Test

Conc. of the pro-duct %	Dilution step	Counts per plate		V_{C1}	V_{C2}	Lg *Na* = Lg ($\bar{x}$ *or* $\bar{x}_{wm}$) + 1	Lg *R* (lg N_W = 7.41)	Contact time
Ready to use	10^0	10+7	11+5	17	16	2.38	5.19	30 sec
	10^{-1}	1+1	1+1	<14	<14			
	10^{-2}	0+0	0+0	<14	<14			
	10^{-3}	0+0	0+0	<14	<14			
Ready to use	10^0	3+2	1+1	<14	<14	<2.15	>5.26	60 min
	10^{-1}	0+1	1+0	<14	<14			
	10^{-2}	0+0	0+0	<14	<14			
	10^{-3}	0+0	0+0	<14	<14			

Explanations

V_c = count per ml (one plate or more)

$\bar{x}$ = average of V_{C1} and V_{C2}

R = reduction (lg *R* = lg N_W – lg N_a)

If N_a <140, lg *R* = > [lg N_W – 2,15]

Appendix 4

Interpretation

The product for instrument disinfection **Bacticid AF** (batch no. 197101017) was tested according to the test method EVS-EN 14561:2006. The test was performed at 20 ± 1 °C, under dirty conditions with the contact time of 30 sec. The dilution – neutralization method was used for testing the products' effectiveness against the reference strains: *Pseudomonas aeruginosa* ATCC 15442; *Enterococcus hirae* ATCC 10541 and *Staphylococcus aureus* ATCC 6538. Under dirty conditions the product was effective against all the reference strains within 30 sec of contact time.

Conclusion

The surviving count of bacterial reference strains showed at least 5 lg reduction meaning that **under dirty conditions the ready to use product Bacticid AF has a bactericidal effect in case of instrument disinfection within 30 sec.**

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Diana Kaare, MSc

Head of laboratory, microbiologist

Nordic Tersus
Laboratory

Test report No. id0518

EVALUATION OF FUNGICIDAL OR YEASTICIDAL ACTIVITY FOR INSTRUMENTS USED IN MEDICAL AREA (EN 14562)

Name of the product:	Bacticid AF
Batch number:	197101017
Order number:	17029
Manufacturer:	Chemi-Pharm Ltd.
Client, representative:	Chemi-Pharm Ltd., Põllu 132, Tallinn, 10917, ESTONIA Maris Millner, +372-51-77-090
Date of delivery:	15.12.2017
Test material conditions:	No specific features, sample in the manufacturers tare
Storage conditions:	In room temperature, dark
Active substance – conc.:	Ethyl alcohol 57.0% wt, isopropyl alcohol 6.0% wt
Appearance of the product:	Transparent liquid
Test concentration:	Ready to use
Contact time:	30 sec, 60 min (obligatory contact time)
Interfering substance:	3 g/l bovine albumin + 3 ml/l sheep blood erythrocytes= dirty conditions
Rinsing liquid:	-
Neutralizer:	Polysorbate 80 30g/l; saponin 30 g/l, lecithin 3 g/l
Test organisms:	*Candida albicans* ATCC 10231
Testing method:	EVS-EN 14562:2006 Quantitative carrier test for the evaluation of fungicidal or yeasticidal activity for instruments used in medical area (phase 2, step 2)
Testing date:	31.01.2018 – 01.02.2018
Results:	look appendix 1-2

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Diana Kaare, MSc

Head of laboratory, microbiologist

Date of test report: 05.02.2018

Nordic Tersus Laboratory OÜ
Harjumaa, Tallinn, Serva tn 44a, 11618
Registrikood: 12561442
Tel: +372 503 0197
E-mail: info@ntl.ee

Appendix 1

TEST RESULTS (yeasticidal carrier test)

EVS-EN 14562; Phase 2, step 2;
Dilution – neutralization method;
Rinsing liquid: -;
Test organism: *Candida albicans* ATCC 1023;
Test temperature: +20° C; Incubation temperature: +30° C
Interfering substance: 3 g/l bovine albumin + 3 ml/l sheep blood erythrocytes= dirty conditions;
Nordic Tersus Laboratory LLC.;
Date of test: 31.01.2018 – 01.02.2018;
Responsible person: Diana Kaare

Validation and controls

Validation suspension (N_{vo})				Experimental Conditions control (A)				Neutralizer control (B)				Method validation (C)			
Counts per plate		V_{C1}	V_{C2}	Counts per plate		V_{C1}	V_{C2}	Counts per plate		V_{C1}	V_{C2}	Counts per plate		V_{C1}	V_{C2}
29+22	26+23	51	49	18+20	20+15	38	35	19+24	28+31	43	59	18+15	21+17	33	38
$\bar{x}$ = 50				$\bar{x}$ = 36.5				$\bar{x}$ = 51				$\bar{x}$ = 35.5			
30 ≤ $\bar{x}$ of N_{vo} ≤ 160? yes ☒ ; no ☐				$\bar{x}$ A is ≥ 0,5 $\bar{x}$ of N_{vo}? yes ☒ ; no ☐				$\bar{x}$ B is ≥ 0,5 $\bar{x}$ of N_{vo}? yes ☒ ; no ☐				$\bar{x}$ C is ≥ 0,5 $\bar{x}$ of N_{vo}? yes ☒ ; no ☐			

Test suspension

Test suspension (N):	N	V_{C1}	V_{C2}	$\bar{x}$ wm = 1.59x 10^8; lg N = 8.20 8.17 ≤ lg N ≤ 8.7 yes ☒ ; no ☐
	10^{-6}	155	162	
	10^{-7}	16	16	

Water control

Water control (N_W):	N_w	Counts per plate		V_{C1}	V_{C2}	$\bar{x}$ x 10 = 1.55 x 10^6 6.15 ≤ lg N_W = 6.19 ≤ (lg N -1.3)? yes ☒ ; no ☐
	10^{-4}	7+9	9+6	16	15	
	10^{-5}	0+1	0+0	<14	<14	

Test

Conc. of the product %	Dilution step	Counts per plate		V_{C1}	V_{C2}	Lg *N*a = Lg ($\bar{x}$ *or* $\bar{x}_{wm}$) + 1	Lg *R* (lg N_W = 6.19)	Contact time
Ready to use	10^0	6+9	8+5	15	14	2.16	4.03	30 sec
	10^{-1}	0+1	0+1	<14	<14			
	10^{-2}	0+0	0+0	<14	<14			
	10^{-3}	0+0	0+0	<14	<14			
Ready to use	10^0	0+0	0+0	<14	<14	<2.15	>4.04	60 min
	10^{-1}	0+0	0+0	<14	<14			
	10^{-2}	0+0	0+0	<14	<14			
	10^{-3}	0+0	0+0	<14	<14			

Explanations

V_c = count per ml (one plate or more)
$\bar{x}$ = average of V_{C1} and V_{C2}

R = reduction (lg *R* = lg N_W – lg N_a)
If N_a <140, lg *R* = > [lg N_W – 2,15]

Appendix 2

Interpretation

The product for instrument disinfection **Bacticid AF** (batch no. 197101017) was tested according to the test method EVS-EN 14562:2006. The test was performed at 20 ± 1 °C, under dirty conditions with the contact time of 30 sec. The dilution – neutralization method was used for testing the products' effectiveness against the reference strain *Candida albicans* ATCC 10231. Under dirty conditions the product was effective against the reference strain within 30 sec of contact time.

Conclusion

The surviving count of the reference strain showed at least 4 lg reduction meaning that **under dirty conditions the ready to use product Bacticid AF has a yeasticidal effect in case of instrument disinfection within 30 sec.**

Diana Kaare, MSc

Head of laboratory, microbiologist

Nordic Tersus
Laboratory

Test report No. id1618

EVALUATION OF MYCOBACTERICIDAL OR TUBERCULOCIDAL ACTIVITY FOR INSTRUMENTS USED IN MEDICAL AREA (EN 14563)

Name of the product:	Bacticid AF
Batch number:	197101017
Order number:	17029
Manufacturer:	Chemi-Pharm Ltd.
Client, representative:	Chemi-Pharm Ltd., Põllu 132, Tallinn, 10917, ESTONIA Maris Millner, +372-51-77-090
Date of delivery:	15.12.2017
Test material conditions:	No specific features, sample in the manufacturers tare
Storage conditions:	In room temperature, dark
Active substance – conc.:	Ethyl alcohol 57.0% wt, isopropyl alcohol 6.0% wt
Appearance of the product:	Transparent liquid
Test concentration:	Ready to use
Contact time:	30 sec, 60 min (obligatory contact time)
Interfering substance:	0.3 g/l bovine albumin = clean conditions
Rinsing liquid:	-
Neutralizer:	Polysorbate 80 30g/l; saponin 30 g/l, lecithin 3 g/l
Test organisms:	*Mycobacterium avium* ATCC 15769 *Mycobacterium terrae* ATCC 15755
Testing method:	EVS-EN 14563:2009 Quantitative carrier test for the evaluation of mycobactericidal or tuberculocidal activity for instruments used in medical area (phase 2, step 2)
Testing date:	30.01.2018 – 20.02.2018
Results:	look appendix 1-3

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Head of laboratory, microbiologist

Date of test report: 22.02.2018

Nordic Tersus Laboratory OÜ
Harjumaa, Tallinn, Serva tn 44a, 11618
Registrikood: 12561442
Tel: +372 503 0197
E-mail: info@ntl.ee

Appendix 1

TEST RESULTS (mycobactericidal carrier test)

EVS-EN 14563; Phase 2, step 2;
Dilution – neutralization method;
Rinsing liquid: -;
Test organism: *Mycobacterium avium* ATCC 15769;
Test temperature: +20° C; Incubation temperature: +37° C
Interfering substance: 0.3 g/l bovine albumin = clean conditions;
Nordic Tersus Laboratory LLC.;
Date of test: 30.01.2018 – 20.02.2018;
Responsible person: Diana Kaare

Validation and controls

Validation suspension (N_{vo})				Experimental Conditions control (A)				Neutralizer control (B)				Method validation (C)			
Counts per plate		V_{C1}	V_{C2}	Counts per plate		V_{C1}	V_{C2}	Counts per plate		V_{C1}	V_{C2}	Counts per plate		V_{C1}	V_{C2}
41+35	44+32	76	76	36+27	31+38	63	69	25+21	29+33	46	62	36+28	32+25	64	57
$\bar{x}$ = 76				$\bar{x}$ = 66				$\bar{x}$ = 54				$\bar{x}$ = 60.5			
30 ≤ $\bar{x}$ of N_{vo} ≤ 160? yes ☒ ; no ☐				$\bar{x}$ A is ≥ 0,5 $\bar{x}$ of N_{vo}? yes ☒ ; no ☐				$\bar{x}$ B is ≥ 0,5 $\bar{x}$ of N_{vo}? yes ☒ ; no ☐				$\bar{x}$ C is ≥ 0,5 $\bar{x}$ of N_{vo}? yes ☒ ; no ☐			

Test suspension

Test suspension (N):	N	V_{C1}	V_{C2}	$\bar{x}$ wm = 2.28 x 10^9; lg N = 9.36 9.17 ≤ lg N ≤ 9.7 yes ☒ ; no ☐
	10^{-7}	217	239	
	10^{-8}	25	21	

Water control

Water control (N_W):	N_W	Counts per plate		V_{C1}	V_{C2}	$\bar{x}$ x 10 = 2.45 x 10^7 7.15 ≤ lg N_W = 7.39 ≤ (lg N -1.3)? yes ☒ ; no ☐
	10^{-5}	13+11	17+7	24	25	

Test

Conc. of the product %	Dilution step	Counts per plate		V_{C1}	V_{C2}	Lg *N*a = Lg ($\bar{x}$ *or* $\bar{x}_{wm}$) + 1	Lg *R* (lg N_W = 7.39)	Contact time
Ready to use	10^0	12+10	13+8	22	21	2.33	5.06	30 sec
	10^{-1}	2+2	1+2	<14	<14			
	10^{-2}	0+0	0+0	<14	<14			
	10^{-3}	0+0	0+0	<14	<14			
Ready to use	10^0	0+2	1+2	<14	<14	<2.15	>5.24	60 min
	10^{-1}	0+0	0+0	<14	<14			
	10^{-2}	0+0	0+0	<14	<14			
	10^{-3}	0+0	0+0	<14	<14			

Explanations

V_c = count per ml (one plate or more)
$\bar{x}$ = average of V_{C1} and V_{C2}

R = reduction (lg R = lg N_W – lg N_a)
If N_a <140, lg R = > [lg N_W – 2,15]

Appendix 2

TEST RESULTS (mycobactericidal carrier test)

EVS-EN 14563; Phase 2, step 2;
Dilution – neutralization method;
Rinsing liquid: -;
Test organism: *Mycobacterium terrae* ATCC 15755;
Test temperature: +20° C; Incubation temperature: +37° C
Interfering substance: 0.3 g/l bovine albumin = clean conditions;
Nordic Tersus Laboratory LLC.;
Date of test: 30.01.2018 – 20.02.2018;
Responsible person: Diana Kaare

Validation and controls

Validation suspension (N_{vo})				Experimental Conditions control (A)				Neutralizer control (B)				Method validation (C)			
Counts per plate		V_{C1}	V_{C2}	Counts per plate		V_{C1}	V_{C2}	Counts per plate		V_{C1}	V_{C2}	Counts per plate		V_{C1}	V_{C2}
51+47	45+49	98	94	31+39	42+26	70	68	28+33	38+37	61	75	25+31	33+38		
$\bar{x}$ = 96				$\bar{x}$ = 69				$\bar{x}$ = 68				$\bar{x}$ = 63.5			
30 ≤ $\bar{x}$ of N_{vo} ≤ 160? yes ☒ ; no ☐				$\bar{x}$ A is ≥ 0,5 $\bar{x}$ of N_{vo}? yes ☒ ; no ☐				$\bar{x}$ B is ≥ 0,5 $\bar{x}$ of N_{vo}? yes ☒ ; no ☐				$\bar{x}$ C is ≥ 0,5 $\bar{x}$ of N_{vo}? yes ☒ ; no ☐			

Test suspension

Test suspension (N):	N	V_{C1}	V_{C2}	$\bar{x}$ wm = 2.65 x 10^9; lg N = 9.42 9.17 ≤ lg N ≤ 9.7 yes ☒ ; no ☐
	10^{-7}	261	272	
	10^{-8}	24	25	

Water control

Water control (N_W):	N_W	Counts per plate		V_{C1}	V_{C2}	$\bar{x}$ x 10 = 2.90 x 10^7 7.15 ≤ lg N_W = 7.46 ≤ (lg N -1.3)? yes ☒ ; no ☐
	10^{-5}	16+10	14+18	26	32	

Test

Conc. of the product %	Dilution step	Counts per plate		V_{C1}	V_{C2}	Lg *N*a = Lg ($\bar{x}$ *or* $\bar{x}_{wm}$) + *1*	Lg *R* (lg N_W = 7.46)	Contact time
Ready to use	10^0	11+15	9+18	22	26	2.38	5.08	30 sec
	10^{-1}	2+2	2+1	<14	<14			
	10^{-2}	0+0	0+0	<14	<14			
	10^{-3}	0+0	0+0	<14	<14			
Ready to use	10^0	1+0	4+4	<14	<14	<2.15	>5.31	60 min
	10^{-1}	0+0	0+1	<14	<14			
	10^{-2}	0+0	0+0	<14	<14			
	10^{-3}	0+0	0+0	<14	<14			

Explanations

V_c = count per ml (one plate or more)
$\bar{x}$ = average of V_{C1} and V_{C2}

R = reduction (lg *R* = lg N_w – lg N_a)
If N_a <140, lg *R* = > [lg N_W – 2,15]

Appendix 3

Interpretation

The product for instrument disinfection **Bacticid AF** (batch no. 197101017) was tested according to the test method EVS-EN 14563:2009. The test was performed at 20 ± 1 °C, under clean conditions with the contact time of 30 sec. The dilution – neutralization method was used for testing the products' effectiveness against following reference strains: *Mycobacterium avium* ATCC 15769, *Mycobacterium terrae* ATCC 15755. Under clean conditions the product was effective against both of reference strains within 30 sec of contact time.

Conclusion

The surviving count of mycobacterial reference strains showed at least 5 lg reduction meaning that **under clean conditions the ready to use product Bacticid AF has a mycobactericidal effect in case of instrument disinfection within 30 sec.**

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Diana Kaare, MSc

Head of laboratory, microbiologist

DR. JOCHEN STEINMANN

C/O DR. BRILL + PARTNER GMBH
INSTITUT FÜR HYGIENE UND MIKROBIOLOGIE
NORDEROOG 2, DE 28259 BREMEN
TELEFON 0049-40/557631-0
TELEFAX 0049-40/557631-11
EMAIL INFO@BRILLHYGIENE.COM
INTERNET WWW.BRILLHYGIENE.COM

DR. J. STEINMANN · C/O DR. BRILL + PARTNER GMBH · NORDEROOG 2 · DE-28259 BREMEN

Chemi-Pharm AS
Pollu 132
EST – TALLINN 10917

Bremen, 07/02/2018

Expert opinion

Activity of Bacticid AF against MNV in a quantitative suspension test according to the EN 14476:2013+A1:2015 under clean conditions

This expert opinion is based on the test report L17/0629aM.1 dating 07/02/2018.

The virus-inactivating properties of the surface disinfectant Bacticid AF of Chemi-Pharm AS against murine norovirus (MNV) were investigated by a quantitative suspension test according to EN 14476 under clean 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 surface disinfectant Bacticid AF was examined undiluted at 20 °C. 20 and 30 seconds were chosen as exposure time. In summary, a virucidal activity against MNV was measured as follows:

	undiluted	**20 seconds**	**clean conditions (0.3 g / l BSA)**

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DR. JOCHEN STEINMANN

C/O DR. BRILL + PARTNER GMBH
INSTITUT FÜR HYGIENE UND MIKROBIOLOGIE
NORDEROOG 2, DE 28259 BREMEN
TELEFON 0049-40/557631-0
TELEFAX 0049-40/557631-11
EMAIL INFO@BRILLHYGIENE.COM
INTERNET WWW.BRILLHYGIENE.COM

DR. J. STEINMANN · C/O DR. BRILL + PARTNER GMBH · NORDEROOG 2 · DE-28259 BREMEN

Chemi-Pharm AS
Pollu 132
EST – TALLINN 10917

Bremen, 07/02/2018

Expert opinion

Activity of Bacticid AF against MNV in a quantitative suspension test according to the EN 14476:2013+A1:2015 under dirty conditions

This expert opinion is based on the test report L17/0629aM.2 dating 07/02/2018.

The virus-inactivating properties of the surface disinfectant Bacticid AF of Chemi-Pharm AS against murine norovirus (MNV) were investigated by a quantitative suspension test according to 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 surface disinfectant Bacticid AF was examined undiluted at 20 °C. 15, 20, 30 and 60 seconds were chosen as exposure time. In summary, a virucidal activity against MNV was measured as follows:

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

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DR. JOCHEN STEINMANN

C/O DR. BRILL + PARTNER GMBH
INSTITUT FÜR HYGIENE UND MIKROBIOLOGIE
NORDEROOG 2, DE 28259 BREMEN
TELEFON 0049-40/557631-0
TELEFAX 0049-40/557631-11
EMAIL INFO@BRILLHYGIENE.COM
INTERNET WWW.BRILLHYGIENE.COM

DR. J. STEINMANN · C/O DR. BRILL + PARTNER GMBH · NORDEROOG 2 · DE-28259 BREMEN

Chemi-Pharm AS
Pollu 132
EST – TALLINN 10917

Bremen, 07/02/2018

Expert opinion

Activity of Bacticid AF against MNV in a quantitative suspension test according to the EN 14476:2013+A1:2015 under dirty conditions

This expert opinion is based on the test report L17/0629aM.2 dating 07/02/2018.

The virus-inactivating properties of the surface disinfectant Bacticid AF of Chemi-Pharm AS against murine norovirus (MNV) were investigated by a quantitative suspension test according to 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 surface disinfectant Bacticid AF was examined undiluted at 20 °C. 15, 20, 30 and 60 seconds were chosen as exposure time. In summary, a virucidal activity against MNV was measured as follows:

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

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DR. JOCHEN STEINMANN

C/O DR. BRILL + PARTNER GMBH
INSTITUT FÜR HYGIENE UND MIKROBIOLOGIE
NORDEROOG 2, DE 28259 BREMEN
TELEFON 0049-40/557631-0
TELEFAX 0049-40/557631-11
EMAIL INFO@BRILLHYGIENE.COM
INTERNET WWW.BRILLHYGIENE.COM

DR. J. STEINMANN · C/O DR. BRILL + PARTNER GMBH · NORDEROOG 2 · DE-28259 BREMEN

Chemi-Pharm AS
Pollu 132
EST – TALLINN 10917

Bremen, 07/02/2018

Expert opinion

Activity of Bacticid AF against MNV in a quantitative suspension test according to the EN 14476:2013+A1:2015 under clean conditions

This expert opinion is based on the test report L17/0629aM.1 dating 07/02/2018.

The virus-inactivating properties of the surface disinfectant Bacticid AF of Chemi-Pharm AS against murine norovirus (MNV) were investigated by a quantitative suspension test according to EN 14476 under clean 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 surface disinfectant Bacticid AF was examined undiluted at 20 °C. 20 and 30 seconds were chosen as exposure time. In summary, a virucidal activity against MNV was measured as follows:

undiluted **20 seconds** **clean conditions (0.3 g / l BSA)**

Dr.

DR. JOCHEN STEINMANN

C/O DR. BRILL + PARTNER GMBH
INSTITUT FÜR HYGIENE UND MIKROBIOLOGIE
NORDEROOG 2, DE 28259 BREMEN
TELEFON 0049-40/557631-0
TELEFAX 0049-40/557631-11
EMAIL INFO@BRILLHYGIENE.COM
INTERNET WWW.BRILLHYGIENE.COM

DR. J. STEINMANN · C/O DR. BRILL + PARTNER GMBH · NORDEROOG 2 · DE-28259 BREMEN

Chemi-Pharm AS
Pollu 132
EST – TALLINN 10917

Bremen, 20/02/2018

Expert opinion

Activity of Bacticid AF 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 L17/0629aMV.2 dating 20/02/2018.

The virus-inactivating properties of the surface disinfectant Bacticid AF 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 surface disinfectant Bacticid AF was examined undiluted at 20 °C. 15, 20, 30 and 60 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 g/l erythrocytes)**

Dr.

DR. JOCHEN STEINMANN

C/O DR. BRILL + PARTNER GMBH
INSTITUT FÜR HYGIENE UND MIKROBIOLOGIE
NORDEROOG 2, DE 28259 BREMEN
TELEFON 0049-40/557631-0
TELEFAX 0049-40/557631-11
EMAIL INFO@BRILLHYGIENE.COM
INTERNET WWW.BRILLHYGIENE.COM

DR. J. STEINMANN · C/O DR. BRILL + PARTNER GMBH · NORDEROOG 2 · DE-28259 BREMEN

Chemi-Pharm AS
Pollu 132
EST – TALLINN 10917

Bremen, 20/02/2018

Expert opinion

Activity of Bacticid AF against modified vaccinia virus Ankara (MVA) in a quantitative suspension test based on the EN 14476:2013+A1:2015 under clean conditions

This expert opinion is based on the test report L17/0629aMV.1 dating 20/02/2018.

The virus-inactivating properties of the surface disinfectant Bacticid AF of Chemi-Pharm AS against modified vaccinia virus Ankara (MVA) were investigated by a quantitative suspension test based on EN 14476 under clean 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 surface disinfectant Bacticid AF was examined undiluted at 20 °C. 15, 20 and 30 seconds were chosen as exposure time. In summary, a virucidal activity against modified vaccinia virus Ankara (MVA) was measured as follows:

undiluted **15 seconds** **clean conditions (0.3 g / l BSA)**

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DR. JOCHEN STEINMANN

C/O DR. BRILL + PARTNER GMBH
INSTITUT FÜR HYGIENE UND MIKROBIOLOGIE
NORDEROOG 2, DE 28259 BREMEN
TELEFON 0049-40/557631-0
TELEFAX 0049-40/557631-11
EMAIL INFO@BRILLHYGIENE.COM
INTERNET WWW.BRILLHYGIENE.COM

DR. J. STEINMANN · C/O DR. BRILL + PARTNER GMBH · NORDEROOG 2 · DE-28259 BREMEN

Chemi-Pharm AS
Pollu 132
EST – TALLINN 10917

Bremen, 20/11/2018

Expert opinion

Activity of Bacticid AF 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 L18/0785R.1 dating 20/11/2018.

The virus-inactivating properties of the surface disinfectant Bacticid AF 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 surface disinfectant Bacticid AF was examined undiluted at 20 °C. 15 and 30 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)**

Dr. Joch

DR. BRILL + DR. STEINMANN
INSTITUTE FOR HYGIENE AND MICROBIOLOGY

ZLG-AP-216.11.02

20/11/2018

Test report L18/0785R.1

Evaluation of the effectiveness of

Bacticid AF

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
EST – TALLINN 10917

TEST REPORT

Norderoog 2, DE - 28259 Bremen
Tel.: +49 40-557631-0, Fax: +49 40-557631-11
info@brillhygiene.com, http://www.brillhygiene.com

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	Bacticid AF
Confirmation no.	207087
Product diluent recommended by the manufacturer	-
Batch number	-
Application	surface disinfection
Production date	25/10/2018
Expiry date	25/10/2021
Active compound (s) (100 g)	57 g ethanol 6 g IPA
Appearance, odour	clear, colorless liquid product specific
pH-values	undiluted: 8.23 (20 °C)
Storage conditions	room temperature in the dark (area with restricted access)
Date of arrival in the laboratory	02/11/2018

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 44) 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 and 30 seconds
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, no precipitation
Virus strain	human rotavirus strain Wa
Date of testing	02/11/2018 – 20/11/2018
End of testing	20/11/2018

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 test product 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 and 30 seconds 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 ≥ 5.75 ± 0.23).

b) The test product showed no 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: 7.50 ± 0.00 (PBS) versus 7.00 ± 0.38 (1:10 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 (7.50 ± 0.35 versus 7.25 ± 0.33 $\log_{10}$ $TCID_{50}$/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 7. Tables 1 to 6 demonstrate the raw data, whereas table 7 (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 (table 1). The reduction factor was ≥ 5.38 ± 0.53 at this time point. This corresponded to an inactivation of ≥ 99.999 %.

The test product as 50.0 % solution was not able to inactivate human rotavirus within 30 seconds of exposure time in this quantitative suspension test (table 2).

The test product as 10.0 % solution was also not able to inactivate human rotavirus within 30 seconds of exposure time in this quantitative suspension test (table 3).

8. Conclusion

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

Therefore, the surface disinfectant Bacticid AF can be declared as active against human rotavirus as follows:

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

Bremen, 20/11/2018

- Dr. Da
Scientifi

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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 Bacticid AF (80.0 %) tested against human rotavirus

Table 2: Raw data for Bacticid AF (50.0 %) tested against human rotavirus

Table 3: Raw data for Bacticid AF (10.0 %) tested against human rotavirus

Table 4: Raw data for formaldehyde solution (0.7 %) tested against human rotavirus

Table 5: Raw data for control of efficacy for suppression of disinfectant activity (80.0 %)

Table 6: Raw data (human rotavirus) for cell sensitivity (80.0 %)

Table 7 (a+b): Summary of results with Bacticid AF and human rotavirus

Legend to the Figures

Figure 1: Virus-inactivating properties of Bacticid AF (80.0 %)

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

Table 1: Raw data for Bacticid AF (80.0 %) tested against human rotavirus at 20 °C (quantal test; 8 wells) (#5776)

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	0004 0020	0020 0000	0000 0000	0000 0000	0000 0000	0000 0000	0000 0000	n.d.	n.d.
			0.5	0030 3000	0000 0000	0000 0000	0000 0000	0000 0000	0000 0000	0000 0000	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.	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	4434 2343	0000 2000	0000 0000	0000 0000
			60	4444 4444	4444 4444	4444 4444	4444 4444	4444 4444	4430 0344	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 Bacticid AF (50.0 %) tested against human rotavirus at 20 °C (quantal test; 8 wells) (#5776)

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.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.
			0.5	4444 4444	4444 4444	4444 4444	4444 4444	4323 4404	4000 3320	0030 0000	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.	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	4434 2343	0000 2000	0000 0000	0000 0000
			60	4444 4444	4444 4444	4444 4444	4444 4444	4444 4444	4430 0344	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 Bacticid AF (10.0 %) tested against human rotavirus at 20 °C (quantal test; 8 wells) (#5776)

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	4444 4444	4444 4444	4444 4444	4444 4444	4444 4444	0330 3334	0000 0000	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	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	4434 2343	0000 2000	0000 0000	0000 0000
			60	4444 4444	4444 4444	4444 4444	4444 4444	4444 4444	4430 0344	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 formaldehyde solution (0.7 %) tested against human rotavirus at 20 °C (quantal test; 8 wells) (#5776)

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	4433 3333	4032 0234	0000 0003	0000 0000	n.d.
			15	tttt tttt	tttt tttt	4444 4444	4444 4444	4420 0322	3000 0304	0000 0000	0000 0000	n.d.
			30	tttt tttt	tttt tttt	4444 4444	4303 3323	3302 2020	3002 0000	0000 0000	0000 0000	n.d.
			60	tttt tttt	tttt tttt	3222 3233	0000 0030	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	4042 0303	0023 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 for control of efficacy for suppression of disinfectant's activity (80.0 %) (#5776)

Product	Interfering substance	dilutions (log_{10})								
		1	2	3	4	5	6	7	8	9
test product	dirty conditions	4444 4444	4444 4444	4444 4444	4444 4444	4444 4444	4324 4240	0000 0400	0000 0000	n.d.
corresponding virus control	dirty conditions	4444 4444	4444 4444	4444 4444	4444 4444	4444 4444	4430 0344	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 6: Raw data (human rotavirus) for cell sensitivity (80.0 %) (#5776)

Product	Dilution	Dilutions (log_{10})								
		1	2	3	4	5	6	7	8	9
PBS	-	4444 4444	4444 4444	4444 4444	4444 4444	4444 4444	4344 4444	0000 0000	0000 0000	n.d.
test product	1:10	4444 4444	4444 4444	4444 4444	4444 4444	4444 4444	0004 0444	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 7a: Summary of results with Bacticid AF 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	80.0 %	dirty conditions	1.50	≤1.88±0.41	≤1.75±0.33	n.d.	n.d.	n.d.	0.25 (RF ≥ 5.38±0.53)
test product	50.0 %	dirty conditions	1.50	n.d.	7.00±0.52	n.d.	n.d.	n.d.	> 0.5 (RF = 0.25±0.61)
test product	10.0 %	dirty conditions	1.50	n.d.	7.25±0.33	n.d.	n.d.	n.d.	> 0.5 (RF = 0.00±0.46)

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

Table 7b: Summary of results with Bacticid AF 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	6.63±0.49	6.25±0.55	4.63±0.25	> 60 (RF = 2.75±0.55)
virus control	n.a.	PBS	n.a.	n.d.	n.d.	n.d.	n.d.	7.38±0.49	n.a.
virus control	n.a.	dirty conditions	n.a.	7.63±0.25	n.d.	n.d.	n.d.	7.25±0.33	n.a.
suppression control	80.0 %	dirty conditions	1.50	n.d.	n.d.	n.d.	7.50±0.35	n.d.	n.a.
sens.control PBS	n.a.	n.a.	n.a.	n.d.	n.d.	n.d.	n.d.	7.50±0.00	n.a.
sens. control test product	80.0 % → 1:10	n.a.	n.a.	n.d.	n.d.	n.d.	n.d.	7.00±0.38	n.a.

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

Figure 1: Virus-inactivating properties of Bacticid AF (80.0 %)

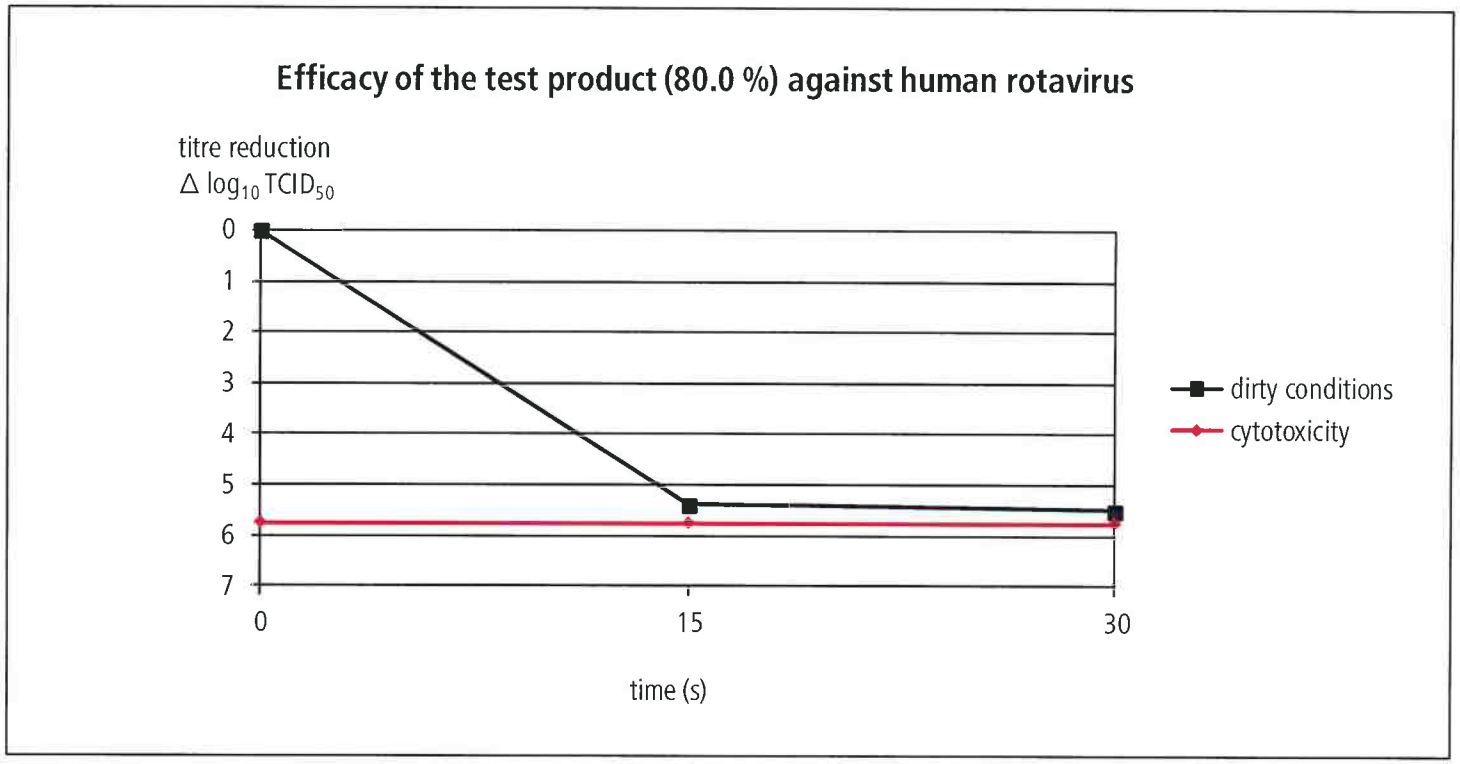
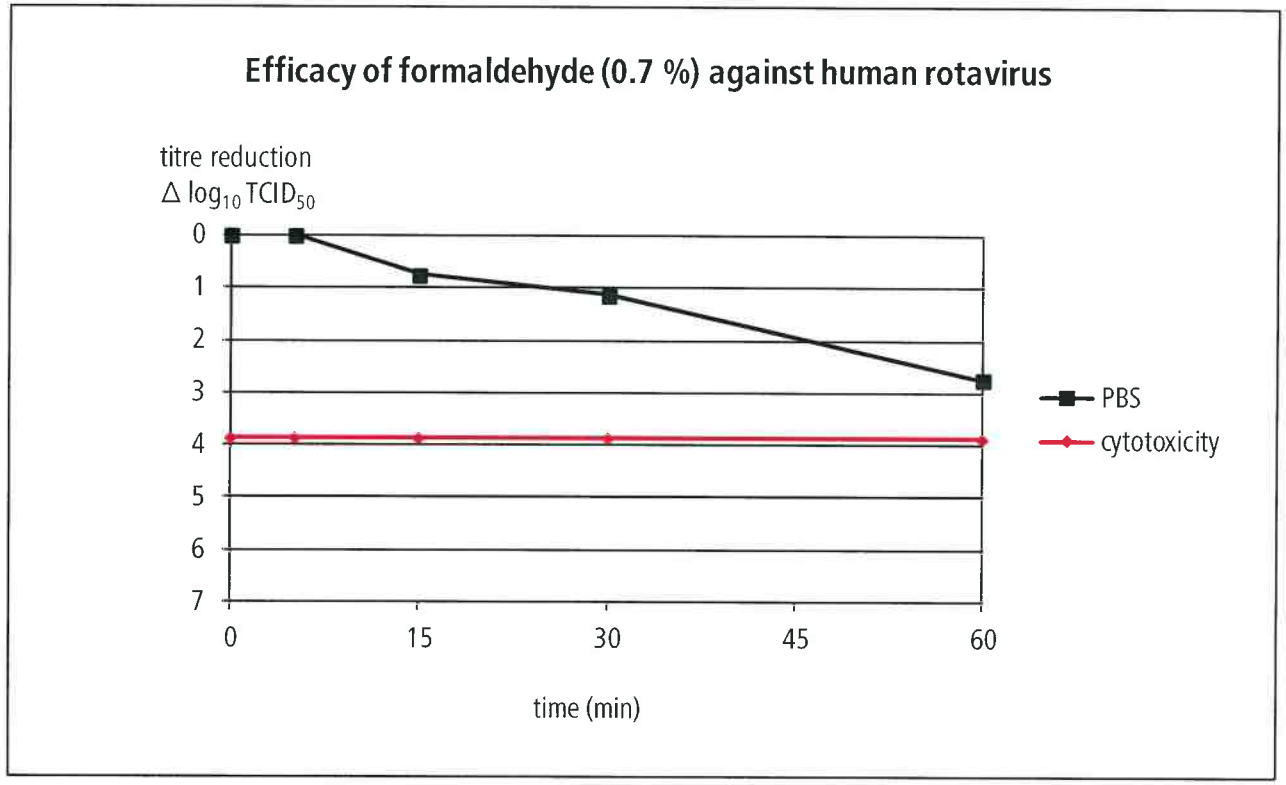


Figure 2: Virus-inactivating properties of formaldehyde (0.7 %)



Nordic Tersus
Laboratory

Test report No. sd0118

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

Name of the product:	Bacticid AF
Batch number:	197101017
Order number:	17028
Manufacturer:	Chemi-Pharm Ltd.
Client, representative:	Chemi-Pharm Ltd., Põllu 132, Tallinn, 10917, ESTONIA Maris Millner, +372-51-77-090
Date of delivery:	15.12.2017
Test material conditions:	No specific features, sample in the manufacturers tare
Storage conditions:	In room temperature, dark
Active substance – conc.:	Ethyl alcohol 57.0% wt, isopropyl alcohol 6.0% wt
Appearance of the product:	Transparent liquid
Test concentration:	Ready to use
Contact time:	30 sec
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:	23.01.2018 – 25.01.2018
Results:	look appendix 1-2

.....

ıa Kaare, MSc

Head of laboratory, microbiologist

Date of test report: 26.01.2018

Nordic Tersus Laboratory OÜ
Harjumaa, Tallinn, Serva tn 44a, 11618
Registrikood: 12561442
Tel: +372 503 0197
E-mail: info@ntl.ee

EESTI AKREDITEERIMISKESKUS
EAK
ESTONIAN ACCREDITATION CENTRE
EN ISO/IEC 17025
L263

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: 23.01.2018 – 25.01.2018.
Responsible person: Diana Kaare

Validation and controls

Dirty conditions

Validation suspension N_{vo}			Experimental conditions control (A)			Filtration control (B)			Method validation (C)		
V_{C1}	45	$\bar{x}$ = 43.5	V_{C1}	38	$\bar{x}$ = 35	V_{C1}	41	$\bar{x}$ = 37	V_{C1}	39	$\bar{x}$ = 41.5
V_{C2}	42		V_{C2}	32		V_{C2}	33		V_{C2}	44	
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}	$\bar{x}_{wm}$ = 1.71 x 10^8; log$N$ = 8.23
N and N_0	10^{-6}	172	164	N_0 = N/100; logN_0 = 6.23
	10^{-7}	19	22	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	>4.08	30 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)

Interpretation:

The ready to use product for surface disinfection **BACTICID AF** (batch no. 197101017) was tested according to the test method EVS-EN 13624:2013. The test was performed at 20 °C ± 1 °C, under dirty conditions with the contact time of 30 s. The membrane filtration method was used for testing the produts effectiveness against the reference strain: *Candida albicans* ATCC 10231. Under dirty conditions the tested product was effective against the reference strain within 30 s of contact time.

Conclusion:

The surviving count of the reference strain showed at least 4 lg reduction meaning that **under dirty conditions the ready to use product BACTICID AF has a yeasticidal effect in case of surface disinfection within 30 s.**

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a Kaare, MSc

Head of laboratory, microbiologist

Nordic Tersus
Laboratory

Test report No. 24021sd

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

Name of the product*:	**BACTICID AF**
Batch number*:	197271120
Order number:	20037
Manufacturer*:	Chemi-Pharm Ltd.
Client, representative*:	Chemi-Pharm Ltd., Tänassilma tee 11, Tänassilma küla, Saku vald, 76406, ESTONIA, Maris Millner, +372 5177090
Date of delivery:	10.12.2020
Test material conditions:	No specific features, sample in the manufacturers tare
Storage conditions:	At room temperature, darkness
Active substance – conc.*:	Ethyl alcohol 57.0% wt, isopropyl alcohol 6.0% wt
Appearance of the product:	Transparent liquid
Test concentration:	80.0%, 50.0%, 25.0%
Contact time:	2 min
Interfering substance:	3g/l bovine albumin + 3 ml/l sheep blood erythrocytes (dirty conditions)
Neutralizer:	-
Rinsing liquid:	Tryptone 1 g/l + NaCl 9 g/l
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:	17.02.2021 – 19.02.2021
Results:	Look appendix 1
Interpretation and conclusion:	Look appendix 2

ACCREDITED LABORATORY NORDIC TERSUS LABORATORY

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eht
Chief specialist
Date of issue: 26.04.2021

** - Data provided by the customer*

Nordic Tersus Laboratory OÜ
Harjumaa, Tallinn, Serva tn 44a, 11618
Registrikood: 12561442
Tel: +372 503 0197
E-mail: info@ntl.ee

EESTI AKREDITEERIMISKESKUS EAK ESTONIAN ACCREDITATION CENTRE
EN ISO/IEC 17025
L263

TEST RESULTS (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 ± 1° C
Interfering substance: 3g/l bovine albumin + 3 ml/l sheep blood erythrocytes
Nordic Tersus Laboratory LLC.;
Date of test: 17.02.2021
Responsible person: Melissa Ingela Bramanis, Allar Laaneleht

Validation and controls

Dirty conditions

Validation suspension N_{vo}			Experimental conditions (A)			Filtration control (B)			Method validation (C)		
V_{C1}	V_{C2}	$\bar{x}$	V_{C1}	V_{C2}	$\bar{x}$	V_{C1}	V_{C2}	$\bar{x}$	V_{C1}	V_{C2}	$\bar{x}$
43	40	41.5	36	30	33	39	42	40.5	45	49	47
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}	$\bar{x}_{wm} = 2.64 \times 10^7$; log$N$ = 7.42
N and N_0	10^{-5}	**247**	**275**	$N_0 = N/10$; logN_0 = 6.42
	10^{-6}	**28**	**28**	6.17≤ logN_0≤6.70; yesX; no □

Experimental results

Concentration of the product %	Dilution step	V_{C1}	V_{C2}	Na (=$\bar{x}$*10)	lgNa	lg$R$	Contact time	Conditions
80.0%	-	19	21	200	2.30	4.12	2 min	Dirty
50.0%	-	>55	>55	>550	>2.74	<3.54	2 min	Dirty
25.0%	-	>55	>55	>550	>2.74	<3.54	2 min	Dirty

Explanations:

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