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Res Pharma Industriale Srl

STUDY REPORT

2020-7887/20 23 00726

Disinfettante mani

SUSPENSION TEST
ACCORDING TO EN 1276:2019
(Phase 2 step 1)

Chemical disinfectants and antiseptics
Quantitative suspension test for the evaluation of bactericidal activity of chemical disinfectants and antiseptics used in food, industrial, domestic and institutional areas – Test method and requirements (phase 2, step 1)

SEPTEMBER 2020

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SUSPENSION TEST ACCORDING TO EN 1276:2019

Chemical disinfectants and antiseptics – Quantitative suspension test for the evaluation of bactericidal activity of chemical disinfectants and antiseptics used in food, industrial, domestic and institutional areas - Test method and requirements (phase 2, step 1)

TEST PRODUCT IDENTIFICATION

PRODUCT NAME	:	Disinfettante mani
SUBSTANCES AND THEIR CONCENTRATIONS	:	Ethanol 70.4% p/p
APPEARANCE OF THE PRODUCT	:	Gel
STORAGE CONDITIONS	:	Room Temperature, Darkness
LOT	:	20204408
METHOD	:	EN 1276:2019
CONTACT TIME	:	1 minute
CONCENTRATION	:	Undiluted (80%), 50%, 1%.
STUDY SPONSOR	:	INT.E.G.RA. srl
PRODUCT SUPPLIER	:	Res Pharma Industriale srl
PRODUCT MANUFACTURER	:	Res Pharma Industriale srl
RECEIPT DATE	:	05/08/2020
STUDY PERIOD	:	18/09/2020-21/09/2020
LAB ID	:	2020-7887/20 23 00726

SCOPE

This document specifies a test method and the minimum requirements for bactericidal activity of chemical disinfectant and antiseptic products that form a homogeneous, physically stable preparation when diluted with hard water or - in the case of ready-to-use products - with water.
Products can only be tested at a concentration of 80 % or less, as some dilution is always produced by adding the test organisms and interfering substance.
This document applies to products that are used in food, industrial, domestic and institutional areas excluding areas and situations where disinfection is medically indicated and excluding products used on living tissues except those for hand hygiene in the above considered areas

PRINCIPLE

A sample of the product as delivered and/or diluted with hard water (or water for ready-to-use products with the exception of handwash products whose first dilution is done in hard water) is added to a test suspension of bacteria in a solution of an interfering substance. The mixture is maintained at the chosen test temperature for the adopted contact time. At the end of this contact time, an aliquot is taken, and the bactericidal and/or the bacteriostatic activity in this portion is immediately neutralized or suppressed by a validated method. The method of choice is dilution-neutralization. If a suitable neutralizer cannot be found, membrane filtration is used. The numbers of surviving bacteria in each sample are determined and the reduction is calculated.
The test is performed using Pseudomonas aeruginosa, Escherichia coli, Staphylococcus aureus and Enterococcus hirae as test organisms. For temperatures ≥ 40 °C only Enterococcus faecium shall be used. For testing of hand hygiene products, Pseudomonas aeruginosa, Escherichia coli K12, Staphylococcus aureus and Enterococcus hirae are used as test organisms.

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TEST CONDITIONS

1. Product type: Hygienic handrub
2. The following procedure was performed in water bath at 20 °C
3. The test product was tested at 1 minute contact time
4. Interfering substance: A final concentration of 0.3g/L bovine albumin was used for testing (clean conditions).
5. Neutralization Method used: Dilution neutralization.
6. Neutralizer used: LPT Dilution Broth containing polysorbate 80.
7. According to EN 1276, products shall be tested at a minimum of three different concentrations to include one concentration in the active range and one concentration in the non-active range. In this case the product was tested: Undiluted (80%), 50%, 1%.

TEST ORGANISMS

Pseudomonas aeruginosa	NCIMB 10421
Staphylococcus aureus	ATCC 6538
Escherichia coli K12	NCTC 10538
Enterococcus hirae	NCIMB 8192

BACTERICIDAL ACTIVITY FOR HAND HYGIENE

The bactericidal concentration for hand hygiene is the concentration of the tested product for which at least a 5 lg reduction for hygienic handrub and 3 lg reduction for hygienic handwash (at 50 % in test concentration or less) is demonstrated in a valid test under the conditions defined by this standard when the test organisms are *Escherichia coli K12, Pseudomonas aeruginosa, Staphylococcus aureus and Enterococcus hirae*.

ASSAY ACCEPTANCE CRITERIA

1. Test Suspension (N) is between 1.5 to 5.0 X 10^8 CFU per mL ($8.17 \leq \log N \leq 8.70$)
2. No (N/10) is between 1.5 to 5.0 X 10^7 CFU per mL ($7.17 \leq \log No \leq 7.70$)
3. Validation Suspension=Nv is between 3.0 x 10^2 and 1.6 x 10^3.
4. Nvo (Nv/10) is between 30 and 160
5. Na is the number of survivors (cells) per ml in the test mixture at the end of contact time.
6. R (log reduction) = No - Na
7. Average recovery values for the experimental conditions control (A) were equal to or greater than 0.5 times the Validation Suspension (Nvo)
8. Average recovery values for the Neutralizer control (B) were equal to or greater than 0.5 times the Validation Suspension (Nvo)
9. Average recovery values for the Method Validation control (C) were equal to or greater than 0.5 times the Validation Suspension (Nvo)
10. Control of weighted mean counts. Quotient is not lower than 5 and not higher than 15

ARCHIVING

The laboratory book which contains all the information (raw data and results) regarding the study and the study reports are kept in the laboratory archives for 5 years.

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TEST RESULTS FOR *Pseudomonas aeruginosa* (BACTERICIDAL SUSPENSION TEST)

Test suspension

Test - suspension (N and No)				
N	Vc1	Vc2	x mean	2.03E+08
10^{-6}	194	213		
10^{-7}	21	19	log N	8.31
			No (N/10)	2.03E+07
			log No	7.31
			7,17 < = logNo < = 7,70	**Yes**

Validation and controls

Validation suspension (Nvo)			Experimental conditions (A)			Neutralizer control (B)			Method validation (C) Undiluted Product conc.: (80%)		
VC 1	40	**x mean**	VC 1	44	**x mean**	VC 1	42	**x mean**	VC 1	45	**x mean**
VC 2	39	**39.5**	VC 2	39	**41.5**	VC 2	46	**44**	VC 2	43	**44**
30<x mean of Nvo < 160? **Yes**			x mean of A is > 0,5*x mean of Nvo? **Yes**			x mean of B is > 0,5*x mean of Nvo or Nvo/1000? **Yes**			x mean of C is > 0,5*x mean of Nvo? **Yes**		

Test Results

Product concentration (%)	Contact time	Dilution step	Vc 1	Vc 2	Average of Vc1 and Vc2	Na= average x10	log Na	log No	log Reduction (No-Na)	Criteria	Result
Undiluted (80%)	1 min	10^{0}	0	0	< 14	< 140	< 2.15	7.31	> 5.16	≥ 5	**PASS TEST**
		10^{-1}	0	0							
50%	1 min	10^{0}	12	14	< 14	< 140	< 2.15	7.31	> 5.16	≥ 5	**PASS TEST**
		10^{-1}	1	2							
1%	1 min	10^{0}	> 330	> 330	> 3300	> 33000	> 4.52	7.31	< 2.79	≥ 5	**FAILS TEST**
		10^{-1}	> 330	> 330							

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TEST RESULTS FOR *Staphylococcus aureus* (BACTERICIDAL SUSPENSION TEST)

Test suspension

Test - suspension (N and No)				
N	Vc1	Vc2	x mean	2.40E+08
10^{-6}	246	237		
10^{-7}	24	22	log N	8.38
			No (N/10)	2.40E+07
			log No	7.38
			7,17 < = logNo < = 7,70	**Yes**

Validation and controls

Validation suspension (Nvo)			Experimental conditions (A)			Neutralizer control (B)			Method validation (C) Undiluted Product conc.: (80%)		
VC 1	49	**x mean**	VC 1	51	**x mean**	VC 1	54	**x mean**	VC 1	50	**x mean**
VC 2	52	**50.5**	VC 2	56	**53.5**	VC 2	56	**55**	VC 2	45	**47.5**
30<x mean of Nvo < 160?			x mean of A is > 0,5*x mean of Nvo?			x mean of B is > 0,5*x mean of Nvo or Nvo/1000?			x mean of C is > 0,5*x mean of Nvo?		
Yes			**Yes**			**Yes**			**Yes**		

Test Results

Product concentration (%)	Contact time	Dilution step	Vc 1	Vc 2	Average of Vc1 and Vc2	Na= average x10	log Na	log No	log Reduction (No-Na)	Criteria	Result
Undiluted (80%)	1 min	10^{0}	0	0	< 14	< 140	< 2.15	7.38	> 5.23	≥ 5	**PASS TEST**
		10^{-1}	0	0							
50%	1 min	10^{0}	3	4	< 14	< 140	< 2.15	7.38	> 5.23	≥ 5	**PASS TEST**
		10^{-1}	0	0							
1%	1 min	10^{0}	> 330	> 330	> 3300	> 33000	> 4.52	7.38	< 2.86	≥ 5	**FAILS TEST**
		10^{-1}	> 330	> 330							

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TEST RESULTS FOR *Escherichia Coli* K12 (BACTERICIDAL SUSPENSION TEST)

Test suspension

Test - suspension (N and No)			
N	Vc1	Vc2	x mean 3.55E+08
10^{-7}	36	34	
10^{-8}	4	4	log N 8.55
			No (N/10) 3.55E+07
			log No 7.55
			7,17 < = logNo < = 7,70 **Yes**

Validation and controls

Validation suspension (Nvo)			Experimental conditions (A)			Neutralizer control (B)			Method validation (C) Undiluted Product conc.: (80%)		
VC 1	79	**x mean**	VC 1	82	**x mean**	VC 1	85	**x mean**	VC 1	91	**x mean**
VC 2	84	**81.5**	VC 2	86	**84**	VC 2	89	**87**	VC 2	82	**86.5**
30<x mean of Nvo < 160? **Yes**			x mean of A is > 0,5*x mean of Nvo? **Yes**			x mean of B is > 0,5*x mean of Nvo or Nvs/1000? **Yes**			x mean of C is > 0,5*x mean of Nvo? **Yes**		

Test Results

Product concentration (%)	Contact time	Dilution step	Vc 1	Vc 2	Average of Vc1 and Vc2	Na= average x10	log Na	log No	log Reduction (No-Na)	Criteria	Result
Undiluted (80%)	1 min	10^{0}	0	0	< 14	< 140	< 2.15	7.55	> 5.40	≥ 5	**PASS TEST**
		10^{-1}	0	0							
50%	1 min	10^{0}	16	24	19.5	195	2.29	7.55	5.26	≥ 5	**PASS TEST**
		10^{-1}	1	2							
1%	1 min	10^{0}	> 330	> 330	> 3300	> 33000	> 4.52	7.55	< 3.03	≥ 5	**FAILS TEST**
		10^{-1}	> 330	> 330							

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TEST RESULTS FOR *Enterococcus hirae* (BACTERICIDAL SUSPENSION TEST)

Test suspension

Test - suspension (N and No)				
N	Vc1	Vc2	x mean	3.36E+08
10^{-7}	31	36		
10^{-8}	4	3	log N	8.53
			No (N/10)	3.36E+07
			log No	7.53
			7,17 < = logNo < = 7,70	**Yes**

Validation and controls

Validation suspension (Nvo)			Experimental conditions (A)			Neutralizer control (B)			Method validation (C) Undiluted Product conc.: (80%)		
VC 1	72	**x mean**	VC 1	74	**x mean**	VC 1	69	**x mean**	VC 1	72	**x mean**
VC 2	68	**70**	VC 2	60	**67**	VC 2	74	**71.5**	VC 2	70	**71**
30<x mean of Nvo < 160? **Yes**			x mean of A is > 0,5*x mean of Nvo? **Yes**			x mean of B is > 0,5*x mean of Nvo or Nvo/1000? **Yes**			x mean of C is > 0,5*x mean of Nvo? **Yes**		

Test Results

Product concentration (%)	Contact time	Dilution step	Vc 1	Vc 2	Average of Vc1 and Vc2	Na= average x10	log Na	log No	log Reduction (No-Na)	Criteria	Result
Undiluted (80%)	1 min	10^{0}	0	0	< 14	< 140	< 2.15	7.53	> 5.38	≥ 5	**PASS TEST**
		10^{-1}	0	0							
50%	1 min	10^{0}	0	0	< 14	< 140	< 2.15	7.53	> 5.38	≥ 5	**PASS TEST**
		10^{-1}	0	0							
1%	1 min	10^{0}	> 330	> 330	> 3300	> 33000	> 4.52	7.53	< 3.01	≥ 5	**FAILS TEST**
		10^{-1}	> 330	> 330							

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CONCLUSION

TEST SUBSTANCE IDENTIFICATION

PRODUCT NAME	:	Disinfettante mani
SUBSTANCES AND THEIR CONCENTRATIONS	:	Ethanol 70.4% p/p
APPEARANCE OF THE PRODUCT	:	Gel
STORAGE CONDITIONS	:	Room Temperature, Darkness
LOT	:	20204408
METHOD	:	EN 1276:2019
CONTACT TIME	:	1 minute
CONCENTRATION	:	Undiluted (80%), 50%, 1%.
STUDY SPONSOR	:	INT.E.G.RA. srl
PRODUCT SUPPLIER	:	Res Pharma Industriale srl
PRODUCT MANUFACTURER	:	Res Pharma Industriale srl
RECEIPT DATE	:	05/08/2020
STUDY PERIOD	:	18/09/2020-21/09/2020
LAB ID	:	2020-7887/20 23 00726

METHODOLOGY ABSTRACT

A sample of the product as delivered and/or diluted with hard water (or water for ready to use products) is added to a test suspension of bacteria in a solution of an interfering substance. The mixture is maintained at 20 °C for 1 minute. At the end of this contact time, an aliquot is taken; the bactericidal and/or the bacteriostatic action in this portion is immediately neutralized or suppressed by a validated method. The numbers of surviving bacteria in each sample are determined and the log reduction is calculated.

RESULT

The product under test: "Disinfettante mani" demonstrated bactericidal activity for hand hygiene disinfection according to EN 1276:2019 (≥ 5 log reduction) under clean conditions at 20 ± 1 °C, when tested:

Undiluted (80%) for 1 minute contact time using as test organisms the reference strains:
Pseudomonas aeruginosa, *Staphylococcus aureus*, *Escherichia coli K12* and *Enterococcus hirae*.

For the QACS Ltd Laboratory,

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STUDY SUMMARY / ABSTRACT

SUSPENSION TEST ACCORDING TO EN 1276:2019

Chemical disinfectants and antiseptics – Quantitative suspension test for the evaluation of bactericidal activity of chemical disinfectants and antiseptics used in food, industrial, domestic and institutional areas - Test method and requirements (phase 2, step 1)

PRODUCT NAME	:	Disinfettante mani
SUBSTANCES AND THEIR CONCENTRATIONS	:	Ethanol 70.4% p/p
APPEARANCE OF THE PRODUCT	:	Gel
STORAGE CONDITIONS	:	Room Temperature, Darkness
LOT	:	20204408
METHOD	:	EN 1276:2019
CONTACT TIME	:	1 minute
CONCENTRATION	:	Undiluted (80%), 50%, 1%.
STUDY SPONSOR	:	INT.E.G.RA. srl
PRODUCT SUPPLIER	:	Res Pharma Industriale srl
PRODUCT MANUFACTURER	:	Res Pharma Industriale srl
RECEIPT DATE	:	05/08/2020
STUDY PERIOD	:	18/09/2020-21/09/2020
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TEST MICROORGANISMS

Pseudomonas aeruginosa	NCIMB 10421
Staphylococcus aureus	ATCC 6538
Escherichia coli K12	NCTC 10538
Enterococcus hirae	NCIMB 8192

RESULT

The product under test: “Disinfettante mani” demonstrated bactericidal activity for hand hygiene disinfection according to EN 1276:2019 (≥ 5 log reduction) under clean conditions at 20 ± 1 °C, when tested:

Undiluted (80%) for 1 minute contact time using as test organisms the reference strains: *Pseudomonas aeruginosa*, *Staphylococcus aureus, Escherichia coli K12* and *Enterococcus hirae*.

Results refer to the samples received and analyzed on the period specified above.
The test report shall not be reproduced except in full, without written approval of the laboratory.
The samples will be stored by the laboratory for 1 month from the end test date.
The study report and raw data will be stored by the laboratory for 5 years.

End of Test Report

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STUDY REPORT

2020-7887/20 23 00727

Disinfettante mani

SUSPENSION TEST
ACCORDING TO EN 1650:2019
(Phase 2 step 1)

Chemical disinfectants and antiseptics
Quantitative suspension test for the evaluation of yeasticidal activity of chemical disinfectants and antiseptics used in food, industrial, domestic and institutional areas - Test method and requirements (phase 2, step 1)

SEPTEMBER 2020

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STUDY REPORT 2020-7887/20 23 00727

SUSPENSION TEST ACCORDING TO EN 1650:2019

Chemical disinfectants and antiseptics – evaluation of yeasticidal activity of chemical disinfectants and antiseptics used in food, industrial, domestic and institutional areas – Test method and requirements (Phase 2 step 1)

TEST PRODUCT IDENTIFICATION

PRODUCT NAME	:	Disinfettante mani
SUBSTANCES AND THEIR CONCENTRATIONS	:	Ethanol 70.4% p/p
APPEARANCE OF THE PRODUCT	:	Gel
STORAGE CONDITIONS	:	Room Temperature, Darkness
LOT	:	20204408
METHOD	:	EN 1650:2019
CONTACT TIME	:	1 minute
CONCENTRATION	:	Undiluted (80%), 50%, 1%.
STUDY SPONSOR	:	INT.E.G.RA. srl
PRODUCT SUPPLIER	:	Res Pharma Industriale srl
PRODUCT MANUFACTURER	:	Res Pharma Industriale srl
RECEIPT DATE	:	05/08/2020
STUDY PERIOD	:	18/09/2020-21/09/2020
LAB ID	:	2020-7887/20 23 00727

SCOPE

This document specifies a test method and the minimum requirements for yeasticidal activity of chemical disinfectant and antiseptic products that form a homogeneous, physically stable preparation when diluted with hard water or - in the case of ready-to-use-products - with water. Products can only be tested at a concentration of 80 % or less as some dilution is always produced by adding the test organisms and interfering substance.
This document applies to products that are used in food, industrial, domestic and institutional areas excluding areas and situations where disinfection is medically indicated and excluding products used on living tissues except those for hand hygiene in the above considered areas.

PRINCIPLE

A sample of the product as delivered and/or diluted with hard water (or water for ready-to-use products) is added to a test suspension of yeasts (yeast cells) in a solution of an interfering substance. The mixture is maintained at the chosen test temperature for the adopted contact time. At the end of this contact time, an aliquot is taken, and the yeasticidal activity in this portion is immediately neutralized or suppressed by a validated method. The method of choice is dilution-neutralization. If a suitable neutralizer cannot be found, membrane filtration is used. The numbers of surviving yeasts in each sample are determined and the reduction is calculated.
The test is performed using only the vegetative cells of *Candida albicans* (yeasticidal activity) as test organisms.

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TEST CONDITIONS

1. Product type: Hygienic handrub
2. The following procedure was performed in water bath at 20 °C
3. The test product was tested at 1 minute contact time
4. Interfering substance: A final concentration of 0.3g/L bovine albumin was used for testing (clean conditions).
5. Neutralization Method used: Dilution neutralization.
6. Neutralizer used: LPT Dilution Broth containing polysorbate 80.
7. According to EN 1650:2019, products shall be tested at a minimum of three different concentrations to include one concentration in the active range and one concentration in the non-active range. In this test product was tested: Undiluted (80%), 50%, 1%

TEST MICROORGANISMS

Candida albicans ATCC 10231

YEASTICIDAL ACTIVITY FOR HAND HYGIENE

The yeasticidal concentration for a hand hygiene is the concentration of the tested product for which a reduction of at least:
• lgR ≥ 4 for handrubs
or
• lgR ≥ 2 for handwashes at 50 % in test concentration or less.
is demonstrated in a valid test under the chosen test conditions in terms of interfering substance.

ASSAY ACCEPTANCE CRITERIA

1. Test Suspension (N) is between 1.5 to 5.0 X 10^7 CFU per mL (7.17≤ log No ≤7.70).
2. No (N/10) is between 1.5 to 5.0 X 10^6 CFU per mL (6.17 ≤ log No ≤ 6.70).
3. Validation Suspension=Nv is between 3.0 x 10^2 and 1.6 x 10^3.
4. Nvo (Nv/10) is between 30 and 160.
5. Na is the number of survivors (cells) per ml in the test mixture at the end of contact time.
6. R (log reduction) = No - Na
7. Average recovery values for the experimental conditions control (A) were equal to or greater than 0.5 times the Validation Suspension (Nvo)
8. Average recovery values for the Neutralizer control (B) were equal to or greater than 0.5 times the Validation Suspension (Nvo)
9. Average recovery values for the Method Validation control (C) were equal to or greater than 0.5 times the Validation Suspension (Nvo)
10. Control of weighted mean counts. Quotient is not lower than 5 and not higher than 15

ARCHIVING

The laboratory book which contains all the information (raw data and results) regarding the study and the study reports are kept in the laboratory archives for 5 years.

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TEST RESULTS FOR Candida albicans (YEASTICIDAL SUSPENSION TEST)

Test suspension

Test - suspension (N and No)				
N	Vc1	Vc2	x mean	3.14E+07
10^{-6}	29	34		
10^{-7}	3	3	log N	7.50
			No (N/10)	3.14E+06
			log No	6.50
			6,17 < = logNo < = 6,70	**Yes**

Validation and controls

Validation suspension (Nvo)			Experimental conditions (A)			Neutralizer control (B)			Method validation (C) undiluted Product conc.: (80%)		
VC 1	59	**x mean**	VC 1	69	**x mean**	VC 1	64	**x mean**	VC 1	69	**x mean**
VC 2	64	**61.5**	VC 2	62	**65.5**	VC 2	67	**65.5**	VC 2	57	**63**
30<x mean of Nvo < 160? **Yes**			x mean of A is > 0,5*x mean of Nvo? **Yes**			x mean of B is > 0,5*x mean of Nvo or Nvo/1000? **Yes**			x mean of C is > 0,5*x mean of Nvo? **Yes**		

Test Results

Product concentration (%)	Contact time	Dilution step	Vc 1	Vc 2	Average of Vc1 and Vc2	Na= average x10	log Na	log No	log Reduction (No-Na)	Criteria	Result
undiluted (80%)	1 min	10^{0}	0	0	< 14	< 140	< 2.15	6.50	> 4.35	≥4	**PASS TEST**
		10^{-1}	0	0							
50%	1 min	10^{0}	0	0	< 14	< 140	< 2.15	6.50	> 4.35	≥4	**PASS TEST**
		10^{-1}	0	0							
1%	1 min	10^{0}	> 330	> 330	> 3300	> 33000	> 4.52	6.50	< 1.98	≥4	**FAILS TEST**
		10^{-1}	> 330	> 330							

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CONCLUSION

TEST PRODUCT IDENTIFICATION

PRODUCT NAME	:	Disinfettante mani
SUBSTANCES AND THEIR CONCENTRATIONS	:	Ethanol 70.4% p/p
APPEARANCE OF THE PRODUCT	:	Gel
STORAGE CONDITIONS	:	Room Temperature, Darkness
LOT	:	20204408
METHOD	:	EN 1650:2019
CONTACT TIME	:	1 minute
CONCENTRATION	:	Undiluted (80%), 50%, 1%.
STUDY SPONSOR	:	INT.E.G.RA. srl
PRODUCT SUPPLIER	:	Res Pharma Industriale srl
PRODUCT MANUFACTURER	:	Res Pharma Industriale srl
RECEIPT DATE	:	05/08/2020
STUDY PERIOD	:	18/09/2020-21/09/2020
LAB ID	:	2020-7887/20 23 00727

METHODOLOGY ABSTRACT

A sample of the product as delivered and/or diluted with hard water (or water for ready to use products) is added to a test suspension of yeasts in a solution of an interfering substance. The mixture is maintained at 20 °C for 1 minute. At the end of this contact time, an aliquot is taken, and the yeasticidal activity in this portion is immediately neutralized or suppressed. The numbers of surviving yeasts in each sample are determined and the reduction is calculated.

RESULT

The product under test: "Disinfettante mani" demonstrated yeasticidal activity for hand hygiene disinfection according to EN 1650:2019 (≥ 4 log reduction), under clean conditions, at 20 ± 1 °C, when tested:

Undiluted (80%) for 1 minute contact time using as test organism the reference strain: *Candida albicans*.

For the QACS Ltd Laboratory,

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Signature date: 30/09/2020
Lagiopoulos Giorgos
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Study Manager

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STUDY SUMMARY / ABSTRACT

SUSPENSION TEST ACCORDING TO EN 1650:2019

Chemical disinfectants and antiseptics – evaluation of yeasticidal activity of chemical disinfectants and antiseptics used in food, industrial, domestic and institutional areas – Test method and requirements (Phase 2 step 1)

TEST PRODUCT IDENTIFICATION

PRODUCT NAME	:	Disinfettante mani
SUBSTANCES AND THEIR CONCENTRATIONS	:	Ethanol 70.4% p/p
APPEARANCE OF THE PRODUCT	:	Gel
STORAGE CONDITIONS	:	Room Temperature, Darkness
LOT	:	20204408
METHOD	:	EN 1650:2019
CONTACT TIME	:	1 minute
CONCENTRATION	:	Undiluted (80%), 50%, 1%.
STUDY SPONSOR	:	INT.E.G.RA. srl
PRODUCT SUPPLIER	:	Res Pharma Industriale srl
PRODUCT MANUFACTURER	:	Res Pharma Industriale srl
RECEIPT DATE	:	05/08/2020
STUDY PERIOD	:	18/09/2020-21/09/2020
LAB ID	:	2020-7887/20 23 00727

TEST MICROORGANISMS

Candida albicans	ATCC 10231

RESULT

The product under test: “Disinfettante mani” demonstrated yeasticidal activity for hand hygiene disinfection according to EN 1650:2019 (≥ 4 log reduction), under clean conditions, at 20 ± 1 °C, when tested:

Undiluted (80%) for 1 minute contact time using as test organism the reference strain: *Candida albicans*.

Results refer to the sample as received and analyzed on the period specified above.

The samples will be stored by the laboratory during 1 month from the end test date.

The study report and raw data will be stored by the laboratory for 5 years.

End of Test Report

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Res Pharma Industriale Srl

STUDY REPORT

2020-7887/20 23 00728

Disinfettante mani

SUSPENSION TEST
ACCORDING TO EN 13727:2012+A2:2015
(Phase 2 step 1)

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)

SEPTEMBER 2020

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STUDY REPORT 2020-7887/20 23 00728

SUSPENSION TEST ACCORDING TO 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)

TEST PRODUCT IDENTIFICATION

PRODUCT NAME	:	Disinfettante mani
SUBSTANCES AND THEIR CONCENTRATIONS	:	Ethanol 70.4% p/p
APPEARANCE OF THE PRODUCT	:	Gel
STORAGE CONDITIONS	:	Room Temperature, Darkness
LOT	:	20204408
METHOD	:	EN 13727:2012+A2:2015
CONTACT TIME	:	1 minute
CONCENTRATION	:	Undiluted (80%), 50%, 1%.
STUDY SPONSOR	:	INT.E.G.RA. srl
PRODUCT SUPPLIER	:	Res Pharma Industriale srl
PRODUCT MANUFACTURER	:	Res Pharma Industriale srl
RECEIPT DATE	:	05/08/2020
STUDY PERIOD	:	18/09/2020-21/09/2020
LAB ID	:	2020-7887/20 23 00728

SCOPE

This European Standard specifies a test method and the minimum requirements for bactericidal activity of chemical disinfectant and antiseptic products that form a homogeneous, physically stable preparation when diluted with hard water, or - in the case of ready-to-use products - with water. Products can only be tested at a concentration of 80 % or less (97 % with a modified method for special cases) as some dilution is always produced by adding the test organisms and interfering substance.
This European Standard applies to products that are used in the medical area in the fields of hygienic handrub, hygienic handwash, surgical handrub, surgical handwash, instrument disinfection by immersion, and surface disinfection by wiping, spraying, flooding or other means.
This European Standard applies to areas and situations where disinfection or antisepsis is medically indicated. Such indications occur in patient care, for example:
— in hospitals, in community medical facilities and in dental institutions;
— in clinics of schools, of kindergartens and of nursing homes;
and may occur in the workplace and in the home. It may also include services such as laundries and kitchens supplying products directly for the patients.

PRINCIPLE

A sample of the product as delivered and/or diluted with hard water (or water for ready-to-use products) is added to a test suspension of bacteria in a solution of an interfering substance. The mixture is maintained at one of the temperatures for the adopted contact time. At the end of this contact time, an aliquot is taken; the bactericidal and/or the bacteriostatic action in this portion is immediately neutralized or suppressed by a validated method. The method of choice is dilution-neutralization. If a suitable neutralizer cannot be found, membrane filtration is used. The numbers of surviving bacteria in each sample are determined and the reduction is calculated. Handwash products are always prediluted with hard water. The resulting solution is regarded as a ready-to-use product.

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TEST CONDITIONS

1. Product type: Hygienic handrub.
2. The following procedure was performed in water bath at 20 °C.
3. The test product was tested at 1 minute contact time.
4. Interfering substance: A final concentration of 0.3g/L bovine albumin was used for testing (clean conditions).
5. Neutralization Method used: Dilution neutralization.
6. Neutralizer used: LPT Dilution Broth containing polysorbate 80.
7. According to EN 13727, products shall be tested at a minimum of three different concentrations to include one concentration in the active range and one concentration in the non-active range. In this case the product was tested: Undiluted, 50% 1%.

TEST ORGANISMS

Pseudomonas aeruginosa	NCIMB 10421
Staphylococcus aureus	ATCC 6538
Escherichia coli K12	NCTC 10538
Enterococcus hirae	NCIMB 8192

BACTERICIDAL ACTIVITY FOR HANDRUB AND HANDWASH PRODUCTS

The product shall be deemed to have passed the EN 13727 standard if it demonstrates in a valid test for handrub and handwash products at 20 °C under the conditions defined by this standard when the test organisms are: *Escherichia coli K12, Pseudomonas aeruginosa, Staphylococcus aureus* and *Enterococcus hirae* at least a:

a) 5 lg reduction within max. 1 min under clean conditions (hygienic handrub);
b) 5 lg reduction within max. 5 min under clean conditions (surgical handrub);
c) 3 lg reduction within max. 1 min under dirty conditions (hygienic handwash);
d) 5 lg reduction within max. 5 min under dirty conditions (surgical handwash).

ASSAY ACCEPTANCE CRITERIA

1. Test Suspension (N) is between 1.5 to 5.0 X 10^8 CFU per mL ($8.17 \leq \log N \leq 8.70$)
2. No (N/10) is between 1.5 to 5.0 X 10^7 CFU per mL ($7.17 \leq \log No \leq 7.70$)
3. Validation Suspension=Nv is between 3.0 x 10^2 and 1.6 x 10^3.
4. Neutralizer control= N_{VB} is between 3.0 x 10^4 and 1.6 x 10^5.
5. N_{VO} (Nv/10) is between 30 and 160.
6. Na is the number of survivors (cells) per ml in the test mixture at the end of contact time.
7. R (log reduction) = No - Na
8. Average recovery values for the experimental conditions control (A) were equal to or greater than 0.5 times the Validation Suspension (Nvo).
9. Average recovery values for the Neutralizer control (B) were equal to or greater than 0.5 times the Validation Suspension (Nvo).
10. Average recovery values for the Method Validation control (C) were equal to or greater than 0.5 times the Validation Suspension (Nvo).

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TEST RESULTS FOR *Pseudomonas aeruginosa* (BACTERICIDAL SUSPENSION TEST)

Test suspension

Test - suspension (N and No)				
N	Vc1	Vc2	x mean	2.03E+08
10^{-6}	194	213		
10^{-7}	21	19	log N	8.31
			No (N/10)	2.03E+07
			log No	7.31
			7,17 < = logNo < = 7,70	**Yes**

Validation and controls

Validation suspension (Nvo)			Experimental conditions (A)			Neutralizer control (B)			Method validation (C) Product conc.: undiluted (80%)		
VC 1	39	**x mean**	VC 1	44	**x mean**	VC 1	47	**x mean**	VC 1	51	**x mean**
VC 2	42	**40.5**	VC 2	49	**46.5**	VC 2	49	**48**	VC 2	50	**50.5**
30<x mean of Nvo < 160?		**Yes**	x mean of A is > 0,5*x mean of Nvo?		**Yes**	x mean of B is > 0,5*x mean of Nvo or NVB/1000?		**Yes**	x mean of C is > 0,5*x mean of Nvo?		**Yes**

Validation suspension (NVB)		
VC 1	44	**x mean**
VC 2	40	**42**
30<x mean of NVB < 160?		**Yes**

Test Results

Product concentration (%)	Contact time	Dilution step	Vc 1	Vc 2	Average of Vc1 and Vc2	Na= average x10	log Na	log No	log Reduction (No-Na)	Criteria	Result
undiluted (80%)	1 min	10^{0}	0	0	< 14	< 140	< 2.15	7.31	> 5.16	≥5	**PASS TEST**
		10^{-1}	0	0							
50%	1 min	10^{0}	10	7	< 14	< 140	< 2.15	7.31	> 5.16	≥5	**PASS TEST**
		10^{-1}	0	1							
1%	1 min	10^{0}	> 330	> 330	> 3300	> 33000	> 4.52	7.31	< 2.79	≥5	**FAILS TEST**
		10^{-1}	> 330	> 330							

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TEST RESULTS FOR *Staphylococcus aureus* (BACTERICIDAL SUSPENSION TEST)

Test suspension

Test - suspension (N and No)				
N	Vc1	Vc2	x mean	2.40E+08
10^{-6}	245	237		
10^{-7}	24	22	log N	8.38
			No (N/10)	2.40E+07
			log No	7.38
			7,17 < = logNo < = 7,70	**Yes**

Validation and controls

Validation suspension (Nvo)			Experimental conditions (A)			Neutralizer control (B)			Method validation (C) Product conc.: undiluted (80%)		
VC 1	49	x mean	VC 1	49	x mean	VC 1	52	x mean	VC 1	50	x mean
VC 2	48	**48.5**	VC 2	54	**51.5**	VC 2	50	**51**	VC 2	59	**54.5**
30<x mean of Nvo < 160?			x mean of A is > 0,5*x mean of Nvo?			x mean of B is > 0,5*x mean of Nvo or NvB/1000?			x mean of C is > 0,5*x mean of Nvo?		
Yes			**Yes**			**Yes**			**Yes**		

Validation suspension (NvB)		
VC 1	46	x mean
VC 2	51	**48.5**
30<x mean of NvB < 160?		**Yes**

Test Results

Product concentration (%)	Contact time	Dilution step	Vc 1	Vc 2	Average of Vc1 and Vc2	Na= average x10	log Na	log No	log Reduction (No-Na)	Criteria	Result
undiluted (80%)	1 min	10^{0}	0	0	< 14	< 140	< 2.15	7.38	> 5.23	≥5	**PASS TEST**
		10^{-1}	0	0							
50%	1 min	10^{0}	0	0	< 14	< 140	< 2.15	7.38	> 5.23	≥5	**PASS TEST**
		10^{-1}	0	0							
1%	1 min	10^{0}	> 330	> 330	> 3300	> 33000	> 4.52	7.38	< 2.86	≥5	**FAILS TEST**
		10^{-1}	> 330	> 330							

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TEST RESULTS FOR *Escherichia coli K12* (BACTERICIDAL SUSPENSION TEST)

Test suspension

Test - suspension (N and No)				
N	Vc1	Vc2	x mean	3.55E+08
10^{-7}	36	34		
10^{-8}	4	4	log N	8.55
			No (N/10)	3.55E+07
			log No	7.55
			7,17 < = logNo < = 7,70	**Yes**

Validation and controls

Validation suspension (Nvo)			Experimental conditions (A)			Neutralizer control (B)			Method validation (C) Product conc.: undiluted (80%)		
VC 1	74	**x mean**	VC 1	75	**x mean**	VC 1	75	**x mean**	VC 1	69	**x mean**
VC 2	79	**76.5**	VC 2	72	**73.5**	VC 2	79	**77**	VC 2	74	**71.5**
30<x mean of Nvo < 160?			x mean of A is > 0,5*x mean of Nvo?			x mean of B is > 0,5*x mean of Nvo or NvB/1000?			x mean of C is > 0,5*x mean of Nvo?		
Yes			**Yes**			**Yes**			**Yes**		

Validation suspension (NvB)		
VC 1	77	**x mean**
VC 2	69	**73**
30<x mean of NvB < 160?		**Yes**

Test Results

Product concentration (%)	Contact time	Dilution step	Vc 1	Vc 2	Average of Vc1 and Vc2	Na= average x10	log Na	log No	log Reduction (No-Na)	Criteria	Result
undiluted (80%)	1 min	10^{0}	0	0	< 14	< 140	< 2.15	7.55	> 5.40	≥5	**PASS TEST**
		10^{-1}	0	0							
50%	1 min	10^{0}	9	12	< 14	< 140	< 2.15	7.55	> 5.40	≥5	**PASS TEST**
		10^{-1}	1	1							
1%	1 min	10^{0}	> 330	> 330	> 3300	> 33000	> 4.52	7.55	< 3.03	≥5	**FAILS TEST**
		10^{-1}	> 330	> 330							

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TEST RESULTS FOR *Enterococcus hirae* (BACTERICIDAL SUSPENSION TEST)

Test suspension

Test - suspension (N and No)				
N	Vc1	Vc2	x mean	3.27E+08
10^{-7}	31	34		
10^{-8}	4	3	log N	8.51
			No (N/10)	3.27E+07
			log No	7.51
			7,17 < = logNo < = 7,70	Yes

Validation and controls

Validation suspension (Nvo)			Experimental conditions (A)			Neutralizer control (B)			Method validation (C) Product conc.: undiluted (80%)		
VC 1	68	x mean	VC 1	72	x mean	VC 1	70	x mean	VC 1	88	x mean
VC 2	62	65	VC 2	76	74	VC 2	67	68.5	VC 2	76	82
30<x mean of Nvo < 160?			x mean of A is > 0,5*x mean of Nvo?			x mean of B is > 0,5*x mean of Nvo or Nvo/1000?			x mean of C is > 0,5*x mean of Nvo?		
Yes			Yes			Yes			Yes		

Validation suspension (Nvo)		
VC 1	69	x mean
VC 2	74	71.5
30<x mean of Nvo < 160?		Yes

Test Results

Product concentration (%)	Contact time	Dilution step	Vc 1	Vc 2	Average of Vc1 and Vc2	Na= average x10	log Na	log No	log Reduction (No-Na)	Criteria	Result
undiluted (80%)	1 min	10^{0}	0	0	< 14	< 140	< 2.15	7.51	> 5.37	≥5	PASS TEST
		10^{-1}	0	0							
50%	1 min	10^{0}	0	0	< 14	< 140	< 2.15	7.51	> 5.37	≥5	PASS TEST
		10^{-1}	0	0							
1%	1 min	10^{0}	> 330	> 330	> 3300	> 33000	> 4.52	7.51	< 3.00	≥5	FAILS TEST
		10^{-1}	> 330	> 330							

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CONCLUSION

SUSPENSION TEST ACCORDING TO 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)

TEST PRODUCT IDENTIFICATION

PRODUCT NAME	:	Disinfettante mani
SUBSTANCES AND THEIR CONCENTRATIONS	:	Ethanol 70.4% p/p
APPEARANCE OF THE PRODUCT	:	Gel
STORAGE CONDITIONS	:	Room Temperature, Darkness
LOT	:	20204408
METHOD	:	EN 13727:2012+A2:2015
CONTACT TIME	:	1 minute
CONCENTRATION	:	Undiluted (80%), 50%, 1%.
STUDY SPONSOR	:	INT.E.G.RA. srl
PRODUCT SUPPLIER	:	Res Pharma Industriale srl
PRODUCT MANUFACTURER	:	Res Pharma Industriale srl
RECEIPT DATE	:	05/08/2020
STUDY PERIOD	:	18/09/2020-21/09/2020
LAB ID	:	2020-7887/20 23 00728

METHODOLOGY ABSTRACT

A sample of the product as delivered and/or diluted with hard water (or water for ready to use products) is added to a test suspension of bacteria in a solution of an interfering substance. The mixture is maintained at 20 °C for 1 minute. At the end of this contact time, an aliquot is taken; the bactericidal and/or the bacteriostatic action in this portion is immediately neutralized or suppressed by a validated method. The numbers of surviving bacteria in each sample are determined and the log reduction is calculated.

RESULT

The product under test: “Disinfettante mani” demonstrated bactericidal activity for hygienic handrub disinfection (≥ 5 log reduction), according to the EN 13727:2012+A2:2015, at 20±1 °C, under clean conditions when tested at concentration:

Undiluted (80%) for 1 minute contact time using as test organisms the reference strains:
Pseudomonas aeruginosa, *Staphylococcus aureus*, *Escherichia coli K12* and *Enterococcus hirae*.

For the QACS Ltd Laboratory,

Signature date: 30/09/2020
Lagiopoulos Giorgos
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Study Manager

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STUDY SUMMARY / ABSTRACT

SUSPENSION TEST ACCORDING TO 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)

TEST PRODUCT IDENTIFICATION

PRODUCT NAME	:	Disinfettante mani
SUBSTANCES AND THEIR CONCENTRATIONS	:	Ethanol 70.4% p/p
APPEARANCE OF THE PRODUCT	:	Gel
STORAGE CONDITIONS	:	Room Temperature, Darkness
LOT	:	20204408
METHOD	:	EN 13727:2012+A2:2015
CONTACT TIME	:	1 minute
CONCENTRATION	:	Undiluted (80%), 50%, 1%.
STUDY SPONSOR	:	INT.E.G.RA. srl
PRODUCT SUPPLIER	:	Res Pharma Industriale srl
PRODUCT MANUFACTURER	:	Res Pharma Industriale srl
RECEIPT DATE	:	05/08/2020
STUDY PERIOD	:	18/09/2020-21/09/2020
LAB ID	:	2020-7887/20 23 00728

TEST ORGANISMS

Pseudomonas aeruginosa	NCIMB 10421
Staphylococcus aureus	ATCC 6538
Escherichia coli K12	NCTC 10538
Enterococcus hirae	NCIMB 8192

RESULT

The product under test: “Disinfettante mani” demonstrated bactericidal activity for hygienic handrub disinfection (≥ 5 log reduction), according to the EN 13727:2012+A2:2015, at 20±1 °C, under clean conditions when tested at concentration:

Undiluted (80%) for 1 minute contact time using as test organisms the reference strains:
Pseudomonas aeruginosa, *Staphylococcus aureus, Escherichia coli K12* and *Enterococcus hirae*.

Results refer to the sample as received and analyzed on the period specified above.
The test report shall not be reproduced except in full, without written approval of the laboratory.
The samples will be stored by the laboratory during 1 month from the end test date.
The study report and raw data will be stored by the laboratory for 5 years.

End of Test report

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Res Pharma Industriale Srl

STUDY REPORT

2020-7887/20 23 00729

Disinfettante mani

SUSPENSION TEST
ACCORDING TO EN 13624:2013
(Phase 2 step 1)

Chemical disinfectants and antiseptics
Quantitative suspension test for the evaluation of fungicidal or yeasticidal activity in the medical area - Test method and requirements (phase 2, step 1)

SEPTEMBER 2020

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STUDY REPORT 2020-7887/20 23 00729

SUSPENSION TEST ACCORDING TO EN 13624:2013

Chemical disinfectants and antiseptics – Quantitative suspension test for the evaluation of yeasticidal activity in the medical area – Test method and requirements
(Phase 2 step 1)

TEST PRODUCT IDENTIFICATION

PRODUCT NAME	:	Disinfettante mani
SUBSTANCES AND THEIR CONCENTRATIONS	:	Ethanol 70.4% p/p
APPEARANCE OF THE PRODUCT	:	Gel
STORAGE CONDITIONS	:	Room temperature, darkness
LOT	:	20204408
METHOD	:	EN 13624:2013
CONTACT TIME	:	1 minute
CONCENTRATION	:	Undiluted (80%), 50%, 1%.
STUDY SPONSOR	:	INT.E.G.RA. srl
PRODUCT SUPPLIER	:	Res Pharma Industriale srl
PRODUCT MANUFACTURER	:	Res Pharma Industriale srl
RECEIPT DATE	:	05/08/2020
STUDY PERIOD	:	18/09/2020-21/09/2020
LAB ID	:	2020-7887/20 23 00729

SCOPE

This European Standard specifies a test method and the minimum requirements for fungicidal or yeasticidal activity of chemical disinfectant and antiseptic products that form a homogeneous, physically stable preparation when diluted with hard water, or - in the case of ready-to-use products - with water. Products can only be tested at a concentration of 80 % or less (97 % with a modified method for special cases) as some dilution is always produced by adding the test organisms and interfering substance.
This European Standard applies to products that are used in the medical area in the fields of hygienic handrub, hygienic handwash, surgical handrub, surgical handwash, instrument disinfection by immersion, and surface disinfection by wiping, spraying, flooding or other means.
This European Standard applies to areas and situations where disinfection or antisepsis is medically indicated. Such indications occur in patient care, for example:
— in hospitals, in community medical facilities and in dental institutions;
— in clinics of schools, of kindergartens and of nursing homes;
and may occur in the workplace and in the home. It may also include services such as laundries and kitchens supplying products directly for the patients.

PRINCIBLE

A sample of the product as delivered and/or diluted with hard water (or water for ready to use products) is added to a test suspension of yeasts in a solution of an interfering substance. The mixture is maintained at the temperature (θ) and and for the chosen contact time (*t*). At the end of this contact time, an aliquot is taken; the yeasticidal action in this portion is immediately neutralized or suppressed by a validated method. The method of choice is dilution-neutralization. If a suitable neutralizer cannot be found, membrane filtration is used. The numbers of surviving yeasts in each sample are determined and the reduction is calculated. Handwash products are always prediluted with hard water. The resulting solution is regarded as a ready-to-use product.

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TEST CONDITIONS

1. Product type: Hygienic handrub.
2. The following procedure was performed in water bath at 20 °C.
3. The test product was tested at 1 minute contact time.
4. Interfering substance: A final concentration of 0.3g/L bovine albumin was used for testing (clean conditions).
5. Neutralization Method used: Dilution neutralization.
6. Neutralizer used: LPT Dilution Broth containing polysorbate 80.
7. According to EN 13624, products shall be tested at a minimum of three different concentrations to include one concentration in the active range and one concentration in the non-active range. In this case the product was tested: Undiluted, 50% 1%.

TEST MICROORGANISMS

Candida albicans ATCC 10231

YEASTICIDAL ACTIVITY FOR HANDRUB AND HANDWASH PRODUCTS

The product shall be deemed to have passed the EN 13624 Standard (yeasticidal activity) if it demonstrates in a valid test for handrub and handwash products at 20 °C under the conditions defined by this standard when the test organism is Candida albicans at least a:

- 4 lg reduction within max. 1 min under clean conditions (hygienic handrub)
- 4 lg reduction within max. 5 min under clean conditions (surgical handrub)
- 2 lg reduction within max. 1 min under dirty conditions (hygienic handwash)
- 4 lg reduction within max. 5 min under dirty conditions (surgical handwash)

ASSAY ACCEPTANCE CRITERIA

1. Test Suspension (N) is between 1.5 to 5.0 X 10^7 CFU per mL ($7.17 \leq \log No \leq 7.70$)
2. No (N/10) is between 1.5 to 5.0 X 10^6 CFU per mL ($6.17 \leq \log No \leq 6.70$)
3. Validation Suspension=Nv is between 3.0 x 10^2 and 1.6 x 10^3.
4. Neutralizer control= N_{VB} is between 3.0 x 10^4 and 1.6 x 10^5.
5. Nvo (Nv/10) is between 30 and 160.
6. Na is the number of survivors (cells) per ml in the test mixture at the end of contact time.
7. R (log reduction) = No - Na
8. Average recovery values for the experimental conditions control (A) were equal to or greater than 0.5 times the Validation Suspension (Nvo)
9. Average recovery values for the Neutralizer control (B) were equal to or greater than 0.5 times the Validation Suspension (Nvo) or N_{VB}/1000.
10. Average recovery values for the Method Validation control (C) were equal to or greater than 0.5 times the Validation Suspension (Nvo)

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TEST RESULTS FOR *Candida albicans* (YEASTICIDAL SUSPENSION TEST)

Test suspension

Test - suspension (N and No)				
N	Vc1	Vc2	x mean	3.14E+07
10^{-6}	29	34		
10^{-7}	3	3	log N	7.50
			No (N/10)	3.14E+06
			log No	6.50
			6,17 < = logNo < = 6,70	**Yes**

Validation and controls

Validation suspension (Nvo)			Experimental conditions (A)			Neutralizer control (B)			Method validation (C) Product conc.: 80% (as is)		
VC 1	66	x mean	VC 1	65	x mean	VC 1	62	x mean	VC 1	67	x mean
VC 2	69	**67.5**	VC 2	72	**68.5**	VC 2	69	**65.5**	VC 2	69	**68**
30<x mean of Nvo < 160?			x mean of A is > 0,5*x mean of Nvo?			x mean of B is > 0,5*x mean of Nvo or NvB/1000?			x mean of C is > 0,5*x mean of Nvo?		
Yes			**Yes**			**Yes**			**Yes**		

Validation suspension (NvB)		
VC 1	64	x mean
VC 2	67	**65.5**
30<x mean of NvB < 160?		**Yes**

Test Results

Product concentration (%)	Contact time	Dilution step	Vc 1	Vc 2	Average of Vc1 and Vc2	Na= average x10	log Na	log No	log Reduction (No-Na)	Criteria	Result
80% (as is)	1 min	10^{0}	0	0	< 14	< 140	< 2.15	6.50	> 4.35	≥4	**PASS TEST**
		10^{-1}	0	0							
50%	1 min	10^{0}	0	0	< 14	< 140	< 2.15	6.50	> 4.35	≥4	**PASS TEST**
		10^{-1}	0	0							
1%	1 min	10^{0}	> 330	> 330	> 3300	> 33000	> 4.52	6.50	< 1.98	≥4	**FAILS TEST**
		10^{-1}	> 330	> 330							

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CONCLUSION
SUSPENSION TEST ACCORDING TO EN 13624:2013

Chemical disinfectants and antiseptics – Quantitative suspension test for the evaluation of yeasticidal activity in the medical area – Test method and requirements
(Phase 2 step 1)

TEST PRODUCT IDENTIFICATION

PRODUCT NAME	:	Disinfettante mani
SUBSTANCES AND THEIR CONCENTRATIONS	:	Ethanol 70.4% p/p
APPEARANCE OF THE PRODUCT	:	Gel
STORAGE CONDITIONS	:	Room temperature, darkness
LOT	:	20204408
METHOD	:	EN 13624:2013
CONTACT TIME	:	1 minute
CONCENTRATION	:	Undiluted (80%), 50%, 1%.
STUDY SPONSOR	:	INT.E.G.RA. srl
PRODUCT SUPPLIER	:	Res Pharma Industriale srl
PRODUCT MANUFACTURER	:	Res Pharma Industriale srl
RECEIPT DATE	:	05/08/2020
STUDY PERIOD	:	18/09/2020-21/09/2020
LAB ID	:	2020-7887/20 23 00729

METHODOLOGY ABSTRACT

A sample of the product as delivered and/or diluted with hard water (or water for ready to use products) is added to a test suspension of fungi in a solution of an interfering substance. The mixture is maintained at 20 °C for 1 minute. At the end of this contact time, an aliquot is taken, and the yeasticidal activity in this portion is immediately neutralized or suppressed. The numbers of surviving yeasts in each sample are determined and the reduction is calculated.

RESULT

The product under test: "Disinfettante mani" demonstrated yeasticidal activity for hygienic handrub disinfection (≥ 4 log reduction) according to EN 13624:2013, at 20±1 °C, under clean conditions when tested at concentration:

Undiluted (80%) for 1 minute contact time using as test organism the reference strain: *Candida albicans*.

For the QACS Ltd Laboratory,

QACS Laboratories
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Signature date: 30/09/2020
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Agronomist-Food Technologist M.Sc.
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STUDY SUMMARY / ABSTRACT

SUSPENSION TEST ACCORDING TO EN 13624:2013

Chemical disinfectants and antiseptics – Quantitative suspension test for the evaluation of yeasticidal activity in the medical area – Test method and requirements
(Phase 2 step 1)

TEST PRODUCT IDENTIFICATION

PRODUCT NAME	:	Disinfettante mani
SUBSTANCES AND THEIR CONCENTRATIONS	:	Ethanol 70.4% p/p
APPEARANCE OF THE PRODUCT	:	Gel
STORAGE CONDITIONS	:	Room temperature, darkness
LOT	:	20204408
METHOD	:	EN 13624:2013
CONTACT TIME	:	1 minute
CONCENTRATION	:	Undiluted (80%), 50%, 1%.
STUDY SPONSOR	:	INT.E.G.RA. srl
PRODUCT SUPPLIER	:	Res Pharma Industriale srl
PRODUCT MANUFACTURER	:	Res Pharma Industriale srl
RECEIPT DATE	:	05/08/2020
STUDY PERIOD	:	18/09/2020-21/09/2020
LAB ID	:	2020-7887/20 23 00729

TEST MICROORGANISMS

Candida albicans	ATCC 10231

RESULT

The product under test: “Disinfettante mani” demonstrated yeasticidal activity for hygienic handrub disinfection (≥ 4 log reduction) according to EN 13624:2013, at 20±1 °C, under clean conditions when tested at concentration:

Undiluted (80%) for 1 minute contact time using as test organism the reference strain: *Candida albicans*.

Results refer to the sample as received and analyzed on the period specified above.
The test report shall not be reproduced except in full, without written approval of the laboratory.
The samples will be stored by the laboratory during 1 month from the end test date.
The study report and raw data will be stored by the laboratory for 5 years.

End of Test Report

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Assessment of the Efficacy of Hygienic Handrub Determined using the European Standard Test Method EN 1500:2013

Chemical disinfectants and antiseptics – Hygienic handrub
Test method and requirements (phase 2/step 2).

TEST PRODUCT IDENTIFICATION

STUDY SPONSOR	:	INT.E.G.RA. srl
MANUFATURER	:	Res Pharma Industriale srl
RECEIPT DATE	:	05/08/2020
STUDY PERIOD	:	25/09/2020-28/09/2020
PRODUCT NAME	:	Disinfettante mani
LAB ID	:	2020-7887/20 23 00730
LOT	:	20204408
STORAGE CONDITIONS	:	Room Temperature, Darkness.
DILUTION (AND IF APPLICABLE DILUENT)	:	Undiluted
APPLICATION CONDITIONS-PEFERENCE	:	2 x 30sec; 2 x 3 ml (60% v/v propan-2-ol)
APPLICATION CONDITIONS-PRODUCT	:	2 x 30sec; 2 x 2.5 ml (5 ml total application quantity)
No of VOLUNTEERS	:	20
ACTIVE SUBSTANCE	:	Ethanol 70.4 % p/p

STUDY REPORT

TABLE OF CONTENTS

Tests Carried Out By: QACS Ltd, 1 Antigonis str, 144-51 Metamorfosis, Greece,
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TEST PRODUCT IDENTIFICATION

STUDY SPONSOR	:	INT.E.G.RA. srl
MANUFATURER	:	Res Pharma Industriale srl
RECEIPT DATE	:	05/08/2020
STUDY PERIOD	:	25/09/2020-28/09/2020
PRODUCT NAME	:	Disinfettante mani
LAB ID	:	2020-7887/20 23 00730
LOT	:	20204408
STORAGE CONDITIONS	:	Room Temperature, Darkness.
DILUTION (AND IF APPLICABLE DILUENT)	:	Undiluted
APPLICATION CONDITIONS-PEFERENCE	:	2 x 30sec; 2 x 3 ml (60% v/v propan-2-ol)
APPLICATION CONDITIONS-PRODUCT	:	2 x 30sec; 2 x 2.5 ml (5 ml total application quantity)
No of VOLUNTEERS	:	20
ACTIVE SUBSTANCE	:	Ethanol 70.4 % p/p

Test Method	:	European Standard EN 1500:2013
Test Procedures	:	Full details of all the test and control procedures used are given in the Test Method
Test Organism	:	Escherichia coli K12 NCTC 10538
Culture Media and Reagents	:	Tryptone Soya Agar, Tryptic Soya Selective Agar, Tryptone Soy Broth
Incubation	:	Plates were incubated at 37 ºC for 24 - 48 h

TEST METHOD

EN 1500:2013 Chemical disinfectants and antiseptics – Hygienic handrub – Test method and requirements (phase 2/step 2).

This European Standard specifies a method of test simulating practical conditions for establishing whether a product for hygienic handrub reduces the release of transient flora according to the requirements when rubbed onto artificially contaminated hands of volunteers.

The method involves applying live test organisms (Escherichia coli K12 NCTC 10538) to the hands, then recovering the test organism in order to obtain a baseline count. The test or reference disinfectant product is then applied to the hands before once again recovering any surviving test organisms in sampling broth containing neutralizers to terminate the effect of any residual disinfectant. Propan-2-ol 60% (V/V) is used as reference. The organisms are enumerated, counts transposed to the Log system and the difference between the numbers recovered from the test or reference, and baseline counts is established and statistically analyzed for significance (WILCOXON'S matched-pairs, Hodges-Lehman). The larger the difference between the two counts, the less effective the product. Each of the volunteers repeats the procedure for the reference first and test product after, or for the product first and the reference after. For the test product to conform to the standard, EN1500:2013, the mean log reduction factor obtained shall be at least not inferior to that achieved by the specified reference hygienic handrub (60% volume concentration of propan-2-ol).

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REQUIREMENTS

When tested in accordance with EN 1500:2013, the mean reduction of the release of the test organism Escherichia coli K12 achieved by the hygienic handrub with the product under test shall be at least not inferior to that achieved by a specified reference hygienic handrub (60 % volume concentration of propan-2-ol).

SUBJECTS

The test was performed on 20 persons (requirement of the Standard 18-22 subjects) who have hands with healthy skin, without cuts or abrasions and with short and clean fingernails. Subject age was at least 18 years of age.

NEUTRILIZATION

A suitable neutralizer was chosen and validated before the test procedure (LPT dilution broth with tween 80 30g/l).

Composition of the neutraliser

Lecithin	3.0g
Sodium thiosulphate	5.0g
Tryptic digest of casein	1.0g
Sodium chloride	8.5g
Disodium hydrogen phosphate	8.0g
Potassium dihydrogen phosphate	1.5g
L-histidine HCL	1.0g
Polysorbate 80	30g

METHOD OF APPLICATION

Application of the test organism: Hands were prepared by washing for 1 minute with 5ml soft soap to remove transients and dried thoroughly on paper towels (Soft soap, 200 g l-1: Linseed oil 50 parts (by weight); Potassium hydroxide 9.5 parts; Ethanol 7 parts in distilled water -as needed-, autoclave to sterilize, pH between 10-11).

The volunteers were randomly divided into two groups of approximately the same size. Group 1 used the reference hygienic handrub and Group 2 the product under test. The test was repeated on the same day with Group 1 using the handrub procedure with the test product and Group 2 using the reference handrub procedure.

Hands were immersed to the mid-metacarpals for 5 sec, fingers apart, in 2 l of cultured test organism, E. coli K12, containing 1.5-5.0 x 10^8 cfu/ml. The same container with the contamination fluid was used for all volunteers. Hands were air dried for 3 minutes in horizontal position with the fingers spread out and rotating to avoid the formation of droplets, either for reference handrub procedure (R) or test product (P) as outlined below.

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PREVALUES

Immediately after treatment, the fingertips were immersed (including the thumb) for 1 min on the base of a petri dish containing 10ml of TSB as sampling fluid in order to assess the release of test micro-organisms before treatment of the hands. A separate petri dish was used for each hand.

REFERENCE PRODUCT:

Three ml of Propan-2-ol 60% (V/V) was poured into the cupped dry hands and rubbed vigorously into the skin for **30 seconds** up to the wrists in accordance with the standard handrub procedure shown in Figure 1. This ensured total coverage of the hands. The technique comprises of five strokes backwards and forwards, palm to palm, right palm over left dorsum and left palm over right dorsum, palm to palm with fingers interlaced, back of fingers to opposing palms with fingers interlocked, rotational rubbing of right thumb clasped in left palm and left thumb clasped in right palm, rotational rubbing with clasped fingers, of right hand in palm of left hand and clasped fingers of left hand in palm of right hand. **The procedure was repeated** with a further **three (3) ml** of Propan-2-ol 60% (V/V) to give a **total rubbing time of 60 seconds.**

TEST PRODUCT:

Two and a half (2.5) ml of product under test was poured into the cupped dry hands and rubbed vigorously into the skin for **30 seconds** up to the wrists in accordance with the standard handrub procedure shown in Figure 1. This ensured total coverage of the hands. The technique comprises of five strokes backwards and forwards, palm to palm, right palm over left dorsum and left palm over right dorsum, palm to palm with fingers interlaced, back of fingers to opposing palms with fingers interlocked, rotational rubbing of right thumb clasped in left palm and left thumb clasped in right palm, rotational rubbing with clasped fingers, of right hand in palm of left hand and clasped fingers of left hand in palm of right hand. The procedure was repeated with a further dose of **two and a half (2.5) ml** of product under test, in order to give a **total rubbing time of 60 seconds.**

POST VALUES

Immediately after treatment, the fingertips were immersed (including the thumb) for 1 min on the base of a petri dish containing 10ml of neutralizer.

The interval between sampling and planting did not exceed 30 min.

INCUBATION

All plates were incubated aerobically at 37°C + 1°C for 20h to 24h; then, the colonies were counted and the plates re-incubated for a further 24h in order to detect slow-growing colonies.

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***E. coli* K12 NCTC 10538 concentration: 3.0 x 10^8 cfu/ml.**

Table 1 Handrub reference procedure Propan-2-ol 60% (V/V). Colony Counts per Plate

No	Hand	Prevalues		Postvalues		
	left or right	10^{-4}	10^{-5}	10^{0}	10^{-1}	10^{-2}
1	l	>330	151	>330	42	4
	r	>330	99	>330	84	6
2	l	>330	235	>330	96	7
	r	>330	195	>330	204	21
3	l	>330	154	>330	154	11
	r	>330	142	>330	46	7
4	l	>330	135	>330	56	7
	r	>330	99	>330	42	5
5	l	>330	80	>330	190	14
	r	>330	74	>330	45	6
6	l	>330	40	>330	39	5
	r	>330	96	>330	41	5
7	l	>330	88	>330	94	16
	r	>330	104	>330	204	24
8	l	>330	85	>330	>330	45
	r	>330	56	>330	>330	39
9	l	>330	45	204	26	2
	r	>330	95	>330	40	4
10	l	>330	74	235	24	4
	r	>330	154	284	27	4
11	l	>330	195	>330	151	15
	r	>330	184	>330	124	14
12	l	>330	94	204	19	3
	r	>330	78	195	14	4
13	l	>330	56	>330	45	6
	r	>330	60	>330	80	8
14	l	>330	45	>330	201	22
	r	>330	27	>330	>330	31
15	l	>330	32	>330	75	7
	r	>330	94	>330	104	14
16	l	>330	104	>330	233	20
	r	>330	96	>330	>330	34
17	l	>330	85	>330	>330	30
	r	>330	132	>330	204	22
18	l	>330	52	>330	45	5
	r	>330	45	>330	>330	38
19	l	>330	74	>330	74	6
	r	>330	91	>330	200	18
20	l	>330	104	>330	40	6
	r	>330	94	>330	159	18

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***E. coli* K12 NCTC 10538 concentration: 3.0 x 10^8 cfu/ml.**

Table 2 Handrub procedure with the test product. Colony Counts per Plate.

No	Hand	Prevalues		Postvalues		
	left or right	10^{-4}	10^{-5}	10^{0}	10^{-1}	10^{-2}
1	l	>330	154	>330	154	16
	r	>330	59	>330	204	15
2	l	>330	45	>330	56	6
	r	>330	88	>330	41	4
3	l	>330	87	>330	36	2
	r	>330	154	188	19	2
4	l	>330	123	206	26	2
	r	>330	99	221	21	3
5	l	>330	74	184	24	2
	r	>330	154	49	4	0
6	l	>330	164	>330	41	7
	r	>330	88	>330	88	8
7	l	>330	106	>330	235	21
	r	>330	124	>330	>330	32
8	l	>330	45	>330	>330	42
	r	>330	68	>330	>330	64
9	l	>330	74	199	19	1
	r	>330	124	203	24	4
10	l	>330	130	199	21	2
	r	>330	75	>330	39	2
11	l	>330	29	>330	254	21
	r	>330	65	>330	>330	29
12	l	>330	235	>330	>330	54
	r	>330	199	>330	>330	42
13	l	>330	203	>330	31	2
	r	>330	154	>330	42	4
14	l	>330	45	>330	36	4
	r	>330	96	>330	60	5
15	l	>330	88	>330	40	4
	r	204	24	>330	45	6
16	l	>330	30	>330	96	7
	r	>330	88	>330	45	6
17	l	>330	96	>330	124	12
	r	144	15	>330	161	14
18	l	204	19	>330	76	6
	r	>330	40	>330	201	21
19	l	>330	64	>330	164	19
	r	>330	164	>330	168	12
20	l	>330	204	>330	42	4
	r	>330	133	>330	28	6

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Table 3 List of computed log_{10} values (mean of left and right hand) and log_{10} reduction

Volunteers	Chronological Sequence	Reference handrub			Handrub with product		
		log prevalues	log postvalues	log R	log prevalues	log postvalues	log R
1	PR -> PP	7.05	2.77	4.28	6.94	3.24	3.69
2	PR -> PP	7.29	3.14	4.15	6.76	2.68	4.08
3	PP -> RP	7.13	2.93	4.20	7.02	2.41	4.62
4	PR -> PP	7.02	2.69	4.33	7.00	2.33	4.67
5	PR -> PP	6.84	2.97	3.88	6.99	1.98	5.01
6	PR -> PP	6.75	2.61	4.14	7.04	2.79	4.25
7	PP -> RP	6.94	3.16	3.78	7.02	3.42	3.60
8	PP -> RP	6.80	3.58	3.22	6.70	3.67	3.03
9	PR -> PP	6.77	2.50	4.27	6.94	2.31	4.63
10	PP -> RP	6.99	2.43	4.56	6.95	2.44	4.52
11	PP -> RP	7.24	3.14	4.10	6.60	3.41	3.19
12	PP -> RP	6.89	2.26	4.63	7.29	3.64	3.66
13	PR -> PP	6.72	2.78	3.94	7.21	2.55	4.66
14	PP -> RP	6.50	3.38	3.12	6.78	2.67	4.11
15	PP -> RP	6.70	2.95	3.75	6.61	2.63	3.98
16	PR -> PP	6.96	3.43	3.53	6.67	2.82	3.85
17	PP -> RP	6.98	3.37	3.61	6.55	3.15	3.40
18	PP -> RP	6.64	3.10	3.55	6.43	3.09	3.35
19	PR -> PP	6.87	3.08	3.79	6.97	3.22	3.75
20	PR -> PP	6.95	2.91	4.04	7.18	2.56	4.62
X	Overall	6.90	2.96	**3.94**	6.88	2.85	**4.03**
s		0.20	0.35	0.41	0.23	0.48	0.57
NN		20	20	20	20	20	20
X	PR -> PP	6.92	2.97	3.96	6.88	2.83	4.05
s		0.18	0.24	0.24	0.22	0.52	0.63
NN		10	10	10	10	10	10
X	PP -> RP	6.88	2.95	3.93	6.88	2.87	4.01
s		0.22	0.45	0.54	0.26	0.46	0.55
NN		10	10	10	10	10	10

logR	: decimal log reduction	X	: Mean
PR -> PP	: Sequence: first RP, second PP	s	: standard deviation
PP -> PR	: Sequence: first PP, second RP	NN	: Number of values

Difference of mean Rs (PR -> PP):	-0.09	
Difference of mean Rs (PR -> PP):	-0.09	
Absolute difference of differences:	0.01	(<2.00)

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CHECK OF ACCEPTANCE CRITERIA

- Complete set of 20 volunteers available (hence, more than the minimum of 18)
- Mean of log prevalues for RP=6.90 and for PP=6.88 (hence both greater than 5.00)
- Individual log reductions less than 3.00: with Reference Product (RP)=0, with Test Product (PP)=0 (hence not more than three individual log reduction factors for each, fewer than 3,00 log)
- Absolute difference of mean differences=0.01(hence less than 2.00)
- All quotients of weighted mean counts between 5 and 15 (in Tables 1 and 2 and in validation of neutralizer)

All acceptance criteria are fulfilled

Table 4 Computation of individual differences of lg Rs of RP-PP

Volunteers	log reduction		Difference RP-PP
	Reference procedure (RP)	Product procedure (PP)	
1	4.28	3.69	0.59
2	4.15	4.08	0.07
3	4.20	4.62	-0.42
4	4.33	4.67	-0.34
5	3.88	5.01	-1.13
6	4.14	4.25	-0.11
7	3.78	3.60	0.18
8	3.22	3.03	0.19
9	4.27	4.63	-0.36
10	4.56	4.52	0.04
11	4.10	3.19	0.91
12	4.63	3.66	0.98
13	3.94	4.66	-0.72
14	3.12	4.11	-0.99
15	3.75	3.98	-0.23
16	3.53	3.85	-0.32
17	3.61	3.40	0.21
18	3.55	3.35	0.20
19	3.79	3.75	0.04
20	4.04	4.62	-0.58

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STATISTICAL EVALUATION (SIGNIFICANCE TESTING)

Since the quality of the data has been found to be acceptable, they will be used for the evaluation of the Product under test by applying the following pass criterion:

PP (procedure with product) shall not be inferior to RP (procedure with reference product propan-2-ol).

In this test, the performance of PP proved to be superior to the performance of the RP. The PP showed log reduction= 4.03 log, while the RP showed log reduction= 3.94 log.

Hence, no further statistical evaluation, like Hodges Lehmann statistical test, is required and it can be concluded that the test preparation PP is not inferior to RP.

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Assessment of the Efficacy of Hygienic Handrub Determined using the European Standard Test Method EN 1500:2013

STUDY SUMMARY

STUDY SPONSOR	:	INT.E.G.RA. srl
MANUFATURER	:	Res Pharma Industriale srl
RECEIPT DATE	:	05/08/2020
STUDY PERIOD	:	25/09/2020-28/09/2020
PRODUCT NAME	:	Disinfettante mani
LAB ID	:	2020-7887/20 23 00730
LOT	:	20204408
STORAGE CONDITIONS	:	Room Temperature, Darkness.
DILUTION (AND IF APPLICABLE DILUENT)	:	Undiluted
APPLICATION CONDITIONS-PEFERENCE	:	2 x 30sec; 2 x 3 ml (60% v/v propan-2-ol)
APPLICATION CONDITIONS-PRODUCT	:	2 x 30sec; 2 x 2.5 ml (5 ml total application quantity)
No of VOLUNTEERS	:	20
ACTIVE SUBSTANCE	:	Ethanol 70.4 % p/p

CONCLUSION

The test product: “Disinfettante mani”, tested at concentration: Undiluted-neat, when applied for total rubbing time of 60sec, using total product quantity of: 5 ml and applied as: 2 doses of 2.5ml per 30sec, conforms to the requirements of EN 1500:2013

RESULTS AUTHENTICITY

The study concerned by this report was carried out under my responsibility, according to the experimental protocol and the quality plan of the QACS Ltd laboratory.

Study Manager:

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Date: 02/10/2020

EN ISO/IEC 17025:2005

ESYD

Cert. No 195/6

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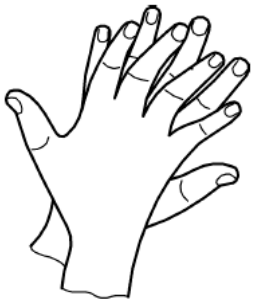
Figure 1. Standard handrub procedure

Pour appropriate volume of handrub product into the cupped dry hands and rub hands 30 s – 60 s in accordance with the standard handrub shown below to ensure total coverage of the hands. The action in each step is repeated five times before proceeding to the next step. After concluding step 6, recommence the series of steps as appropriate to complete the washing time.

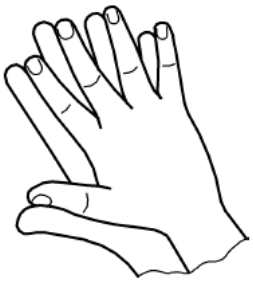
Step 1

Palm to palm



Step 2

Right palm over left dorsum and left palm over right dorsum (five times)

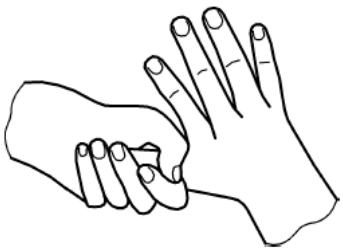


Step 3

Palm to palm with fingers interlaced (five times)

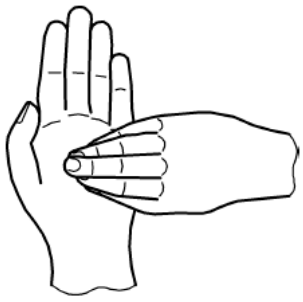
Step 4

Backs of fingers to opposing palms with fingers interlocked (five times)



Step 5

Rotational rubbing of right thumb clasped in left palm and vice versa (five times)



Step 6

Rotational rubbing, backwards and forwards with clasped fingers of right hand in left palm and vice versa (five times)

Adapted from EN 1500:2013 Chemical disinfectants and antiseptics – hygienic handrub - Test method and requirements (phase 2/step2)

Results refer to the sample as received and analyzed in the period specified above.
The test report shall not be reproduced except in full, without written approval of the laboratory.
The samples will be stored by the laboratory for 2 months from the end test date.
The study report and raw data will be stored by the laboratory for 2 years.

End of Study Report

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Res Pharma Industriale srl

STUDY REPORT

2020 – 7887 / 20 23 00731

Disinfettante mani

ANTIVIRAL ACTIVITY ACCORDING TO THE STANDARD EN 14476

OF THE PRODUCT "DISINFETTANTE MANI " AGAINST POLIOVIRUS,

ADENOVIRUS, MURINE NOROVIRUS AND INFLUENZA VIRUS A(H1N1) pdm09

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PRODUCT IDENTIFICATION

Product code/name	Disinfettante mani
Study Sponsor	Res Pharma Industriale srl
Date of receipt	September 01st, 2020
Product Appearance	Clear, colorless liquid
Storage	At room temperature (20°C)
Concentration used in the test	97.0% of the received product
Active compounds	Not provided by manufacturer

EXPERIMENTAL CONDITIONS

Test Method	**14476:2013+A2:2019: "Chemical disinfectants and antiseptics – Quantitative suspension test for the evaluation of virucidal activity in the medical area - Test method and requirements (Phase2/Step 1)"**
Test period	September 2020
Strains of viruses	Adenovirus type 5 (ATCC VR-5) Poliovirus 1 Sabin strain, LSc-2ab (WHO) Murine Norovirus (Strain S99 Berlin) Influenza virus A(H1N1) pdm09 (The vaccine strain: A/Brisbane/02/2018)
Cell lines	Human epithelial type 2 (Hep-2) cells Human Rhabdomyosarcoma cells (RD) RAW 264.7 macrophage (ATCC) Madin-Darby canine kidney (MDCK)
Culture medium	DMEM (Dulbecco's Minimal Essential Medium)
Contact time	2 min
Test temperature	Water bath, 20±1°C
Interfering substance	BSA 0.3 g/L (clean conditions)
Inactivation process	Dilution 1/10 in ice cold maintenance medium
Technical supervisor	Pogka Vasiliki, Ph.D, Antonios Kalliaropoulos
Facilities	BSL-2 facility, Public Health Laboratories, Hellenic Pasteur Institute.

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1. Principle of the test

A 97% dilution of the product "Disinfettante mani " was added to a test suspension of titrated viruses in bovine serum albumin solutions of 0.3 g/L (clean conditions). The mixtures were maintained at 20°C for 2 min. At the end of contact time, an aliquot was taken and the virucidal activity was suppressed by dilutions in ice-cold maintenance medium. The dilutions were then inoculated onto cell monolayers in 96-well culture plates for the titration of the remaining viruses. The titers of the viruses expressed in $TCID_{50}$ values, after 5-days incubation, were determined and expressed in log scale. Reduction of the viruses' infectivity was calculated from the differences of the log virus titers before (control) and after treatment with the product. According to the EN 14476 standard a product has antiviral activity when the reduction of the virus is at least 4 log.

2. Titration of the test viruses

The antiviral activity of the product was tested against three virus strains proposed by the Standard EN 14476, Adenovirus type 5, Poliovirus type 1 and Murine norovirus as well as against the influenza A(H1N1) pdm09. The viruses were propagated in the appropriate cell culture system to produce a high titer: Hep-2 monolayers for adenovirus titration, RD for poliovirus, RAW cell monolayers for M. norovirus titration and MDCK cell monolayers for influenza virus titration. Each virus was tested in decimal dilutions 10^{-3} up to 10^{-10}. Each dilution was inoculated 10x in wells of 96-well culture plates with the appropriate cell monolayer. The infected cells were incubated at 37°C in a 5% CO_2 atmosphere for 5 days. The Tissue Culture Infectious Dose ($TCID_{50}$) i.e. the infection dose of a virus suspension inducing a Cytopathic Effect (CPE) in 50% of cell culture units was estimated by the end-point Spearman-Karber method:

$$\textbf{Log } TCID_{50} = L - d(S - 0.5),$$

where *L* is the highest virus concentration used, *d* is the log difference of dilutions, *S* is the sum of % affected (CPE) at each dilution.

The standard error was calculated as follows:

$$\sigma_m^2 = d_f^2 \Sigma p_i(1-p_i)/(n_i-1)$$

where *d* is the logarithm of dilution factor, p_i was the observed reaction rate, *n* the number of test objects per dilution and σ_m standard error of the logarithmic titer.

CPE results of each virus on the appropriate cell line are presented in tables 1a, 1b, 1c and 1d respectively.

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Table 1a. Titration of the adenovirus on Hep-2 cells

Virus dilutions	CPE in cell culture wells of culture plate (*)										Cell control	
10^{-3}	4	4	4	4	4	4	4	4	4	4	0	0
10^{-4}	4	4	4	4	4	4	4	4	4	4	0	0
10^{-5}	4	4	4	4	4	4	4	4	4	4	0	0
10^{-6}	4	4	4	4	4	4	4	4	4	4	0	0
10^{-7}	4	4	4	4	4	4	4	4	4	4	0	0
10^{-8}	4	4	4	4	4	4	4	4	4	4	0	0
10^{-9}	0	0	0	4	0	0	0	0	4	0	0	0
10^{-10}	0	0	0	0	0	0	0	0	0	0	0	0

(*) CPE grading in each well: 0= no CPE, 1= 25% CPE, 2=50% CPE, 3= 75% CPE 4= 100% CPE

Table 1b. Titration of the Poliovirus on RD cells

Virus dilutions	CPE in cell culture wells of culture plate (*)										Cell control	
10^{-3}	4	4	4	4	4	4	4	4	4	4	0	0
10^{-4}	4	4	4	4	4	4	4	4	4	4	0	0
10^{-5}	4	4	4	4	4	4	4	4	4	4	0	0
10^{-6}	4	4	4	4	4	4	4	4	4	4	0	0
10^{-7}	4	4	4	4	4	4	4	4	4	4	0	0
10^{-8}	4	4	4	4	4	4	4	4	4	4	0	0
10^{-9}	0	4	0	4	4	0	4	0	0	4	0	0
10^{-10}	0	0	0	0	0	0	0	0	0	0	0	0

(*) CPE grading in each well: 0= no CPE, 1= 25% CPE, 2=50% CPE, 3= 75% CPE 4= 100% CPE

Table 1c. Titration of the Murine norovirus on RAW cells

Virus dilutions	CPE in cell culture wells of culture plate (*)										Cell control	
10^{-3}	4	4	4	4	4	4	4	4	4	4	0	0
10^{-4}	4	4	4	4	4	4	4	4	4	4	0	0
10^{-5}	4	4	4	4	4	4	4	4	4	4	0	0
10^{-6}	4	4	4	4	4	4	4	4	4	4	0	0
10^{-7}	4	4	4	4	4	4	4	4	4	4	0	0
10^{-8}	4	4	4	4	0	4	4	0	4	4	0	0
10^{-9}	0	4	4	0	0	0	0	0	0	4	0	0
10^{-10}	0	0	0	0	0	0	0	0	0	0	0	0

(*) CPE grading in each well: 0= no CPE, 1= 25% CPE, 2=50% CPE, 3= 75% CPE 4= 100% CPE

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Table 1d. Titration of the influenza A(H1N1) pdm09 virus on MDCK cells

Virus dilutions	CPE in cell culture wells of culture plate (*)										Cell control	
10^{-3}	4	4	4	4	4	4	4	4	4	4	0	0
10^{-4}	4	4	4	4	4	4	4	4	4	4	0	0
10^{-5}	4	4	4	4	4	4	4	4	4	4	0	0
10^{-6}	4	4	4	4	4	4	4	4	4	4	0	0
10^{-7}	4	4	4	4	4	4	4	4	4	4	0	0
10^{-8}	4	4	4	4	4	4	4	4	4	4	0	0
10^{-9}	4	4	4	0	4	4	0	0	4	4	0	0
10^{-10}	0	0	0	0	0	0	0	0	0	0	0	0

(*) CPE grading in each well: 0= no CPE, 1= 25% CPE, 2=50% CPE, 3= 75% CPE 4= 100% CPE

By using of the Spearman-Karber formula on the aforementioned CPE results, the calculated $TCID_{50}$ of the Adenovirus, Poliovirus, the Norovirus and Influenza virus A(H1N1) pdm09 strains were $10^{-7.7}$, $10^{-8.0}$, $10^{-7.6}$ and $10^{-8.0}$ respectively. Taking into account the standard error of the above calculation, the titers of the four strains used in the tests were:

Initial titer of Adenovirus type 5	**Log $TCID_{50}$/0.1mL = 7.7±0.133**
Initial titer of Poliovirus type 1	**Log $TCID_{50}$/0.1mL = 8.0±0.167**
Initial titer of Murine norovirus	**Log $TCID_{50}$/0.1mL = 7.6±0.203**
Initial titer of influenza A(H1N1) pdm09	**Log $TCID_{50}$/0.1mL = 8.2±0.153**

3. Cytotoxic effect of the product

We determined the highest concentration of the product (97.0% final concentrations) not having toxic effect on the cells used for the virus culture. Dilutions 10^{-1} to 10^{-8} of the product in culture medium with 0.3 g/L BSA were incubated in ice-cold water for 30 min and then 100 µL of each dilution were inoculated onto monolayers of Hep-2, RD, RAW and MDCK cells in the wells of culture plates. Any microscopic changes in the cells after 5-days incubation were recorded.

No cytotoxic effect was observed on Hep-2, RD and RAW cells in all dilutions of a 97.0% final concentration of the product.

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4. Reference test for virus inactivation

Formaldehyde 0.7% (w/v) was included as reference for test validation according to the Standard EN 14476. Cytotoxicity test as well as antiviral activity determination was performed on RD cells using serial dilutions of up to 10^{-8} of the aforementioned formaldehyde test solution. Contact times were 30 min and 60 min. The results of the cytotoxicity and the virus inactivation tests are presented in the tables 2 and 3 respectively (only the results for 30 min contact time are shown).

Table 2: Cytotoxicity test of formaldehyde solution tested on RD cells

	Product Dilutions	Presence or absence of cell cytotoxicity of the product (*)										Cell control	
	10^{-1}	tox	tox	tox	tox	tox	tox	tox	tox	tox	tox	0	0
	10^{-2}	tox	tox	tox	tox	tox	tox	tox	tox	tox	tox	0	0
	10^{-3}	tox	tox	tox	tox	tox	tox	tox	tox	tox	tox	0	0
	10^{-4}	tox	tox	tox	tox	tox	tox	tox	tox	tox	tox	0	0
	10^{-5}	0	0	0	0	0	0	0	0	0	0	0	0
	10^{-6}	0	0	0	0	0	0	0	0	0	0	0	0
	10^{-7}	0	0	0	0	0	0	0	0	0	0	0	0
	10^{-8}	0	0	0	0	0	0	0	0	0	0	0	0

(*) tox= cytotoxicity, 0 = absence of cytotoxicity

Table 3: Data of formaldehyde solution inactivation tested against Poliovirus

	Virus Dilutions	CPE in cell culture wells of culture plate (*)										Cell control	
	10^{-3}	tox	tox	tox	tox	tox	tox	tox	tox	tox	tox	0	0
	10^{-4}	tox	tox	tox	tox	tox	tox	tox	tox	tox	tox	0	0
	10^{-5}	tox	tox	tox	tox	tox	tox	tox	tox	tox	tox	0	0
	10^{-6}	tox	tox	tox	tox	tox	tox	tox	tox	tox	tox	0	0
	10^{-7}	0	0	0	0	0	0	0	0	0	0	0	0
	10^{-8}	0	0	0	0	0	0	0	0	0	0	0	0
	10^{-9}	0	0	0	0	0	0	0	0	0	0	0	0
	10^{-10}	0	0	0	0	0	0	0	0	0	0	0	0

(*) CPE grading in each well: 0= no CPE, 1= 25% CPE, 2=50% CPE, 3= 75% CPE 4= 100% CPE. Tox=cytotoxicity

A reduction of at least 2.5 log of the poliovirus titer was recorded in the presence of 0.7% (w/v) formaldehyde. Higher log reduction could not be observed due to toxicity of formaldehyde on

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RD cells. According to the EN 14476 standard, the difference between the logarithmic titer of the virus control and the logarithmic titer of the test organism in the reference inactivation test should be between 2 and 4.5 log after 30 min for poliovirus to verify the method.

5. Antiviral activity of the product

The antiviral activity of the product against the adenovirus, poliovirus, murine norovirus and influenza virus A(H1N1) pdm09 strains was determined for 2 min at 20±1°C in 0.3 g/L (clean conditions). Immediately at the end of contact time, a 1/10 dilution was made in ice-cold cell maintenance medium and 30 min later, subsequent serial dilutions (step 1:10) were inoculated onto cell culture monolayers. After incubation, the titer of each virus was calculated, and the reduction of the virus infectivity was determined from the log differences of virus titers before and after treatment with the product. Results are presented in tables 4, 5, 6 and 7 for adenovirus, poliovirus, murine norovirus and influenza virus A(H1N1) pdm09, respectively.

Table 4: Adenovirus titration after a 2 min contact with 97.0% final concentration of the product in 0.3 % BSA

	Virus Dilutions	CPE in cell culture wells of culture plate (*)										Cell control	
	10^{-3}	4	4	4	0	4	0	0	4	4	0	0	0
	10^{-4}	0	0	0	0	0	0	0	0	0	0	0	0
	10^{-5}	0	0	0	0	0	0	0	0	0	0	0	0
	10^{-6}	0	0	0	0	0	0	0	0	0	0	0	0
	10^{-7}	0	0	0	0	0	0	0	0	0	0	0	0
	10^{-8}	0	0	0	0	0	0	0	0	0	0	0	0
	10^{-9}	0	0	0	0	0	0	0	0	0	0	0	0
	10^{-10}	0	0	0	0	0	0	0	0	0	0	0	0

(*) CPE grading in each well: 0= no CPE, 1= 25% CPE, 2=50% CPE, 3= 75% CPE 4= 100% CPE. Tox=cytotoxicity

The titer of the Adenovirus remaining after the treatment with the product is:

Log $TCID_{50}$after treatment: 2.1

Log difference=initial virus titer – virus titer after treatment = 7.7-2.1 = 5.6

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Table 5: Poliovirus titration after a 2 min contact with 97.0% final concentration of the product in 0.3 % BSA

Virus Dilutions	CPE in cell culture wells of culture plate (*)										Cell control	
10^{-3}	4	4	4	4	4	4	4	4	4	4	0	0
10^{-4}	0	0	0	0	0	0	0	0	0	0	0	0
10^{-5}	0	0	0	0	0	0	0	0	0	0	0	0
10^{-6}	0	0	0	0	0	0	0	0	0	0	0	0
10^{-7}	0	0	0	0	0	0	0	0	0	0	0	0
10^{-8}	0	0	0	0	0	0	0	0	0	0	0	0
10^{-9}	0	0	0	0	0	0	0	0	0	0	0	0
10^{-10}	0	0	0	0	0	0	0	0	0	0	0	0

(*) CPE grading in each well: 0= no CPE, 1= 25% CPE, 2=50% CPE, 3= 75% CPE 4= 100% CPE. Tox=cytotoxicity

The titer of the Poliovirus remaining after the treatment with the product is:

Log $TCID_{50}$after treatment: 2.5

Log difference=initial virus titer – virus titer after treatment = 8.0-2.5 = 5.5

Table 6: M. norovirus titration after a 2 min contact with 97% final concentration of the product in 0.3 % BSA

Virus Dilutions	CPE in cell culture wells of culture plate (*)										Cell control	
10^{-3}	0	4	0	4	0	0	0	0	0	0	0	0
10^{-4}	0	0	0	0	0	0	0	0	0	0	0	0
10^{-5}	0	0	0	0	0	0	0	0	0	0	0	0
10^{-6}	0	0	0	0	0	0	0	0	0	0	0	0
10^{-7}	0	0	0	0	0	0	0	0	0	0	0	0
10^{-8}	0	0	0	0	0	0	0	0	0	0	0	0
10^{-9}	0	0	0	0	0	0	0	0	0	0	0	0
10^{-10}	0	0	0	0	0	0	0	0	0	0	0	0

(*) CPE grading in each well: 0= no CPE, 1= 25% CPE, 2=50% CPE, 3= 75% CPE 4= 100% CPE. Tox=cytotoxicity

The titer of the murine norovirus remaining after the treatment with the product is:

Log $TCID_{50}$ after treatment: 1.7

Log difference=initial virus titer – virus titer after treatment = 7.6-1.7= 5.9

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Table 7: Influenza virus A(H1N1) pdm09 titration after a 2 min contact with 97% final concentration of the product in 0.3 % BSA

	Virus Dilutions	CPE in cell culture wells of culture plate (*)										Cell control	
	10^{-3}	0	0	4	0	4	0	4	4	4	0	0	0
	10^{-4}	0	0	0	0	0	0	0	0	0	0	0	0
	10^{-5}	0	0	0	0	0	0	0	0	0	0	0	0
	10^{-6}	0	0	0	0	0	0	0	0	0	0	0	0
	10^{-7}	0	0	0	0	0	0	0	0	0	0	0	0
	10^{-8}	0	0	0	0	0	0	0	0	0	0	0	0
	10^{-9}	0	0	0	0	0	0	0	0	0	0	0	0
	10^{-10}	0	0	0	0	0	0	0	0	0	0	0	0

(*) CPE grading in each well: 0= no CPE, 1= 25% CPE, 2=50% CPE, 3= 75% CPE 4= 100% CPE. Tox=cytotoxicity

The titer of the influenza virus A(H1N1) pdm09 remaining after the treatment with the product is:

Log $TCID_{50}$ after treatment: 2.0

Log difference=initial virus titer – virus titer after treatment = 8.2-2.0 = 6.2

6. Method Requirements

The product under test shall demonstrate at least a decimal log (lg) reduction of 4 in virus titre when tested in accordance with EN 14476.

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7. Conclusion

The antiviral activity of the product " PT1 - PMC - Disinfettante mani" against the Adenovirus type 5, Poliovirus type 1, Murine norovirus and Influenza virus A(H1N1)pdm09 was tested according to the EN 14476 standard: “Chemical disinfectants and antiseptics - Quantitative suspension test for the evaluation of virucidal activity in the medical area- test method and requirements (Phase2/Step1).” According to the EN 14476 standard a product has antiviral activity when a reduction of at least 4 log of the virus is observed.

The product under test tested when tested undiluted (97.0% final concentration), demonstrated:

- a 5.6 log reduction of the Adenovirus type 5 (ATCC VR-5) after 2 min contact time in the presence of 0.3 g/L BSA, at 20^{o}C
- a 5.5 log reduction of the Poliovirus 1 Sabin strain, LSc-2ab (WHO) after 2 min contact time in the presence of 0.3 g/L BSA, at 20^{o}C
- a 5.9 log reduction of the Murine Norovirus (Strain S99 Berlin) after 2 min contact time in the presence of 0.3 g/L BSA, at 20^{o}C
- a 6.2 log reduction of the Influenza virus A(H1N1) pdm09 after a 2 min contact time in the presence of 0.3 g/L BSA, at 20^{o}C

The product demonstrated antiviral activity against the non-enveloped DNA adenovirus, the non-enveloped RNA poliovirus, the non-enveloped RNA murine norovirus and the enveloped influenza virus A(H1N1) pdm09.

According to the EN 14476 standard, products that have antiviral activity against the adenovirus, the poliovirus and the murine norovirus are considered active against all viruses (enveloped and non-enveloped).

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End of Study Report