

B. Braun Medical AG
Seesatz 17
CH - 6204 Sempach

Hamburg, 21 September 2017

Expert opinion

Mycobactericidal Activity of **Hexaquart XL** in the quantitative suspension test according to DIN EN 14348:2005 (Phase 2, Step 1)

The disinfectant **Hexaquart XL** was tested and evaluated according to DIN EN 14348:2005 "Chemical Disinfectants and Antiseptics - Quantitative Suspension Test for the Evaluation of Mycobactericidal Activity of Chemical Disinfectants Used in the Medical Area Including Instrument Disinfectants – Test Methods and Requirements (Phase 2, Step 1)".

According to the test report no. L17/0050.1 dated 21/09/2017 of Dr. Brill + Partner GmbH the preparation showed mycobactericidal activity under dirty conditions (3.0 g/L bovine albumin + 3.0 mL/L sheep erythrocytes) at 20°C ± 1°C.

Hexaquart XL complies with the requirements of DIN EN 14348:2005 (phase 2, step 1) with the following concentration-time relationship:

Mycobactericidal:	dirty conditions	2.0 %	15 minutes
		1.0 %	60 minutes



Dr. Florian H. H. Brill

Test report no L17/0050.1

Quantitative suspension test for the evaluation of Mycobactericidal Activity of **Hexaquart XL** in the Medical Area (DIN EN 14348:2005; Phase 2, Step 1*)

In accordance with your order, we tested the preparation **Hexaquart XL** for its activity in the quantitative suspension test according to DIN EN 14348:2005* under dirty conditions.

1 General Information and Material

1.1 Client

Client: B. Braun Medical AG, Dr Michael Kurz, Seesatz 17, CH - 6204 Sempach
Date of order: 29/05/2017
Confirmation no.: 201395

1.2 Identification of Test Laboratory

Location: Dr. Brill + Partner GmbH · Institute for Hygiene and Microbiology,
Stiegstück 34, DE-22339 Hamburg, Germany
Study manager: Dipl.-Ing. Dr. rer. nat. Andreas Kampe
Scientific assistant: Dipl.-Biol. Dr. rer. nat. Jan-Hendrik Klock
Laboratory technicians: Carmela Jänicke

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1.4 Identification of Sample

Name of product: **Hexaquart XL**
Batch no.: R-40_25102016
Manufacturer: B. Braun Medical AG, CH - 6204 Sempach

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Date of delivery:	26/01/2017
Storage conditions:	room temperature and darkness
Appearance of product:	red clear liquid
Odour:	characteristic
Recommended diluent:	Tap water
Diluent used:	water of standardised hardness (WSH, pH 7.0)
pH value, concentrate:	11.8
pH value, 2.0 % (measured in diluent):	10.5
pH value, 1.0 % (measured in diluent):	10.3
pH value, 0.5 % (measured in diluent):	10.1
pH value, 0.1 % (measured in diluent):	9.2
Active agents (Manufacturer's data):	9.9 % N,N-Bis(3-Aminopropyl)dodecylamin 6.6 % DDAC

1.5 Test Conditions

Test period:	24/04/ - 15/05/2017
Lab task no.:	L17/0050.2
Product test concentrations:	0.5 + 1.0 + 2.0 %
Exposure time:	15 + 30 + 60 minutes
Test temperature:	20°C ± 1°C
Incubation temperature:	36°C ± 1°C
Organic load:	dirty conditions (3.0 g/L bovine albumin + 3.0 mL/L sheep erythrocytes)
Neutraliser:	filter conditioning pre-rinse 100 ml 1 g/L Tween 80 + 0.5 g/L Sodium oleate; rinse solution 200 ml 0.5 g/L Tween 80 + 0.5 g/L Tween + 0.5 g/L lecithine followed by 200 ml bidest (Membrane filtration)
Test organisms:	<i>Mycobacterium terrae</i> ATCC 15755 <i>Mycobacterium avium</i> ATCC 15769

2 Methods

The tests were carried out according to DIN EN 14348:2005 "Chemical Disinfectants and Antiseptics - Quantitative Suspension Test for the Evaluation of Mycobactericidal Activity of Chemical Disinfectants Used in the Medical Area Including Instrument Disinfectants – Test Methods and Requirements (Phase 2, Step 1)".

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Because of inactivation problems with dilution neutralisation method with products containing amines as actives a modified membrane filtration method was chosen using filter membranes suitable for growth of mycobacteria (Merck-Millipore EZ-PAK, EZHAG474). Filters were preconditioned by pre-rinse with 100 ml rinse of 1 g/L Tween 80 and 0.5 g/L Sodium oleate.

After contact time 1 ml of product test mixture in 9 ml rinsing fluid (ml 0.5 g/L Tween 80 + 0.5 g/L Tween + 0.5 g/L lecithine) in a ST-20 Homogenisation Tube with glass beads. The mixture was then homogenised for 2 min at 400 UPm by an Ultra Turrax Tube Drive (IKA).

Then membrane filtration was performed by transferring samples into 50 ml rinsing fluid. After filtration the filter was rinsed with 200 ml rinsing fluid and a final rinse with 200 ml bidistilled water. Testing was done with two dilutions 10^0 and 10^{-2} in two parallels. Finally membranes were transferred to 7H10 agar plates and incubated.

3 Results

The test results based on DIN EN 14348: 2005 are summarised in tables 1 and 2.

The test bacteria were sufficiently (RF >4) inactivated with the following concentration-time relationship:

Mycobactericidal:	dirty conditions	2.0 %	15 minutes
		1.0 %	60 minutes

Hamburg, 21/09/2017

Dipl.-Ing. Dr. rer. nat. Andreas Kampe
Study Manager

Dipl.-Biol. Dr. rer. nat. Jan-Hendrik Klock
Quality control

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Table 1.1: Validation, Controls and Evaluation

Product name: **Hexaquart XL** Batch: R-40_25102016
Test organism: *Mycobacterium terrae* Temperature: 20°C ± 1°C
Organic load: dirty conditions Neutraliser: Membrane filtration
Contact time: **15 minutes** Lab task no.: L17/0050.2

Suspension for validation (N _{v0})			Control of test conditions (A)			Control of neutraliser (B)			Validation procedure (C) Product conc.: 2,00 %			
	microbial count	\bar{x}		microbial count	\bar{x}		microbial count	\bar{x}		microbial count	\bar{x}	
V _{c1}	8	22	32,5	V _{c1}	35	40	V _{c1}	31	33,5	V _{c1}	24	27,5
V _{c2}	13	22		V _{c2}	45		V _{c2}	36		V _{c2}	31	
30 ≤ \bar{x} of N _{v0} ≤ 160			Yes	\bar{x} of A(15') is ≥ 0,5 x \bar{x} of N _{v0} ?		Yes	\bar{x} of B is ≥ 0,5 x \bar{x} of N _{v0} ?		Yes	\bar{x} of C(15') is ≥ 0,5 x \bar{x} of N _{v0} ?		Yes

Prüfsuspension (N und N ₀)	N	microbial counts of plates				V _{c1}	V _{c2}	$\bar{x}_{wm} /$ lg N	N ₀ =N/10; lg N ₀	8,17 ≤ N ₀ ≤ 8,70 ?
	1,00E-07	144	129	131	114	273	245	2,59E+09	8,41	Yes
	1,00E-08	10	7	11	9	17	20	9,41		

Product concentration [%]	N	microbial counts of plates				V _{c1}	V _{c2}	N _a = \bar{x} x 10	lg N _a	lg R
										(lg N ₀ =8,41)
0,5	1,00E+00	>330		>330		>330	>330	>6,60E+06	> 6,52	≤ 1,89
	1,00E-01									
	1,00E-02	>330		>330		>330	>330			
	1,00E-03									
1,0	1,00E+00	>330		>330		>330	>330	5,75E+04	4,76	3,65
	1,00E-01									
	1,00E-02	55		60		55	60			
	1,00E-03									
2,0	1,00E+00	77		91		77	91	8,40E+02	2,92	5,49
	1,00E-01									
	1,00E-02	1		0		<14	<14			
	1,00E-03									

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Table 1.2: Validation, Controls and Evaluation

Product name: **Hexaquart XL** Batch: R-40_25102016
Test organism: *Mycobacterium terrae* Temperature: 20°C ± 1°C
Organic load: dirty conditions Neutraliser: Membrane filtration
Contact time: **30 minutes** Lab task no.: L17/0050.2

Suspension for validation (N _{vo})				Control of test conditions (A)				Control of neutraliser (B)				Validation procedure (C) Product conc.: 2,00 %							
	microbial count			∑		microbial count			∑		microbial count			∑					
V _{c1}	8	22	32,5	V _{c1}	44		39	V _{c1}	31		33,5	V _{c1}	33		30,5				
V _{c2}	13	22		V _{c2}	34			V _{c2}	36			V _{c2}	28						
30 ≤ ∑ of N _{vo} ≤ 160				Yes	∑ of A(30') is ≥ 0,5 x ∑ of N _{vo} ?				Yes	∑ of B is ≥ 0,5 x ∑ of N _{vo} ?				Yes	∑ of C(30') is ≥ 0,5 x ∑ of N _{vo} ?				Yes

Prüfsuspension (N und N ₀)		N		microbial counts of plates				V _{c1}	V _{c2}	∑ _{wm} / lg N		N ₀ =N/10; lg N ₀		8,17 ≤ N ₀ ≤ 8,70 ?	
		1,00E-07		144	129	131	114	273	245	2,59E+09		8,41		Yes	
		1,00E-08		10	7	11	9	17	20	9,41					

Product concentration [%]		N		microbial counts of plates				V _{c1}	V _{c2}	N _a = ∑ x 10		lg N _a		lg R (lg N ₀ =8,41)	
0,5		1,00E+00	>330		>330		>330	>330	>6,60E+06	> 6,52	≤ 1,89				
		1,00E-01													
		1,00E-02	>330		>330		>330	>330							
		1,00E-03													
1,0		1,00E+00	>330		>330		>330	>330	4,75E+04	4,68	3,74				
		1,00E-01													
		1,00E-02	54		41		54	41							
		1,00E-03													
2,0		1,00E+00	24		31		24	31	2,75E+02	2,44	5,97				
		1,00E-01													
		1,00E-02	0		0		<14	<14							
		1,00E-03													

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Table 1.3: Validation, Controls and Evaluation

Product name: **Hexaquart XL** Batch: R-40_25102016
Test organism: *Mycobacterium terrae* Temperature: 20°C ± 1°C
Organic load: dirty conditions Neutraliser: Membrane filtration
Contact time: **60 minutes** Lab task no.: L17/0050.2

Suspension for validation (N _{v0})			Control of test conditions (A)			Control of neutraliser (B)			Validation procedure (C)		
microbial count			microbial count			microbial count			microbial count		
V _{c1}	8	22	V _{c1}	22		V _{c1}	31		V _{c1}	43	
V _{c2}	13	22	V _{c2}	33		V _{c2}	36		V _{c2}	27	
30 ≤ \bar{x} of N _{v0} ≤ 160			\bar{x} of A(60') is ≥ 0,5 x \bar{x} of N _{v0} ?			\bar{x} of B is ≥ 0,5 x \bar{x} of N _{v0} ?			\bar{x} of C(60') is ≥ 0,5 x \bar{x} of N _{v0} ?		
Yes			Yes			Yes			Yes		

Prüfsuspension (N und N ₀)	N	microbial counts of plates				V _{c1}	V _{c2}	$\bar{x}_{wm} / \lg N$	N ₀ =N/10; lg N ₀	8,17 ≤ N ₀ ≤ 8,70 ?
	1,00E-07	144	129	131	114	273	245	2,59E+09		
	1,00E-08	10	7	11	9	17	20	9,41	8,41	Yes

Product concentration [%]	N	microbial counts of plates				V _{c1}	V _{c2}	N _a = \bar{x} x 10	lg N _a	lg R (lg N ₀ = 8,41)
0,5	1,00E+00	>330		>330		>330	>330	1,25E+05	5,10	3,32
	1,00E-01									
	1,00E-02	120		130		120	130			
	1,00E-03									
1,0	1,00E+00	100		110		100	110	1,05E+03	3,02	5,39
	1,00E-01									
	1,00E-02	0		0		<14	<14			
	1,00E-03									
2,0	1,00E+00	0		4		<14	<14	<1,40E+02	<2,15	≥ 6,27
	1,00E-01									
	1,00E-02	0		0		<14	<14			
	1,00E-03									

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Table 2.1: Validation, Controls and Evaluation

Product name: **Hexaquart XL** Batch: R-40_25102016
Test organism: *Mycobacterium avium* Temperature: 20°C ± 1°C
Organic load: dirty conditions Neutraliser: Membrane filtration
Contact time: **15 minutes** Lab task no.: L17/0050.2

Suspension for validation (N _{v0})				Control of test conditions (A)				Control of neutraliser (B)				Validation procedure (C) Product conc.: 2,00 %			
	microbial count		\bar{x}		microbial count		\bar{x}		microbial count		\bar{x}		microbial count		\bar{x}
V _{c1}	35	27	62,5	V _{c1}	64		55	V _{c1}	60		56	V _{c1}	52		57,5
V _{c2}	34	29		V _{c2}	46			V _{c2}	52			V _{c2}	63		
30 ≤ \bar{x} of N _{v0} ≤ 160			Yes	\bar{x} of A(15') is ≥ 0,5 x \bar{x} of N _{v0} ?			Yes	\bar{x} of B is ≥ 0,5 x \bar{x} of N _{v0} ?			Yes	\bar{x} of C(15') is ≥ 0,5 x \bar{x} of N _{v0} ?			Yes

Prüfsuspension (N und N ₀)	N	microbial counts of plates				V _{c1}	V _{c2}	\bar{x}_{wm} / lg N	N ₀ =N/10; lg N ₀	8,17 ≤ N ₀ ≤ 8,70 ?
	1,00E-07	>330	>330	>330	>330	>660	>660	4,75E+09	8,68	Yes
	1,00E-08	17	24	31	23	41	54	9,68		

Product concentration [%]	N	microbial counts of plates				V _{c1}	V _{c2}	N _a = \bar{x} x 10	lg N _a	lg R (lg N ₀ =8,68)
0,5	1,00E+00	>330		>330		>330	>330	>6,60E+06	> 6,52	≤ 2,16
	1,00E-01									
	1,00E-02	>330		>330		>330	>330			
	1,00E-03									
1,0	1,00E+00	>330		>330		>330	>330	4,10E+04	4,61	4,06
	1,00E-01									
	1,00E-02	35		47		35	47			
	1,00E-03									
2,0	1,00E+00	>330		>330		>330	>330	8,95E+03	3,95	4,72
	1,00E-01									
	1,00E-02	0		0		<14	<14			
	1,00E-03									



Table 2.2: Validation, Controls and Evaluation

Product name: **Hexaquart XL** Batch: R-40_25102016
Test organism: *Mycobacterium avium* Temperature: 20°C ± 1°C
Organic load: dirty conditions Neutraliser: Membrane filtration
Contact time: **30 minutes** Lab task no.: L17/0050.2

Suspension for validation (N _{v0})				Control of test conditions (A)				Control of neutraliser (B)				Validation procedure (C) Product conc.: 2,00 %			
	microbial count		̄		microbial count		̄		microbial count		̄		microbial count		̄
V _{c1}	35	27	62,5	V _{c1}	55		64	V _{c1}	60		56	V _{c1}	76		70
V _{c2}	34	29		V _{c2}	73			V _{c2}	52			V _{c2}	64		
30 ≤ ̄ of N _{v0} ≤ 160			Yes	̄ of A(30') is ≥ 0,5 x ̄ of N _{v0} ?			Yes	̄ of B is ≥ 0,5 x ̄ of N _{v0} ?			Yes	̄ of C(30') is ≥ 0,5 x ̄ of N _{v0} ?			Yes

Prüfsuspension (N und N ₀)	N	microbial counts of plates				V _{c1}	V _{c2}	$\bar{x}_{wm} / \lg N$	N ₀ =N/10; lg N ₀	8,17 ≤ N ₀ ≤ 8,70 ?
	1,00E-07	>330	>330	>330	>330	>660	>660	4,75E+09	8,68	Yes
	1,00E-08	17	24	31	23	41	54	9,68		

Product concentration [%]	N	microbial counts of plates				V _{c1}	V _{c2}	N _a = \bar{x} x 10	lg N _a	lg R (lg N ₀ = 8,68)
	1,00E+00	>330		>330		>330	>330	>6,60E+06	> 6,52	≤ 2,16
0,5	1,00E-01									
	1,00E-02	>330		>330		>330	>330			
	1,00E-03									
1,0	1,00E+00	>330		>330		>330	>330	1,95E+04	4,29	4,39
	1,00E-01									
	1,00E-02	25		14		25	14			
	1,00E-03									
2,0	1,00E+00	43		67		43	67	5,50E+02	2,74	5,94
	1,00E-01									
	1,00E-02	0		0		<14	<14			
	1,00E-03									



Table 2.3: Validation, Controls and Evaluation

Product name: **Hexaquart XL** Batch: R-40_25102016
Test organism: *Mycobacterium avium* Temperature: 20°C ± 1°C
Organic load: dirty conditions Neutraliser: Membrane filtration
Contact time: **60 minutes** Lab task no.: L17/0050.2

Suspension for validation (N ₀)				Control of test conditions (A)				Control of neutraliser (B)				Validation procedure (C) Product conc.: 2,00 %			
	microbial count		\bar{x}		microbial count		\bar{x}		microbial count		\bar{x}		microbial count		\bar{x}
V _{c1}	35	27	62,5	V _{c1}	74		67,5	V _{c1}	60		56	V _{c1}	51		60,5
V _{c2}	34	29		V _{c2}	61			V _{c2}	52			V _{c2}	70		
30 ≤ \bar{x} of N ₀ ≤ 160			Yes	\bar{x} of A(60') is ≥ 0,5 x \bar{x} of N ₀ ?			Yes	\bar{x} of B is ≥ 0,5 x \bar{x} of N ₀ ?			Yes	\bar{x} of C(60') is ≥ 0,5 x \bar{x} of N ₀ ?			Yes

Prüfsuspension (N und N ₀)	N	microbial counts of plates				V _{c1}	V _{c2}	$\bar{x}_{wm} /$ lg N	N ₀ =N/10; lg N ₀	8,17 ≤ N ₀ ≤ 8,70 ?
	1,00E-07	>330	>330	>330	>330	>660	>660	4,75E+09	8,68	Yes
	1,00E-08	17	24	31	23	41	54	9,68		
Product concentration [%]	N	microbial counts of plates				V _{c1}	V _{c2}	N _a = \bar{x} x 10	lg N _a	lg R
										(lg N ₀ = 8,68)
0,5	1,00E+00	>330		>330		>330	>330	2,75E+04	4,44	4,24
	1,00E-01									
	1,00E-02	31		24		31	24			
	1,00E-03									
1,0	1,00E+00	90		66		90	66	7,80E+02	2,89	5,78
	1,00E-01									
	1,00E-02	1		4		<14	<14			
	1,00E-03									
2,0	1,00E+00	5		3		<14	<14	<1,40E+02	<2,15	≥ 6,53
	1,00E-01									
	1,00E-02	0		0		<14	<14			
	1,00E-03									

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4 List of Abbreviations

A	=	control of test conditions
B	=	control of neutraliser
C	=	validation of method at highest product concentration
N	=	test suspension
N _{vo}	=	suspension for validation
n.t.	=	not tested
N ₀	=	microbial count of test suspension N / 10 (microbial count at time index 0)
R	=	germ reduction in log ₁₀ -steps
V _c	=	viable microbial count per ml
\bar{x}	=	weighted mean of N

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