus Laboratory LLC.;
Date of test: 03.01.2019 – 04.01.2019
Responsible person: Allar Laaneleht

Validation and controls

Dirty conditions

Validation suspension N_{vo}			Experimental conditions control (A)			Filtration control (B)			Method validation (C)		
V_{C1}	44	$\bar{x}$ = 47	V_{C1}	36	$\bar{x}$ = 33	V_{C1}	33	$\bar{x}$ = 37	V_{C1}	40	$\bar{x}$ = 43
V_{C2}	50		V_{C2}	30		V_{C2}	41		V_{C2}	46	
30 ≤ $\bar{x}$ N_{vo} ≤160? yes X; no□			$\bar{x}$ A is ≥ 0,5 $\bar{x}$ N_{vo}? yes X; no□			$\bar{x}$ B is ≥ 0,5 $\bar{x}$ N_{vo}? yesX; no□			$\bar{x}$ C is ≥ 0,5 $\bar{x}$ N_{vo}? yes X; no□		

Test suspension and test

Testsuspension:	N	V_{C1}	V_{C2}	$\bar{x}_{wm}$ = 1.85 x 10^9; log$N$ = 9.27
N and N_0	10^{-7}	197	175	N_0 = N/100; logN_0 =7.27
	10^{-8}	20	15	7,17≤ logN_0≤7,70; yesX; no □

Experimental results

Concentration of the product %	Dilution step	V_{C1}	V_{C2}	Na (=$\bar{x}$*10)	log Na	logR	Contact time	Conditions
Ready to use	-	<14	<14	<140	<2.15	>5.12	15 s	dirty

Explanations:
V_C = count per ml (one plate or more)
$\bar{x}$ = average of V_{C1} and V_{C2} (1. + 2. duplicate)
N = cfu/ml microbes in testsuspension
N_0 = cfu/ml at the start of the contact time (t=0)
N_{vo} = cfu/ml in the validation suspension (t=0)
Na = surviving microbes after the test
R = reduction factor (R= N_0/ Na; LogR=LogN_0 - Log Na)

Appendix 5

Interpretation:

The product for hand disinfection **CHEMISEPT WIPES MD** (batch no. 79040818) was tested according to the test method EVS-EN 13727:2012+A2:2015. The test was performed at 20 °C ± 1 °C, under dirty conditions with the contact time of 15 s. The membrane filtration method was used for testing the products' effectiveness against the reference strains: *Pseudomonas aeruginosa* ATCC 15442, *Enterococcus hirae* ATCC 10541, *Staphylococcus aureus* ATCC 6538, Escherichia coli K12 NTCT 10538. Under dirty conditions the tested product was effective against all the reference strains within 15 sec of contact time.

Conclusion:

The surviving count of bacterial reference strains showed at least 5lg reduction meaning that **under dirty conditions the ready to use product Chemisept Wipes MD has a bactericidal effect in case of hand disinfection within 15 s.**

Nordic Tersus
Laboratory

Test report No. hd0319

EVALUATION OF MYCOBACTERICIDAL ACTIVITY (EN 14348)

Name of the product:	CHEMISEPT WIPES MD
Batch number:	79040818
Order number:	18023
Manufacturer:	Chemi-Pharm Ltd.
Client, representative:	Chemi-Pharm Ltd., Põllu 132, Tallinn, 10917, Eston ', +372-51-77-090
Date of delivery:	05.12.2018
Test material conditions:	No specific features, sample in the manufacturers tare
Storage conditions:	In room temperature, dark;
Active substance – conc.:	Ethyl alcohol 72% wt
Appearance of the product:	Transparent liquid
Test concentration:	Ready to use
Test conditions:	Dirty conditions
Contact time:	30 s, 60 min (obligatory)
Interfering substance:	3.0 g/l bovine albumin + 3ml/l sheep blood erythrocytes = dirty conditions
Test neutralizer:	-
Rinsing liquid:	Tryptone 1 g/l + NaCl 9 g/l
Test organisms:	*Mycobacterium terrae* ATCC 15755; *Mycobacterium avium* ATCC 15769
Testing method base:	**EVS-EN 14348:2005 – Chemical disinfectants and antiseptics - Quantitative suspension test for the evaluation of mycobactericidal activity of chemical disinfectants in the medical area including instrument disinfectants - Test methods and requirements (phase 2, step 1)**
Testing date:	06.12.2018 – 27.12.2018
Results:	look appendix 1-3

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Appendix 1

TEST RESULTS (mycobactericidal suspension test)

EVS-EN 14348:2005; Phase 2, step 1;
Membrane filtration method; Spread plate;
Rinsing liquid: Polysorbate 80, 5g/l + L-histidine, 0,5g/l;
Test organism: *Mycobacterium terrae* ATCC 15755;
Test temperature: +20° C; Incubation temperature: +37° C
Solvents: diluent, water;
Interfering substance: 3.0 g/l bovine albumin + 3ml/l sheep blood erythrocytes = dirty conditions
Nordic Tersus Laboratory LLC.;
Date of test: 06.12.2018 – 27.12.2018
Responsible person: Allar Laaneleht

Validation and controls

Dirty conditions

Validation suspension N_{vo}			Experimental conditions (A)			Filtration control (B)			Method validation (C)		
V_{C1}	82	$\bar{x}$ = 77.5	V_{C1}	55	$\bar{x}$ = 57	V_{C1}	48	$\bar{x}$ = 50.5	V_{C1}	64	$\bar{x}$ = 66
V_{C2}	73		V_{C2}	59		V_{C2}	53		V_{C2}	68	
30 ≤ $\bar{x}$ N_{vo} ≤160?yes X; no □			$\bar{x}$ A is ≥ 0.5 $\bar{x}$ N_{vo}?yes X;no □			$\bar{x}$ B is ≥ 0.5 $\bar{x}$ N_{vo}? yesX; no □			$\bar{x}$ C is ≥ 0.5 $\bar{x}$ N_{vo}? yes X;no □		

Test suspension and test

Testsuspension:	N	V_{C1}	V_{C2}	$\bar{x}_{wm}$ = 2.46 x 10^9; log$N$ = 9.39
	10^{-7}	251	240	$N_0 = N/10$; logN_0 = 8.39
N and N_0	10^{-8}	28	22	8.17≤ logN_0≤8.7; yes X; no □

Experimental results

Concentration of the product.%	Dilution step	V_{C1}	V_{C2}	Na (=x̄*10)	log Na	logR	Contact time	Conditions
RTU	10^0	117	104	1105	3.04	5.35	30 s	dirty
	10^{-1}	<14	<14					
	10^{-2}	<14	<14					
	10^{-3}	<14	<14					
RTU	10^0	<14	<14	<140	<2.15	>6.15	60 min	Dirty
	10^{-1}	<14	<14					
	10^{-2}	<14	<14					
	10^{-3}	<14	<14					

Explanations:

V_C = count per ml (one plate or more)

x̄ = average of V_{C1} and V_{C2} (1. + 2. duplicate)

N = cfu/ml microbes in testsuspension

N_0 = cfu/ml at the start of the contact time (t=0)

N_{vo} = cfu/ml in the validation suspension (t=0)

Na = surviving microbes after the test

R = reduction factor (R= N_0/ Na; LogR=LogN_0 - Log Na)

Appendix 2

TEST RESULTS (mycobactericidal suspension test)

EVS-EN 14348:2005; Phase 2, step 1;
Membrane filtration method; Spread plate;
Rinsing liquid: Polysorbate 80, 5g/l + L-histidine, 0,5g/l;
Test organism: *Mycobacterium avium* ATCC 15769;
Test temperature: +20° C; Incubation temperature: +37° C
Solvents: diluent, water;
Interfering substance: 3.0 g/l bovine albumin + 3ml/l sheep blood erythrocytes = dirty conditions
Nordic Tersus Laboratory LLC.;
Date of test: 06.12.2018 – 27.12.2018
Responsible person: Allar Laaneleht

Validation and controls

Dirty conditions

Validation suspension N_{vo}			Experimental conditions (A)			Filtration control (B)			Method validation (C)		
V_{C1}	61	$\bar{x}$ = 54	V_{C1}	44	$\bar{x}$ = 40	V_{C1}	37	$\bar{x}$ = 35	V_{C1}	39	$\bar{x}$ = 42
V_{C2}	47		V_{C2}	36		V_{C2}	33		V_{C2}	45	
30 ≤ $\bar{x}$ N_{vo} ≤160?yes X; no □			$\bar{x}$ A is ≥ 0.5 $\bar{x}$ N_{vo}?yes X;no □			$\bar{x}$ B is ≥ 0.5 $\bar{x}$ N_{vo}? yesX; no □			$\bar{x}$ C is ≥ 0.5 $\bar{x}$ N_{vo}? yes X;no □		

Test suspension and test

Testsuspension:	N	V_{C1}	V_{C2}	$\bar{x}_{wm}$ = x 10^9; log$N$ = 9.30
	10^{-7}	197	206	$N_0 = N/10$; logN_0 = 8.30
N and N_0	10^{-8}	23	17	8.17≤ logN_0≤8.7; yes X; no □

Experimental results

Concentration of the product.%	Dilution step	V_{C1}	V_{C2}	Na (=x̄*10)	log Na	logR	Contact time	Conditions
RTU	10^0	102	94	980	2.99	5.31	30 s	dirty
	10^{-1}	<14	<14					
	10^{-2}	<14	<14					
	10^{-3}	<14	<14					
RTU	10^0	<14	<14	<140	<2.15	>6.15	60 min	dirty
	10^{-1}	<14	<14					
	10^{-2}	<14	<14					
	10^{-3}	<14	<14					

Explanations:

V_C = count per ml (one plate or more)

x̄ = average of V_{C1} and V_{C2} (1. + 2. duplicate)

N = cfu/ml microbes in testsuspension

N_0 = cfu/ml at the start of the contact time (t=0)

N_{vo} = cfu/ml in the validation suspension (t=0)

Na = surviving microbes after the test

R = reduction factor (R= N_0/ Na; LogR=LogN_0 - Log Na)

Appendix 3

Interpretation

Using the EN 14348 standard, there was tested a ready to use product – **CHEMISEPT WIPES MD** (Batch No. 79040818) at 20 °C ± 1 °C, with the contact times of 30 s and 60 min (obligatory) under dirty conditions. The membrane filtration method was used for testing products' effectiveness against the reference strains: *Mycobacterium terrae* ATCC 15755, *Mycobacterium avium* ATCC 15769. Under dirty conditions the tested product was active against both of the testorganisms at contact times tested.

Conclusion

By the test results can be concluded that as treated by the product the surviving microorganisms count showed at least 4lg reducion, meaning that **under dirty conditions the ready to use product CHEMISEPT WIPES MD is mycobactericidal in case of hand disinfection, during conctact time of 30 s.**

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Chemi-Pharm AS
Pollu 132
EST – TALLINN 10917

Bremen, 09/01/2019

Expert opinion

Activity of CHEMISEPT WIPES MD against modified vaccinia virus Ankara (MVA) in a quantitative suspension test based on the EN 14476:2013+A1:2015 under dirty conditions

This expert opinion is based on the test report L18/0890MV.1 dating 09/01/2019.

The virus-inactivating properties of the surface disinfectant CHEMISEPT WIPES MD of Chemi-Pharm AS against modified vaccinia virus Ankara (MVA) were investigated by a quantitative suspension test based on EN 14476 under dirty conditions.

According to EN 14476, a disinfectant or a disinfectant solution at a particular concentration is considered as having virus-inactivating properties if within the recommended exposure period the titre is reduced by ≥ 4 $\log_{10}$ (inactivation ≥ 99.99 %).

The soaking solution of the surface disinfectant CHEMISEPT WIPES MD was examined undiluted at 20 °C. 15 seconds were chosen as exposure time. In summary, a virucidal activity against modified vaccinia virus Ankara (MVA) was measured as follows:

undiluted **15 seconds** **dirty conditions (3.0 g/l BSA + 3.0 ml/l erythrocytes)**

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ilac-MRA DAkkS Deutsche Akkreditierungsstelle D-PL-13412-01-01 D-PL-13412-01-02
Anerkannt durch/Recognized by Zentralstelle der Länder für Gesundheitsschutz bei Arzneimitteln und Medizinprodukten www.zlg.de
ZLG-AP-216.11.02

TEST REPORT

09/01/2019

Test report L18/0890MV.1

Evaluation of the effectiveness of

CHEMISEPT WIPES MD

Test virus: modified vaccinia virus Ankara (MVA)

Method: based on EN 14476:2013+A1:2015 (dirty conditions)

quantitative suspension test for the evaluation of virucidal activity of chemical disinfectants and antiseptics used in human medicine

Sponsor:
Chemi-Pharm AS
Pollu 132
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Tel.: +49 40-557631-0, Fax: +49 40-557631-11
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1. Identification of test laboratory

Dr. Brill + Partner GmbH Institute for Hygiene and Microbiology, Norderoog 2, DE - 28259 Bremen

2. Identification of sample

Manufacturer	Chemi-Pharm AS
Name of product	CHEMISEPT WIPES MD
Confirmation no.	207453
Product diluent recommended by the manufacturer	-
Batch number	79040818
Application	surface disinfection
Production date	04/08/2018
Expiry date	04/08/2021
Active compound (s) (100 g)	72 g ethanol
Appearance, odour	clear, colorless liquid (soaking solution) product specific
pH-values	undiluted: 6.27 (20 °C)
Storage conditions	room temperature in the dark (area with restricted access)
Date of arrival in the laboratory	05/12/2018

3. Materials

3.1 Culture medium and reagents

- Eagle`s Minimum Essential Medium with Hank`s BSS (MEM, Biozym Scientific GmbH, catalogue no. 880144)
- fetal calf serum (Biochrom AG, article no. S 0115)
- 1.4 % formaldehyde solution (dilution of Roti®-Histofix 4 %, Carl Roth GmbH)
- Aqua bidest. (SG ultrapure water system, type Ultra Clear; serial no. 86996-1)
- PBS (Invitrogen, article no. 18912-014)
- BSA (Sigma-Aldrich-Chemie GmbH, article no. CA-2153)

- sheep erythrocytes (Fiebig Nährstofftechnik).

3.2 Virus and cells

The modified vaccinia virus Ankara (MVA) originated from Dr. Manteufel, Institut für Tierhygiene und Öffentliches Veterinärwesen, DE - 04103 Leipzig. Before inactivation assays, virus had been passaged three times in *BHK 21-cells* (Baby Hamster Kidney).

BHK 21-cells (passage 104) originated from the Friedrich-Löffler-Institut, Bundesforschungsinstitut für Tiergesundheit (formerly Bundesforschungsanstalt für Viruskrankheiten der Tiere, isle of Riems).

The cells were inspected regularly for morphological alterations and for contamination by mycoplasmas. No morphological alterations of cells and no contamination by mycoplasmas could be detected.

3.3 Apparatus, glassware and small items of equipment

- CO_2 incubator, Nunc GmbH & Co. KG, model QWJ 350
- Agitator (Vortex Genie Mixer, type G 560E)
- pH measurement 315i (WTW, article no. 2A10-100)
- Centrifuge (Sigma-Aldrich-Chemie GmbH, type 113)
- Microscope (Olympus, type CK 30)
- Centrifuge 5804 R (Eppendorf AG)
- Water bath (JULABO, Julabo U 3)
- Adjustable and fixed-volume pipettes (Eppendorf AG)
- Polysterol 96-well microtitre plate (Nunc GmbH & Co. KG, Wiesbaden)
- Cell culture flask (Nunc GmbH & Co. KG, Wiesbaden)
- Sealed test tubes (Sarstedt AG & Co., Nümbrecht).

4. Experimental conditions

Test temperature	20 °C ± 1.0 °C
Concentration of test product	undiluted (80.0 %) and as 50.0 % and 10.0 % (demonstration of non-active range) solutions
Appearance of product dilutions	no precipitation
Contact times	15 seconds and 30 minutes
Interfering substance	3.0 g/l bovine serum albumin + 3.0 ml/l erythrocytes (dirty conditions, EN 14476)
Procedure to stop action of disinfectant	immediate dilution
Diluent	Aqua bidest.
Stability of product in the mix with virus and interfering substance (80.0 % solution)	medium clouding, no precipitation
Virus strain	modified vaccinia virus Ankara (MVA) (ATCC VR-1508)
Date of testing	05/12/2018 – 09/01/2019
End of testing	09/01/2019

5. Methods

5.1 Preparation of test virus suspension

For preparation of test virus suspension, *BHK 21-cells* were cultivated with MEM and 10 % or 2 % fetal calf serum. *Cells* were infected with a multiplicity of infection of 0.1. After cells showed a cytopathic effect, they were subjected to a freeze/thaw procedure followed by a low speed centrifugation in order to sediment cell debris. After aliquotation, test virus suspension was stored at – 80 °C.

5.2 Preparation of disinfectant (dilutions)

The soaking solution of CHEMISEPT WIPES MD was tested undiluted. Due to the addition of interfering substance and test virus suspension an 80.0 % solution resulted.

Furthermore, the product was evaluated as 50.0 % and 10.0 % solutions (demonstrating of non-active range). These solutions were prepared with Aqua bidest. immediately before the inactivation tests.

5.3 Infectivity assay

Infectivity was determined as endpoint titration according to EN 5.5 transferring 0.1 ml of each dilution into eight wells of a microtitre plate to 0.1 ml of freshly trypsinised *BHK 21-cells* (10-15 x 10^3 cells per well), beginning with the highest dilution. Microtitre plates were incubated at 37 °C in a 5 % CO_2-atmosphere. The cytopathic effect was read by using an inverted microscope after six days. Calculation of the infective dose $TCID_{50}$/ml was calculated with the method of Spearman (2) and Kärber (3) with the following formula:

$$- \log_{10}TCID_{50} = X_0 - 0.5 + \sum r/n$$

meaning

X_0 = $\log_{10}$ of the lowest dilution with 100 % positive reaction

r = number of pos. determinations of lowest dilution step with 100 % positive and all higher positive dilution steps

n = number of determinations for each dilution step.

5.4 Calculation and verification of virucidal activity

The virucidal activity of the test disinfectant was evaluated by calculating the decrease in titre in comparison with the control titration without disinfectant. The difference is given as reduction factor (RF).

According to the EN 14476, a disinfectant or a disinfectant solution at a particular concentration is having virus-inactivating efficacy if the titre is reduced at least by 4 $\log_{10}$ steps within the recommended exposure period. This corresponds to an inactivation of ≥ 99.99 %.

5.5 Inactivation assay (end point titration)

Determination of virucidal activity has been carried out according to EN 5.5. The test product was examined undiluted (80.0 %) and as 50.0 % and 10.0 % (demonstration of non-active range) solutions in Aqua bidest. at 20 °C based on EN 14476. 15 seconds and 30 minutes were chosen as contact times.

Immediately at the end of a chosen contact time, activity of the disinfectant was stopped by dilution to 10^{-8}.

Titrations of the virus control were performed at the beginning of the test and after the longest exposure time (EN 5.5.7). One part by volume of test virus suspension was mixed with one part interfering substance and eight parts by volume of WSH or Aqua bidest. (RTU products).

Furthermore, a cell control (only addition of medium) was incorporated.

Inactivation tests were carried out in sealed test tubes in a water bath at 20 °C ± 1.0 °C. Aliquots were retained after appropriate exposure times and residual infectivity was determined.

5.6 Inactivation assay following the large volume plating method (LVP)

Following the large volume plating method (4) the inactivation assays were further diluted 1:500 (80.0 % solution) in cell culture medium. The total volume was added (without any further dilution) to the permissive cells. By introducing such a huge dilution it is possible to eliminate cytotoxicity of the test product in order to demonstrate a 4 $\log_{10}$ reduction of virus titre. Calculation of virus titre follows formula of Taylor or Poisson (5, 6). This method is necessary for those products which demonstrate a great cytotoxicity.

125 µl of the inactivation assays were added to 62.5 ml medium (total dilution of 1:500) and then the total volume was distributed in 6 microtitre plates (108 µl / well, 576 wells total). After 6 days of inoculation cultures were observed for cytopathic effects.

The calculation of virus titre without residual virus followed the formula of Poisson:

$$c = \ln p / -V$$

c = number of virus particles

p = the probability to find no virus. The probability to find no virus should not greater than 5 % (p=0.05). By doing so, the number of virus particles can be calculated with a probability of 95 %.

V = test volume (ml)

The titre to be used for calculating the reduction factor (RF) was finally calculated as followed: the determined number of virus particle is first converted with the aid of the dilution factor in the number of particle per ml. Subsequently, the numbers of particles per ml have to be converted in the tissue culture infectious dose per ml ($TCID_{50}$/ml) (1.0 $TCID_{50}$

corresponds to 0.69 infectious virus particles). The common logarithm of this value results in the virus titre (log_{10} $TCID_{50}$/ml) used for calculating the reduction factor (RF).

In assays with residual virus, formula according to Taylor was used for calculating the virus titre:

$$c/ml = \frac{D}{Vw} \times \left(-\ln \frac{n - n_p}{n}\right)$$

c = number of virus particles

D = dilution

V_W = volume per well

n = number of inoculated wells

n_p = number of virus-positive wells

For calculating the reduction factor using the formula according to Taylor the number of virus particles is converted to the logarithmic titre ($log_{10}TCID_{50}$/ml) as described above.

5.7 Determination of cytotoxicity

Determination of cytotoxicity was performed according to EN 5.5.4.1.

5.8 Cell sensitivity to virus

For the control of cell sensitivity to virus two parts by volume of water were mixed with eight parts by volume of the lowest apparently non-cytotoxic dilution of the product. These mixtures or PBS as control were added to a volume of double concentrated cell suspension. After 1 h at 37 °C the cells were centrifuged and re-suspended in cell culture medium (EN 5.5.4.2b).

Finally, a comparative titration of the test virus suspension was performed on the pre-treated (disinfectant) and non-pre-treated (PBS) cells as described above.

5.9 Control of efficacy for suppression of disinfectant's activity

Furthermore, a control of efficiency for suppression of disinfectant's activity was included (EN 5.5.5).

5.10 Reference virus inactivation test

As reference for test validation a 0.7 % formaldehyde solution according to EN 5.5.6 was included. 5, 15, 30 and 60 minutes were chosen as contact times. In addition, cytotoxicity of formaldehyde test solution was determined based on EN 5.5.6.2 with dilutions up to 10^{-5}.

6. Verification of the methodology

The following criteria as mentioned in EN 5.7 were fulfilled:

a) The titre of the test virus suspension allowed the determination of a ≥ 4 $\log_{10}$ reduction (maximal virus reduction $\geq 5.02 \pm 0.13$, LVP)

b) The test product (80.0 %) showed cytotoxicity in the 1:100 dilutions thus allowing the detection of a 4 $\log_{10}$ reduction of virus titre.

c) The comparative titration on pre-treated (disinfectant) and non-pre-treated (PBS) *BHK 21-cells* showed no significant difference (< 1 $\log_{10}$; EN 5.7) of virus titre: 6.75 ± 0.43 (PBS, LVP) versus 6.75 ± 0.33 (1:500 dilutions of disinfectant as 80.0 % solution, LVP) $\log_{10}$ $TCID_{50}$/ml.

d) The control of efficacy for suppression of disinfectant's activity (80.0 %) showed no decrease ($\leq$ 0.5 $\log_{10}$; EN 5.5.5.1) in virus titre (6.25 ± 0.33 versus 6.50 ± 0.00 $\log_{10}$ $TCID_{50}$/ml).

e) One concentration demonstrated a 4 $\log_{10}$ reduction and (at least) one concentration demonstrated a $\log_{10}$ reduction of less than 4.

Since all criteria according EN 5.7 were fulfilled, examination with MVA based on EN 14476 is valid.

7. Results

Results of examination are shown in tables 1 to 9. Tables 1 to 7 demonstrate the raw data, whereas tables 8 (a+b) and 9 give a summary of results.

Since it was not possible to show a reduction in virus titre of 4 $\log_{10}$-steps testing the undiluted test product using the endpoint dilution method due to cytotoxicity, this concentration was tested using the large volume plating method. The further dilutions were examined using the end point dilution method.

The 50.0 % solution was not active within 15 seconds of exposure time (table 1).

The 10.0 % solution was not active within 30 minutes of exposure time (table 2).

In parallel to the end point dilution method the large volume plating method (LVP) was introduced testing the undiluted test product with 15 seconds of exposure time. The mean virus titre was $\log_{10}$ $TCID_{50}$/ml = 6.56 ± 0.13.

The undiluted test product in an 80.0 % assay was active after 15 seconds of exposure time (table 7). Since no residual virus was found in 576 cell culture units, the result according to the formula of Poisson was ≤ 1.54 $\log_{10}$ $TCID_{50}$. The reduction factor was therefore ≥ 5.02 ± 0.13 (6.56 ± 0.13 $\log_{10}$ $TCID_{50}$ minus ≤ 1.54 $\log_{10}$ $TCID_{50}$) after 15 seconds of exposure time. This corresponded to an inactivation of ≥ 99.999 %.

8. Conclusion

The surface disinfectant CHEMISEPT WIPES MD tested undiluted demonstrated activity against MVA after an exposure time of 15 seconds under dirty conditions.

Therefore, the surface disinfectant CHEMISEPT WIPES MD can be declared as active against MVA as follows:

undiluted 15 seconds dirty conditions

Bremen, 09/01/2019

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9. Quality control

The Quality Assurance of the results was maintained by performing the determination of the virus-inactivating properties of the disinfectant in accordance with Good Laboratory Practice regulations:

1) Chemicals Act of Germany, Appendix 1, dating of 01.08 1994 (BGBI. I, 1994, page 1703). Appendix revised at 14. 05. 1997 (BGBI. I, 1997, page 1060).
2) OECD Principles of Good Laboratory Practice (revised 1997); OECD Environmental Health and Safety Publications; Series on Principles of Good Laboratory Practice and Compliance Monitoring – Number 1. Environment Directorate, Organization for Economic Co-operation and Development, Paris 1998.

The plausibility of the results was additionally confirmed by controls incorporated in the inactivation assays.

10. Records to be maintained

All testing data, protocol, protocol modifications, the final report, and correspondence between Dr. Brill + Partner GmbH and the sponsor will be stored in the archives at Dr. Brill + Partner GmbH.

11. Literature

1. EN 14476:2013+A1:2015: Chemical disinfectants and antiseptics – Quantitative suspension test for the evaluation of virucidal activity of chemicals disinfectants and antiseptics in human medicine test - Test method and requirements (phase 2, step 1)

2. Spearman, C.: The method of `right or wrong cases` (constant stimuli) without Gauss's formulae. Brit J Psychol; 2 1908, 227-242

3. Kärber, G.: Beitrag zur kollektiven Behandlung pharmakologischer Reihenversuche. Arch Exp Path Pharmak; 162, 1931, 480-487

4. Rabenau HF., Schwebke I., Blümel J., Eggers M., Glebe D., Rapp I., Sauerbrei A., Steinmann E., Steinmann, J., Willkommen H. Wutzler P.: Leitlinie der Deutschen Vereinigung zur Bekämpfung der Viruskrankheiten (DVV) e.V. und des Robert Koch-Instituts (RKI) zur Prüfung von chemischen Desinfektionsmitteln auf Wirksamkeit gegen Viren in der Humanmedizin (Fassung vom 1. Dezember 2014). Bundesgesundheitsbl; 58, 2015, 493–504

5. Bekanntmachung über die Zulassung von Arzneimitteln, Anforderungen an Validierungsstudien zum Nachweis der Virussicherheit von Arzneimitteln aus menschlichem Blut oder Plasma vom 20. Dezember 1993/21. Januar 1994. Bundesanzeiger Nr. 84: 4740-4744 bzw. CPMP/BWP/268/95: Note for Guidance on virus validation studies: the design, contribution and interpretation of studies validating the inactivation and removal of viruses. http://www.ema.europa.eu

6. Taylor JR.: An Introduction to Error Analysis: The study of Uncertainties in Physical Measurements. 2nd ed. University Science Books, 1997, 327 pp

Appendix:

Legend to the Tables

Legend to the Figures

Table 1: Raw data for CHEMISEPT WIPES MD (50.0 %) tested against MVA at 20 °C (quantal test; 8 wells) (#5840)

Product	Concentration	Interfering substance	Contact time (min)	Dilutions (log_{10})								
				1	2	3	4	5	6	7	8	9
test product	50.0 %	dirty conditions	0.25	n.d.	4444 4444	4444 4444	4232 3433	3402 2020	0000 0000	0000 0000	n.d.	n.d.
			5	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.
			15	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.
			30	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.
test product cytotoxicity	50.0 %	dirty conditions	n.a.	0000 0000	0000 0000	0000 0000	0000 0000	0000 0000	n.d.	n.d.	n.d.	n.d.
virus control	n.a.	dirty conditions	0	4444 4444	4444 4444	4444 4444	4444 4444	2043 0344	0000 0023	0000 0000	0000 0000	0000 0000
			60	4444 4444	4444 4444	4444 4444	4444 4444	2243 4333	0000 0000	0000 0000	0000 0000	0000 0000

n.a. = not applicable
n.d. = not done

0 = no virus present; t = cytotoxic
1 to 4 = virus present (degree of CPE in 8 cell culture units) (wells of microtitre plates)

Table 2: Raw data for CHEMISEPT WIPES MD (10.0 %) tested against MVA at 20 °C (quantal test; 8 wells) (#5840)

Product	Concentration	Interfering substance	Contact time (min)	Dilutions (log_{10})								
				1	2	3	4	5	6	7	8	9
test product	10.0 %	dirty conditions	1	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.
			5	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.
			15	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.
			30	4444 4444	4444 4444	4444 4444	4444 4444	3332 3223	4220 0000	0000 0000	n.d.	n.d.
test product cytotoxicity	10.0 %	dirty conditions	n.a.	0000 0000	0000 0000	0000 0000	0000 0000	0000 0000	n.d.	n.d.	n.d.	n.d.
virus control	n.a.	dirty conditions	0	4444 4444	4444 4444	4444 4444	4444 4444	2043 0344	0000 0023	0000 0000	0000 0000	0000 0000
			60	4444 4444	4444 4444	4444 4444	4444 4444	2243 4333	0000 0000	0000 0000	0000 0000	0000 0000

n.a. = not applicable
n.d. = not done

0 = no virus present; t = cytotoxic
1 to 4 = virus present (degree of CPE in 8 cell culture units) (wells of microtitre plates)

Table 3: Raw data for formaldehyde solution (0.7 %) tested against MVA at 20 °C (quantal test; 8 wells) (#5840)

Product	Concentration	Interfering substance	Contact time (min)	Dilutions (log_{10})								
				1	2	3	4	5	6	7	8	9
formaldehyde	0.7 % (m/V)	PBS	5	tttt tttt	tttt tttt	tttt tttt	3000 0000	0000 0000	0000 0000	0000 0000	0000 0000	n.d.
			15	tttt tttt	tttt tttt	tttt tttt	0000 0000	0000 0000	0000 0000	0000 0000	0000 0000	n.d.
			30	tttt tttt	tttt tttt	tttt tttt	0000 0000	0000 0000	0000 0000	0000 0000	0000 0000	n.d.
			60	tttt tttt	tttt tttt	tttt tttt	0000 0000	0000 0000	0000 0000	0000 0000	0000 0000	n.d.
formaldehyde cytotoxicity	0.7 % (m/V)	PBS	n.a.	tttt tttt	tttt tttt	tttt tttt	0000 0000	0000 0000	n.d.	n.d.	n.d.	n.d.
virus control	n.a.	PBS	0	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.
			60	4444 4444	4444 4444	4444 4444	4444 4444	3443 2332	0000 3000	0000 0000	0000 0000	0000 0000

n.a. = not applicable
n.d. = not done

0 = no virus present; t = cytotoxic
1 to 4 = virus present (degree of CPE in 8 cell culture units) (wells of microtitre plates)

Table 4: Raw data for control of efficacy for suppression of disinfectant's activity (80.0 %) (#5840)

Product	Interfering substance	dilutions (log_{10})								
		1	2	3	4	5	6	7	8	9
test product	dirty conditions	n.d.	n.d.	4444 4444	4444 4444	4323 2002	0000 0000	0000 0000	0000 0000	n.d.
corresponding virus control	dirty conditions	4444 4444	4444 4444	4444 4444	4444 4444	2243 4333	0000 0000	0000 0000	0000 0000	0000 0000

n.a. = not applicable
n.d. = not done

0 = no virus present; t = cytotoxic
1 to 4 = virus present (degree of CPE in 8 cell culture units) (wells of microtitre plates)

Table 5: Raw data (MVA) for cell sensitivity (80.0 % solution) (#5840) (LVP)

Product	Dilution	Dilutions (log_{10})								
		1	2	3	4	5	6	7	8	9
PBS	-	4444 4444	4444 4444	4444 4444	4444 4444	2322 3402	0000 3220	0000 0000	0000 0000	n.d.
test product	1:500	4444 4444	4444 4444	4444 4444	4444 4444	4234 3323	0000 3030	0000 0000	0000 0000	n.d.

n.a. = not applicable
n.d. = not done

0 = no virus present; t = cytotoxic
1 to 4 = virus present (degree of CPE in 8 cell culture units) (wells of microtitre plates)

Table 6: Determination of virus titre (LVP) at 20 °C (#5840)

Virus titration	Interfering substance	dilutions (log_{10})								
		1	2	3	4	5	6	7	8	9
1st control	dirty conditions	4444 4444	4444 4444	4444 4444	4444 4444	2243 4333	0000 0000	0000 0000	0000 0000	0000 0000
2nd control	dirty conditions	4444 4444	4444 4444	4444 4444	4444 4444	3342 3332	0000 3000	0000 0000	0000 0000	n.d.

n.a. = not applicable
n.d. = not done

t = cytotoxic 0 = no virus detectable
1 to 4 = virus detectable (degree of CPE in 8 wells of a microtitre plate)

Table 7: Inactivation of MVA by CHEMISEPT WIPES MD (80.0 %) at 20 °C (15 seconds) (LVP, 1:500) (#5840)

Interfering substance	Row	1	2	3	4	5	6	7	8	9	10	11	12
dirty conditions	plate 1/6	0000 0000	0000 0000	0000 0000	0000 0000	0000 0000	0000 0000	0000 0000	0000 0000	0000 0000	0000 0000	0000 0000	0000 0000
	plate 2/6	0000 0000	0000 0000	0000 0000	0000 0000	0000 0000	0000 0000	0000 0000	0000 0000	0000 0000	0000 0000	0000 0000	0000 0000
	plate 3/6	0000 0000	0000 0000	0000 0000	0000 0000	0000 0000	0000 0000	0000 0000	0000 0000	0000 0000	0000 0000	0000 0000	0000 0000
	plate 4/6	0000 0000	0000 0000	0000 0000	0000 0000	0000 0000	0000 0000	0000 0000	0000 0000	0000 0000	0000 0000	0000 0000	0000 0000
	plate 5/6	0000 0000	0000 0000	0000 0000	0000 0000	0000 0000	0000 0000	0000 0000	0000 0000	0000 0000	0000 0000	0000 0000	0000 0000
	plate 6/6	0000 0000	0000 0000	0000 0000	0000 0000	0000 0000	0000 0000	0000 0000	0000 0000	0000 0000	0000 0000	0000 0000	0000 0000

t = cytotoxic

0 = no virus detectable
1 to 4 = virus detectable (degree of CPE in 8 wells of a microtitre plate)

Table 8a: Summary of results (end point dilution method) with CHEMISEPT WIPES MD and MVA

Product	Con-centration	Interfering substance	Level of cytotoxicity	log_{10} $TCID_{50}$/ml after ....min					> 4 log_{10} reduction after ...min
				0.25	5	15	30	60	
test product	50.0 %	dirty conditions	1.50	6.13±0.37	n.d.	n.d.	n.d.	n.d.	> 0.25 (RF = 0.38±0.37)
test product	10.0 %	dirty conditions	1.50	n.d.	n.d.	n.d.	6.88±0.37	n.d.	> 30 (RF = 0.00±0.37)

n.a. = not applicable n.d. = not done

Table 8b: Summary of results (end point dilution method) with CHEMISEPT WIPES MD and MVA

Product	Con-centration	Interfering substance	Level of cytotoxicity	log_{10} $TCID_{50}$/ml after ....min					> 4 log_{10} reduction after ... min
				0	5	15	30	60	
formaldehyde	0.7 % (w/v)	PBS	4.50	n.d.	≤ 4.63±0.25	≤ 4.50±0.00	≤ 4.50±0.00	≤ 4.50±0.00	≥ 15 (RF ≥ 2.13±0.18)
virus control	n.a.	PBS	n.a.	n.d.	n.d.	n.d.	n.d.	6.63±0.25	n.a.
virus control	n.a.	dirty conditions	n.a.	6.50±0.46	n.d.	n.d.	n.d.	6.50±0.00	n.a.
suppression control	80.0 %	dirty conditions	3.50	n.d.	n.d.	n.d.	6.25±0.33	n.d.	n.a.

n.a. = not applicable n.d. = not done sens. = sensitivity

Table 9: Summary of results (LVP, 1:500) with CHEMISEPT WIPES MD and MVA

Product	Con-centration	Interfering substance	Level of cytotoxicity	log_{10} $TCID_{50}$/ml after ....min					> 4 log_{10} reduction after ...min
				0.25	5	15	30	60	
test product	80.0 %	dirty conditions	n.a.	≤ 1.54	n.d.	n.d.	n.d.	n.d.	0.25 (RF ≥ 5.02±0.13)
virus control	n.a.	dirty conditions	n.a.	n.d.	n.d.	n.d.	n.d.	6.50±0.00 6.63±0.25 (Ø6.56±0.13)	n.a.
sens. PBS	n.a.	n.a.	n.a.	n.d.	n.d.	n.d.	n.d.	6.75±0.44	n.a.
sens. product	80.0 % → 1:500	n.a.	n.a.	n.d.	n.d.	n.d.	n.d.	6.75±0.33	n.a.

n.a. = not applicable n.d. = not done sens. = sensitivity n.c. = not calculable

Figure 1: Virus-inactivating properties of CHEMISEPT WIPES MD (80.0 %) (LVP)

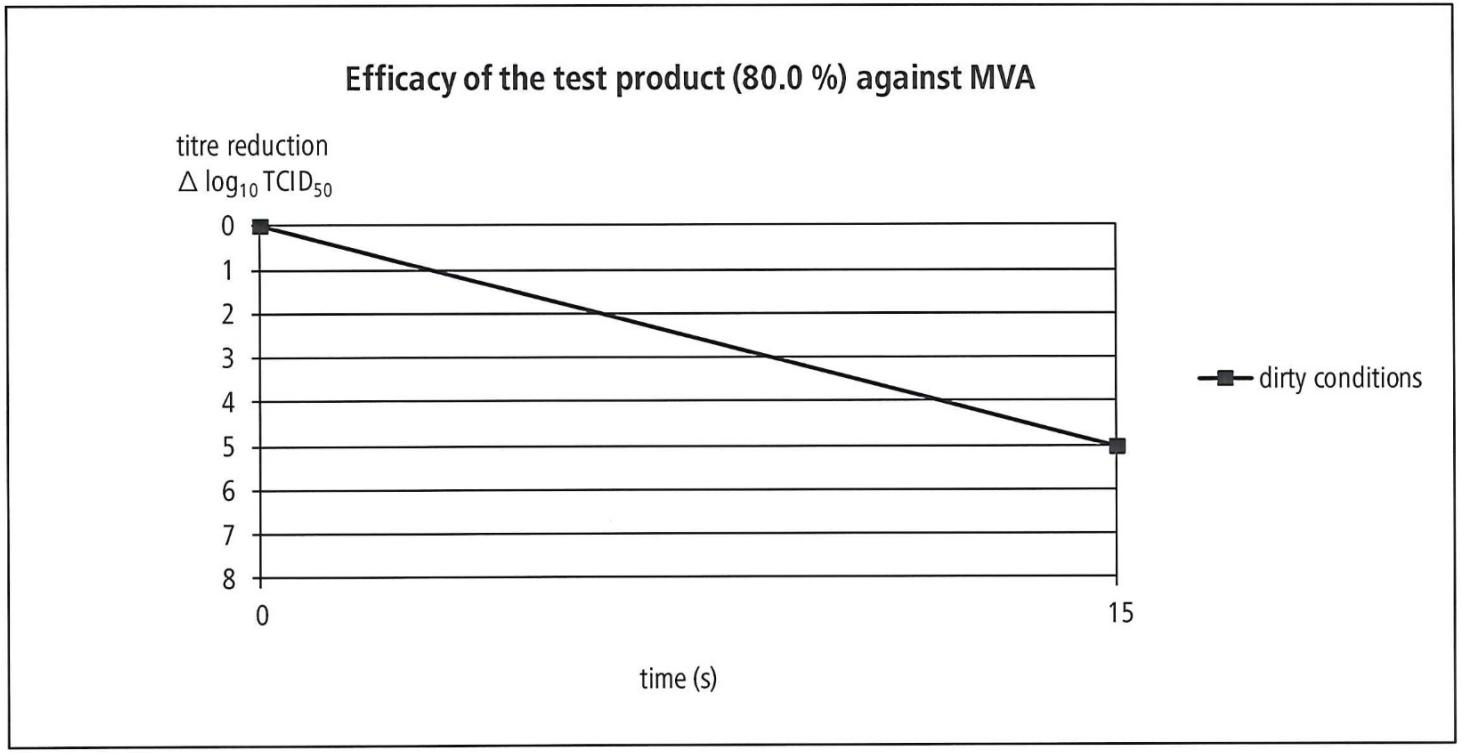
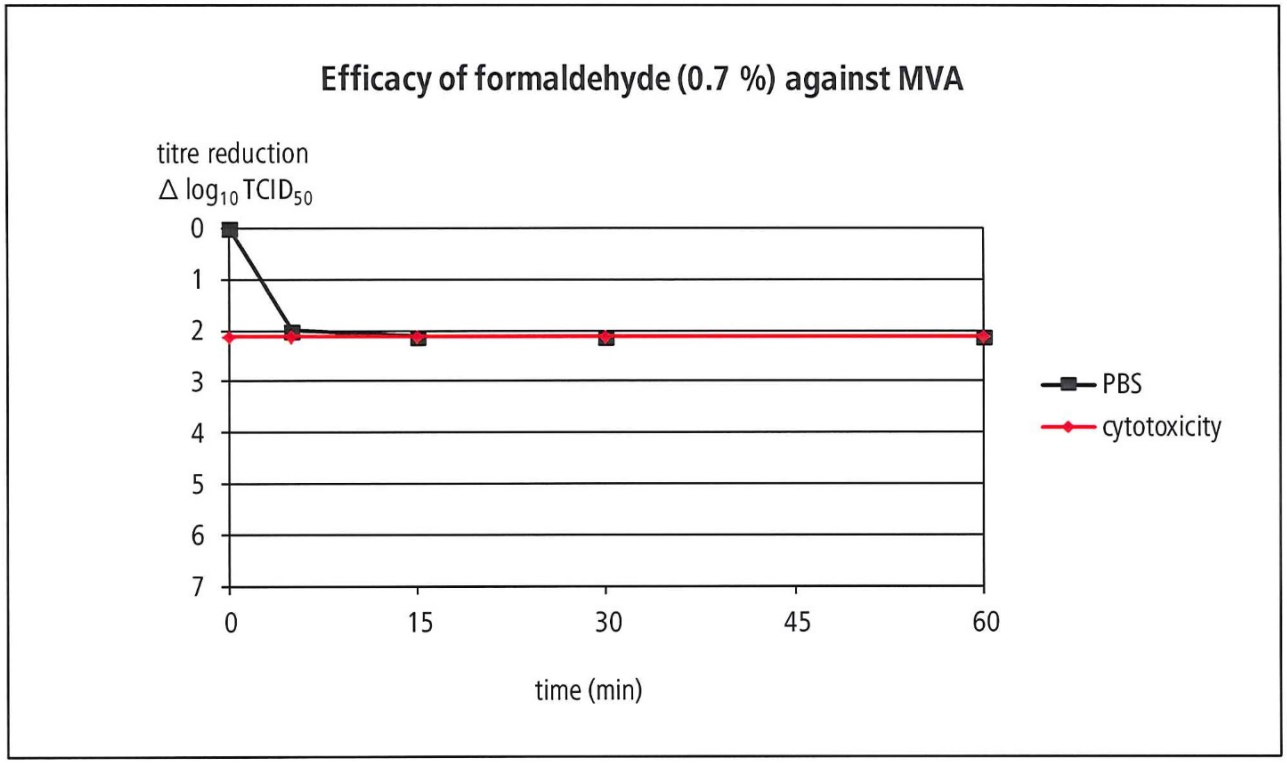


Figure 2: Virus-inactivating properties of formaldehyde (0.7 %)



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Chemi-Pharm AS
Pollu 132
EST – TALLINN 10917

Bremen, 09/01/2019

Summary: Virus-inactivating properties (virucidal activity against enveloped viruses) of CHEMISEPT WIPES MD of Chemi-Pharm AS according to EN 14476:2013+A1:2015/prA2:2016 under dirty conditions

This summary is based on the following test report of Dr. Brill + Partner GmbH for surface disinfectant CHEMISEPT WIPES MD produced by Chemi-Pharm AS:

modified vaccinia virus Ankara test report (L18/0890MV.1) dating 09/01/2019

The following concentration and exposure time are necessary for the inactivation of the test virus:

undiluted 15 seconds

in order to achieve a 4 $\log_{10}$ reduction (inactivation ≥ 99.99 %) under dirty conditions in a quantitative suspension test according to EN 14476:2013+A1:2015/prA2:2016.

After evaluation with modified vaccinia virus Ankara the soaking solution of the surface disinfectant CHEMISEPT WIPES MD can be declared as having **"virucidal activity against all enveloped viruses"** according to EN 14476:2013+A1:2015/prA2:2016.

The declaration **"virucidal activity against all enveloped viruses"** covers all enveloped viruses (Annex A) like HBV, HCV, HIV and Ebola virus.

From Annex A in EN 14476
Examples of viruses which may contaminate human medical instruments, hands, surfaces (*Enveloped viruses are in bold*)

NOTE This list is not exhaustive.

Blood

Enterovirus
Filoviridae
Flavivirus
Herpesviridae
Hepatitis A Virus (HAV)
Hepatitis B virus (HBV)
Hepatitis C virus (HCV)
Hepatitis Delta virus (HDV)
Human Immunodeficiency Virus (HIV)
Human T Cell Leukemia Virus (HTLV)
Parvovirus B 19

Respiratory tract

Adenovirus (Mast-)
Coronavirus
Enterovirus
Herpesviridae
Influenza Virus
Paramyxoviridae
Rhinovirus
Rubella Virus

Neural tissue, ear & nose, eye

Adenovirus (Mast-)
Enterovirus
Herpesviridae
Measles Virus
Human Immunodeficiency Virus (HIV)
Polyomavirus
Rabies Virus
Rubella Virus

Gastro-intestinal

Adenovirus (Mast-)
Caliciviridae
Coronavirus
Astrovirus
Enterovirus
Hepatitis A Virus (HAV)
Hepatitis E Virus (HEV)
Rotavirus

Skin, breast and/or milk

Enterovirus
Herpesviridae
Human Immunodeficiency Virus (HIV)
Human T Cell Leukemia Virus (HTLV)
Papillomavirus
Poxviridae

Spleen and lymph nodes (see also „Blood")

Human T Cell Leukemia Virus (HTLV)
Human Immunodeficiency Virus (HIV)

Dental procedure

Adenovirus (Mast-)
Enterovirus
Herpesviridae
Hepatitis B virus (HBV)
Hepatitis C Virus (HCV)
Hepatitis Delta Virus (HDV)
Human Immunodeficiency Virus (HIV)

Urogenital tract

Hepatitis B Virus (HBV)
Herpesviridae
Human Immunodeficiency Virus (HIV)
Human T Cell Leukemia Virus (HTLV)
Papillomavirus
Polyomavirus

Reference:
Van Regenmortel MHV et al., Eds.: Virus Taxonomy, Classification and Nomenclature of Viruses, seventh report of the international committee on taxonomy of viruses.
Academic Press, San Diego, 2000

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Chemi-Pharm AS
Pollu 132
EST – TALLINN 10917

Bremen, 20/12/2018

Expert opinion

Activity of CHEMISEPT WIPES MD against human rotavirus strain Wa in a quantitative suspension test based on EN 14476:2013+A1:2015 under dirty conditions

This expert opinion is based on the test report L18/0890R.1 dating 20/12/2018.

The virus-inactivating properties of the surface disinfectant CHEMISEPT WIPES MD of Chemi-Pharm AS against human rotavirus strain Wa were investigated by a quantitative suspension test based on EN 14476 under dirty conditions.

According to this norm, a disinfectant or a disinfectant solution at a particular concentration is considered as having virus-inactivating properties if within the recommended exposure period the titre is reduced by ≥ 4 $\log_{10}$ (inactivation ≥ 99.99 %).

The soaking solution of the surface disinfectant CHEMISEPT WIPES MD was examined undiluted at 20 °C. 15 seconds were chosen as exposure time. In summary, a virucidal activity against human rotavirus was measured as follows:

undiluted **15 seconds** **dirty conditions (3.0 g/l BSA + 3.0 ml/l erythrocytes)**

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DAkkS Deutsche Akkreditierungsstelle D-PL-13412-01-01 D-PL-13412-01-02
Anerkannt durch/Recognized by Zentralstelle der Länder für Gesundheitsschutz bei Arzneimitteln und Medizinprodukten www.zlg.de
ZLG-AP-216.11.02

TEST REPORT

20/12/2018

Test report L18/0890R.1

Evaluation of the effectiveness of

CHEMISEPT WIPES MD

Test virus: human rotavirus strain Wa

Method: based on EN 14476:2013+A1:2015 (dirty conditions)

quantitative suspension test for the evaluation of virucidal activity of chemical disinfectants and antiseptics used in human medicine

Sponsor:
Chemi-Pharm AS
Pollu 132
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1. Identification of test laboratory

Dr. Brill + Partner GmbH Institute for Hygiene and Microbiology, Norderoog 2, DE - 28259 Bremen

2. Identification of sample

Manufacturer	Chemi-Pharm AS
Name of product	CHEMISEPT WIPES MD
Confirmation no.	207453
Product diluent recommended by the manufacturer	-
Batch number	79040818
Application	surface disinfection
Production date	04/08/2018
Expiry date	04/08/2021
Active compound (s) (100 g)	72 g ethanol
Appearance, odour	clear, colorless liquid (soaking solution) product specific
pH-values	undiluted: 6.27 (20 °C)
Storage conditions	room temperature in the dark (area with restricted access)
Date of arrival in the laboratory	05/12/2018

3. Materials

3.1 Culture medium and reagents

- Eagle`s Minimum Essential Medium with Earle`s BSS (EMEM, Biozym Scientific GmbH, catalogue no. 880121)
- Fetal calf serum (Biochrom AG, article no. S 0115)
- 1.4 % formaldehyde solution (dilution of Roti®-Histofix 4 %, Carl Roth GmbH)
- Aqua bidest. (SG ultrapure water system, type Ultra Clear; serial no. 86996-1)
- PBS (Invitrogen, article no. 18912-014)
- BSA (Sigma-Aldrich-Chemie GmbH, article no. CA-2153)
- sheep erythrocytes (Fiebig Nährstofftechnik)

- Trypsin (SERVA Electrophoresis GmbH, article no. 37290).

3.2 Virus and cells

The human rotavirus strain Wa (serotype 1, subgroup II) was obtained by Prof. Dr. Holger Rabenau, Institute of Medical Virology of the Johann Wolfgang Goethe University of Frankfurt, DE - 60596 Frankfurt. Before the described tests, the virus had been passaged in *MA-104 cells* (embryonic rhesus monkey kidney cell line).

The cells (passage 49) were inspected regularly for morphological alterations and for contamination by mycoplasmas. No morphological alterations of cells and no contamination by mycoplasmas could be detected.

3.3 Apparatus, glassware and small items of equipment

- CO_2 incubator, Nunc GmbH & Co. KG, model QWJ 350
- Agitator (Vortex Genie Mixer, type G 560E)
- pH measurement 315i (WTW, article no. 2A10-100)
- Centrifuge (Sigma-Aldrich-Chemie GmbH, type 113)
- Microscope (Olympus, type CK 30)
- Centrifuge 5804 R (Eppendorf AG)
- Water bath (JULABO, Julabo U 3)
- Adjustable and fixed-volume pipettes (Eppendorf AG)
- Polysterol 96-well microtitre plate (Nunc GmbH & Co. KG, Wiesbaden)
- Cell culture flask (Nunc GmbH & Co. KG, Wiesbaden)
- Sealed test tubes (Sarstedt AG & Co., Nümbrecht).

4. Experimental conditions

Test temperature	20 °C ± 1.0 °C
Concentration of test product	undiluted (80.0 %) and as 50.0 % and 10.0 % (demonstration of non-active range) solutions
Appearance of product dilutions	no precipitation
Contact times	15 seconds and 30 minutes
Interfering substance	3.0 g/l bovine serum albumin + 3.0 ml/l erythrocytes (dirty conditions, EN 14476)
Procedure to stop action of disinfectant	immediate dilution
Diluent	Aqua bidest.
Stability of product in the mix with virus and interfering substance (80.0 % solution)	strong clouding, minor precipitation
Virus strain	human rotavirus strain Wa
Date of testing	05/12/2018 – 20/12/2018
End of testing	20/12/2018

5. Methods

5.1 Preparation of test virus suspension

After washing with serum-free Eagle's Minimum Essential Medium twice, cells were incubated with EMEM without fetal calf serum for three hours to eliminate all FCS. This was followed by the addition of virus (stock virus suspension) to *MA-104 cells* in the presence of trypsin for two hours ± 10 minutes at 37 °C. After this time, medium with trypsin was added. If 90 % of the cells showed a cytopathic effect, cells were subjected to a rapid two-fold freeze-thawing procedure followed by a centrifugation at 1.620 g for 30 minutes at 4 °C in order to sediment cell debris. After aliquotation the supernatant was stored as test virus suspension at –80 °C.

5.2 Preparation of disinfectant (dilutions)

The soaking solution of CHEMISEPT WIPES MD was tested undiluted. Due to the addition of interfering substance and test virus suspension an 80.0 % solution resulted.

Furthermore, the product was evaluated as 50.0 % and 10.0 % solutions (demonstrating of non-active range). These solutions were prepared with Aqua bidest. immediately before the inactivation tests.

5.3 Infectivity assay

Infectivity was determined by means of end point dilution titration in a micro-procedure. For this, samples were serially diluted with ice-cold EMEM with trypsin and 100 µl of each dilution were placed after aspiration of the medium in eight wells of a sterile polystyrene flat bottom 96-well microtitre plate with a preformed *MA-104* monolayer. After one hour at 37 °C, 100 µl EMEM with trypsin were added. Incubation took place at 37 °C in a CO_2-atmosphere (5.0 % CO_2 - content). Finally, cultures were observed for cytopathic effects for six days of inoculation. The infective dose ($TCID_{50}$) was calculated according to the method of Spearman (2) and Kärber (3) with the following formula:

$$- \log_{10}TCID_{50} = X_0 - 0.5 + \sum r/n$$

meaning

X_0 = $\log_{10}$ of the lowest dilution with 100 % positive reaction

r = number of pos. determinations of lowest dilution step with 100 % positive and all higher positive dilution steps

n = number of determinations for each dilution step.

5.4 Calculation and verification of virucidal activity

The virucidal activity of the test disinfectant was evaluated by calculating the decrease in titre in comparison with the control titration without disinfectant. The difference is given as reduction factor (RF).

According to the EN 14476, a disinfectant or a disinfectant solution at a particular concentration is having virus-inactivating efficacy if the titre is reduced at least by four $\log_{10}$ steps within the recommended exposure period. This corresponds to an inactivation of ≥ 99.99 %.

5.5 Inactivation assay

Determination of virucidal activity has been carried out in accordance to EN 5.5. The test product was examined undiluted (80.0 %) and as 50.0 % and 10.0 % (demonstration of non-active range) solutions in Aqua bidest. at 20 °C based on EN 14476. 15 seconds and 30 minutes were chosen as contact times.

Immediately at the end of a chosen contact time, activity of the disinfectant was stopped by dilution to 10^{-8}.

Titrations of the virus control were performed at the beginning of the test and after the longest exposure time (EN 5.5.7). One part by volume of test virus suspension was mixed with one part interfering substance and eight parts by volume of WSH or Aqua bidest. (RTU products).

Furthermore, a cell control (only addition of medium) was incorporated.

Inactivation tests were carried out in sealed test tubes in a water bath at 20 °C ± 1.0 °C. Aliquots were retained after appropriate exposure times, and residual infectivity was determined.

5.6 Determination of cytotoxicity

Determination of cytotoxicity was performed according to EN 5.5.4.1.

5.7 Cell sensitivity to virus

For the control of cell sensitivity to virus two parts by volume hard water were mixed with eight parts by volume of the lowest apparently non-cytotoxic dilution of the product. This mixture or PBS as control was added to the wells of the microtitre plates with a preformed monolayer of *MA-104-cells*.
Finally, a comparative titration of the test virus suspension was performed on the pre-treated (disinfectant) and non-pre-treated (PBS) cells as described above.

5.8 Control of efficacy for suppression of disinfectant's activity

Furthermore, a control of efficiency for suppression of disinfectant's activity was included (EN 5.5.5).

5.9 Reference virus inactivation test

As reference for test validation a 0.7 % formaldehyde solution according to EN 5.5.6 was included. 5, 15, 30 and 60 minutes were chosen as contact times. In addition, cytotoxicity of formaldehyde test solution was determined based on EN 5.5.6.2 with dilutions up to 10^{-5}.

6. Verification of the methodology

The following criteria as mentioned in EN 5.7 were fulfilled:

a) The titre of the test virus suspension allowed the determination of a ≥ 4 $\log_{10}$ reduction (maximal virus reduction ≥ 4.88 ± 0.29).

b) The test product showed cytotoxicity in the 1:10 dilutions (80.0 %) thus allowing the detection of a 4 $\log_{10}$ reduction of virus titre.

c) The comparative titration on pre-treated (disinfectant) and non-pre-treated (PBS) *MA-104 cells* showed no significant difference (< 1 $\log_{10}$; EN 5.7) of virus titre: 7.00 ± 0.44 (PBS) versus 6.88 ± 0.45 (1:100 dilution of disinfectant as 80.0 % solution) $\log_{10}$ $TCID_{50}$/ml.

d) The control of efficacy for suppression of disinfectant's activity (80.0 % solution) showed no decrease (≤ 0.5 $\log_{10}$; EN 5.5.5.1) in virus titre (7.13 ± 0.51 versus 7.38 ± 0.41 $\log_{10}$ $TCID_{50}$/ml).

e) One concentration demonstrated a 4 $\log_{10}$ reduction and (at least) one concentration demonstrated a $\log_{10}$ reduction of less than 4.

Since all criteria according EN 5.7 were fulfilled, examination with human rotavirus based on EN 14476 is valid.

7. Results

Results of examination are shown in tables 1 to 8. Tables 1 to 7 demonstrate the raw data, whereas table 8 (a+b) gives a summary of results.

The undiluted test product (80.0 %) was able to inactivate human rotavirus after 15 seconds of exposure time in this quantitative suspension test (tables 1 and 2). The reduction factors were ≥ 4.75 ± 0.31 and ≥ 4.88 ± 0.29 at this time point. The mean value was ≥ 4.81 ± 0.21. This corresponded to an inactivation of ≥ 99.99 %.

The test product as 50.0 % solution was not able to inactivate human rotavirus within 15 seconds of exposure time in this quantitative suspension test (table 3).

The test product as 10.0 % solution was also not able to inactivate human rotavirus within 30 minutes of exposure time in this quantitative suspension test (table 4).

8. Conclusion

The surface disinfectant CHEMISEPT WIPES MD tested undiluted demonstrated effectiveness against human rotavirus after an exposure time of 15 seconds under dirty conditions.

Therefore, the surface disinfectant CHEMISEPT WIPES MD can be declared as active against human rotavirus as follows:

undiluted **15 seconds** **dirty conditions**

Bremen, 20/12/2018

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9. Quality control

The Quality Assurance of the results was maintained by performing the determination of the virus-inactivating properties of the disinfectant in accordance with Good Laboratory Practice regulations:

1) Chemicals Act of Germany, Appendix 1, dating of 01.08 1994 (BGBI. I, 1994, page 1703). Appendix revised at 14. 05. 1997 (BGBI. I, 1997, page 1060).
2) OECD Principles of Good Laboratory Practice (revised 1997); OECD Environmental Health and Safety Publications; Series on Principles of Good Laboratory Practice and Compliance Monitoring – Number 1. Environment Directorate, Organization for Economic Co-operation and Development, Paris 1998.

The plausibility of the results was additionally confirmed by controls incorporated in the inactivation assays.

10. Records to be maintained

All testing data, protocol, protocol modifications, the final report, and correspondence between Dr. Brill + Partner GmbH and the sponsor will be stored in the archives at Dr. Brill + Partner GmbH.

11. Literature

1. EN 14476:2013+A1:2015: Chemical disinfectants and antiseptics – Quantitative suspension test for the evaluation of virucidal activity of chemicals disinfectants and antiseptics in human medicine test - Test method and requirements (phase 2, step 1)

2. Spearman, C.: The method of `right or wrong cases` (constant stimuli) without Gauss's formulae. Brit J Psychol; 2 1908, 227-242

3. Kärber, G.: Beitrag zur kollektiven Behandlung pharmakologischer Reihenversuche. Arch Exp Path Pharmak; 162, 1931, 480-487

Appendix:

Legend to the Tables

Table 1: Raw data for CHEMISEPT WIPES MD (80.0 %) tested against human rotavirus (1st assay)

Table 2: Raw data for CHEMISEPT WIPES MD (80.0 %) tested against human rotavirus (2nd assay)

Table 3: Raw data for CHEMISEPT WIPES MD (50.0 %) tested against human rotavirus

Table 4: Raw data for CHEMISEPT WIPES MD (10.0 %) tested against human rotavirus

Table 5: Raw data for formaldehyde solution (0.7 %) tested against human rotavirus

Table 6: Raw data for control of efficacy for suppression of disinfectant activity (80.0 %)

Table 7: Raw data (human rotavirus) for cell sensitivity (80.0 %)

Table 8 (a+b): Summary of results with CHEMISEPT WIPES MD and human rotavirus

Legend to the Figures

Figure 1: Virus-inactivating properties of CHEMISEPT WIPES MD (80.0 %)

Figure 2: Virus-inactivating properties of formaldehyde (0.7 %)

Table 1: Raw data for CHEMISEPT WIPES MD (80.0 %) tested against human rotavirus at 20 °C (quantal test; 8 wells) (#5817) (1st assay)

Product	Concentration	Interfering substance	Contact time (min)	Dilutions (log_{10})								
				1	2	3	4	5	6	7	8	9
test product	80.0 %	dirty conditions	0.25	tttt tttt	0000 0000	0000 0000	0000 0000	0000 0000	0000 0000	0000 0000	n.d.	n.d.
			0.5	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.
			2	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.
			30	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.
test product cytotoxicity	80.0 %	dirty conditions	n.a.	tttt tttt	0000 0000	0000 0000	0000 0000	0000 0000	n.d.	n.d.	n.d.	n.d.
virus control	n.a.	dirty conditions	0	4444 4444	4444 4444	4444 4444	4444 4444	4444 4444	4444 4442	0000 0203	0000 0000	0000 0000
			60	4444 4444	4444 4444	4444 4444	4444 4444	4444 4444	3440 0404	0003 0000	0000 0000	0000 0000

n.a. = not applicable
n.d. = not done

0 = no virus present; t = cytotoxic
1 to 4 = virus present (degree of CPE in 8 cell culture units) (wells of microtitre plates)

Table 2: Raw data for CHEMISEPT WIPES MD (80.0 %) tested against human rotavirus at 20 °C (quantal test; 8 wells) (#5830) (2nd assay)

Product	Concentration	Interfering substance	Contact time (min)	Dilutions (log_{10})								
				1	2	3	4	5	6	7	8	9
test product	80.0 %	dirty conditions	0.25	tttt tttt	0000 0000	0000 0000	0000 0000	0000 0000	0000 0000	0000 0000	n.d.	n.d.
			0.5	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.
			2	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.
			30	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.
test product cytotoxicity	80.0 %	dirty conditions	n.a.	tttt tttt	0000 0000	0000 0000	0000 0000	0000 0000	n.d.	n.d.	n.d.	n.d.
virus control	n.a.	dirty conditions	0	4444 4444	4444 4444	4444 4444	4444 4444	4444 4444	4423 4444	0000 0000	0000 0000	0000 0000
			60	4444 4444	4444 4444	4444 4444	4444 4444	4444 4444	0404 4444	0000 0400	0000 0000	0000 0000

n.a. = not applicable
n.d. = not done

0 = no virus present; t = cytotoxic
1 to 4 = virus present (degree of CPE in 8 cell culture units) (wells of microtitre plates)

Table 3: Raw data for CHEMISEPT WIPES MD (50.0 %) tested against human rotavirus at 20 °C (quantal test; 8 wells) (#5830)

Product	Concentration	Interfering substance	Contact time (min)	Dilutions (log_{10})								
				1	2	3	4	5	6	7	8	9
test product	50.0 %	dirty conditions	0.25	tttt tttt	4444 4444	4444 4444	4444 4444	4444 4244	0040 0004	0000 0000	n.d.	n.d.
			0.5	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.
			2	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.
			30	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.
test product cytotoxicity	50.0 %	dirty conditions	n.a.	tttt tttt	0000 0000	0000 0000	0000 0000	0000 0000	n.d.	n.d.	n.d.	n.d.
virus control	n.a.	dirty conditions	0	4444 4444	4444 4444	4444 4444	4444 4444	4444 4444	4423 4444	0000 0000	0000 0000	0000 0000
			60	4444 4444	4444 4444	4444 4444	4444 4444	4444 4444	0404 4444	0000 0400	0000 0000	0000 0000

n.a. = not applicable
n.d. = not done

0 = no virus present; t = cytotoxic
1 to 4 = virus present (degree of CPE in 8 cell culture units) (wells of microtitre plates)

Table 4: Raw data for CHEMISEPT WIPES MD (10.0 %) tested against human rotavirus at 20 °C (quantal test; 8 wells) (#5830)

Product	Concentration	Interfering substance	Contact time (min)	Dilutions (log_{10})								
				1	2	3	4	5	6	7	8	9
test product	10.0 %	dirty conditions	0.25	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.
			0.5	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.
			2	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.
			30	4444 4444	4444 4444	4444 4444	4444 4444	4444 4444	4434 4344	1003 0000	0000 0000	n.d.
test product cytotoxicity	10.0 %	dirty conditions	n.a.	0000 0000	0000 0000	0000 0000	0000 0000	0000 0000	n.d.	n.d.	n.d.	n.d.
virus control	n.a.	dirty conditions	0	4444 4444	4444 4444	4444 4444	4444 4444	4444 4444	4423 4444	0000 0000	0000 0000	0000 0000
			60	4444 4444	4444 4444	4444 4444	4444 4444	4444 4444	0404 4444	0000 0400	0000 0000	0000 0000

n.a. = not applicable
n.d. = not done

0 = no virus present; t = cytotoxic
1 to 4 = virus present (degree of CPE in 8 cell culture units) (wells of microtitre plates)

Table 5: Raw data for formaldehyde solution (0.7 %) tested against human rotavirus at 20 °C (quantal test; 8 wells) (#5830)

Product	Concentration	Interfering substance	Contact time (min)	Dilutions (log_{10})								
				1	2	3	4	5	6	7	8	9
formaldehyde	0.7 % (m/V)	PBS	5	tttt tttt	tttt tttt	4444 4444	4444 4444	4313 3333	3033 3201	0000 0004	0000 0000	n.d.
			15	tttt tttt	tttt tttt	4444 4444	4444 4444	4014 4424	4000 0032	0000 0000	0000 0000	n.d.
			30	tttt tttt	tttt tttt	4444 4444	4444 4444	0033 3230	0000 0000	0000 0000	0000 0000	n.d.
			60	tttt tttt	tttt tttt	3331 3331	0321 0300	0000 0000	0000 0000	0000 0000	0000 0000	n.d.
formaldehyde cytotoxicity	0.7 % (m/V)	PBS	n.a.	tttt tttt	tttt tttt	0000 0000	0000 0000	0000 0000	n.d.	n.d.	n.d.	n.d.
virus control	n.a.	PBS	0	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.
			60	4444 4444	4444 4444	4444 4444	4444 4444	4444 4444	0223 0243	0400 0000	0000 0001	0000 0000

n.a. = not applicable
n.d. = not done

0 = no virus present; t = cytotoxic
1 to 4 = virus present (degree of CPE in 8 cell culture units) (wells of microtitre plates)

Table 6: Raw data for control of efficacy for suppression of disinfectant's activity (80.0 %) (#5830)

Product	Interfering substance	dilutions (log_{10})								
		1	2	3	4	5	6	7	8	9
test product	dirty conditions	tttt tttt	4444 4444	4444 4444	4444 4444	4440 4444	1240 4003	0400 0000	0000 0000	n.d.
corresponding virus control	dirty conditions	4444 4444	4444 4444	4444 4444	4444 4444	4444 4444	0404 4444	0000 0400	0000 0000	0000 0000

n.a. = not applicable
n.d. = not done

0 = no virus present; t = cytotoxic
1 to 4 = virus present (degree of CPE in 8 cell culture units) (wells of microtitre plates)

Table 7: Raw data (human rotavirus) for cell sensitivity (80.0 %) (#5830)

Product	Dilution	Dilutions (log_{10})								
		1	2	3	4	5	6	7	8	9
PBS	-	4444 4444	4444 4444	4444 4444	4444 4444	4444 4443	0400 1040	0000 4000	0000 0000	n.d.
test product	1:100	4444 4444	4444 4444	4444 4444	4444 4444	4444 4444	4402 3444	1400 0110	0000 0000	n.d.

n.a. = not applicable
n.d. = not done

0 = no virus present; t = cytotoxic
1 to 4 = virus present (degree of CPE in 8 cell culture units) (wells of microtitre plates)

Table 8a: Summary of results with CHEMISEPT WIPES MD and human rotavirus

Product*	Con-centration	Interfering substance	Level of cytotoxicity	log_{10} $TCID_{50}$/ml after ....min					> 4 log_{10} reduction after … min
				0.25	0.5	2	30	60	
test product (1)	80.0 %	dirty conditions	2.50	≤2.50±0.00	n.d.	n.d.	n.d.	n.d.	0.25 (RF ≥ 4.75±0.31)
test product (2)	80.0 %	dirty conditions	2.50	≤2.50±0.00	n.d.	n.d.	n.d.	n.d.	0.25 (RF ≥ 4.88±0.29)
test product (2)	50.0 %	dirty conditions	2.50	6.75±0.33	n.d.	n.d.	n.d.	n.d.	> 0.25 (RF = 0.63±0.53)
test product (2)	10.0 %	dirty conditions	1.50	n.d.	n.d.	n.d.	7.25±0.33	n.d.	> 30 (RF = 0.00±0.53)

*the number in the brackets gives the number of the corresponding virus control (see table 8b)

n.a. = not applicable n.d. = not done

Table 8b: Summary of results with CHEMISEPT WIPES MD and human rotavirus

Product	Con-centration	Interfering substance	Level of cytotoxicity	log_{10} $TCID_{50}$/ml after ....min					> 4 log_{10} reduction after … min
				0	5	15	30	60	
formaldehyde	0.7 % (w/v)	PBS	3.50	n.d.	7.38±0.41	6.75±0.44	6.13±0.37	5.00±0.38	> 60 (RF = 2.50±0.61)
virus control	n.a.	PBS	n.a.	n.d.	n.d.	n.d.	n.d.	7.50±0.48	n.a.
virus control (1)	n.a.	dirty conditions	n.a.	7.75±0.33	n.d.	n.d.	n.d.	7.25±0.44	n.a.
virus control (2) (+ suppression)	n.a.	dirty conditions	n.a.	7.50±0.00	n.d.	n.d.	n.d.	7.38±0.41	n.a.
suppression control	80.0 %	dirty conditions	2.50	n.d.	n.d.	n.d.	7.13±0.51	n.d.	n.a.
sens.control PBS	n.a.	n.a.	n.a.	n.d.	n.d.	n.d.	n.d.	7.00±0.44	n.a.
sens. control test product	80.0 % → 1:100	n.a.	n.a.	n.d.	n.d.	n.d.	n.d.	6.88±0.45	n.a.

n.a. = not applicable n.d. = not done sens. = sensitivity

Figure 1: Virus-inactivating properties of CHEMISEPT WIPES MD (80.0 %)

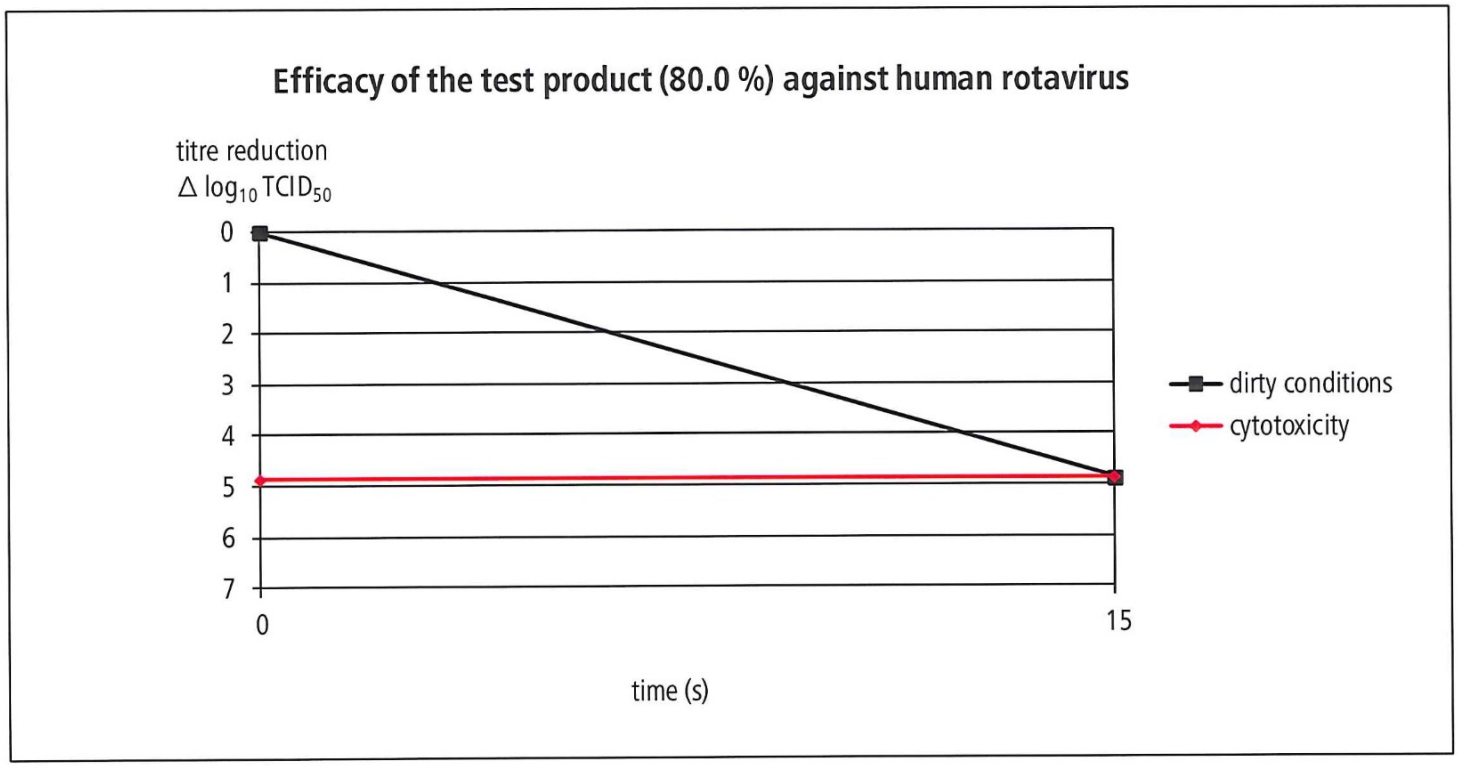

Figure 2: Virus-inactivating properties of formaldehyde (0.7 %)