TEST REPORT S-2017-02179 SAM

SPONSOR	CLEANSERVICE GROUP AG
	WILERSTRASSE 2180
	CH-9230 FLAWIL
	SWITZERLAND
STUDY MONITOR	EUROFINS SCIENTIFIC AG
	PARKSTRASSE 10
	CH-5012 SCHÖNENWERD
	SWITZERLAND
TEST METHOD	AFNOR NF T 72-281, 2014 – Methods of airborne disinfection of surfaces - Determination of bactericidal, fungicidal, yeasticidal, mycobactericidal, tuberculicidal, sporicidal and virucidal activity, including bacteriophages.

TEST SAMPLE

IDENTIFICATION	*CLEANLINE 15 (SANOSIL S015)*		
SAMPLE TYPOLOGY	Biocide and Antimicrobials		
BATCH N.	Z0504B7	CODE	//
MANUFACTURING DATE	Not provided	EXPIRY DATE	Oct-19
MANUFACTURER	Sanosil AG		
COMPOSITION	Hydrogen peroxide 7.5% Silver 0.0075%		
SAMPLE ID	ACE-2017-00081432		
RECEIVING N.	EUITVI-93691	RECEIVING DATE	1-Jun-17

MICRO-NEBULIZER MODULATOR (SPONSOR'S MACHINE)

IDENTIFICATION	*CLEANCUBE PRO SERIE II*
MANUFACTURER	Cleanservice Group AG
SERIAL #	Not provided
ENGINE POWER	1200 W
ENGINE ROTATION SPEED	19000 rpm
TECHNICAL DATA	Electrical data 220-240V (50-60 Hz) Amperage max. 6.3 A Auxiliary Voltage 24 V
TANK CAPACITY	5 liters
CASING DIMENSIONS	370 x 370 x 400 mm

FIGURE			
EUROFINS ID NUMBER	ACE-2017-00081445		
EUROFINS RECEIVING N.	EUITVI-93691		
EUROFINS RECEIVING DATE	1-Jun-17		
ANALYSIS STARTING DATE	3-Aug-17	**ANALYSIS ENDING DATE**	29-Aug-17
EXPERIMENTAL CONDITIONS			
TEST TEMPERATURE	20°C ±2°C		
RELATIVE HUMIDITY	Between 40% and 80%		
CONCENTRATION	Neat (the disinfectant was directly inserted into the tank of the device)		
NEBULIZATION TIME	20 minutes (first run) – 10 minutes (second run) – 10 minutes (third run)		
CONTACT TIME	30 minutes after the end of each nebulization run (for three runs)		
DISTANCE DISINFECTION APPARATUS/CARRIER	The test carriers were placed in the opposite side of the disinfection system at a distance of about 3.0 m from the device		
VOLUME OF THE INDOOR TEST ROOM	50 m^3 (cubic meters)		
AIRBORNE M^3 SETTING	50 mc		
TEST SURFACE	Stainless steel carriers (4 cm in diameter)		
INACTIVATION OF THE PRODUCT	*Diluent/Rinsing liquid* The following rinsing liquid has been used: Tryptone of casein 1.0 g NaCl 8.5 g Polysorbate 80 5.0 g Distilled water q. s. to 1000 ml		
INTERFERING SUBSTANCE	Skim milk solution with a final concentration of 0.5%		
INCUBATION TEMPERATURE	37°C±1°C (for bacterial strain)		
TEST STRAINS	*Staphylococcus aureus* ATCC 6538 *Pseudomonas aeruginosa* ATCC 15442 *Escherichia coli* ATCC 10536 *Enterococcus hirae* ATCC 10541		

TEST METHOD (SUMMARY DESCRIPTION)	
CARRIER PREPARATION	For each bacterial strain, the carriers were inoculated with 0.05 ml of the microbial suspension diluted 20/1 v/v with the interfering substance and then were left to dry until visible dry within 120 minutes at 37°C ±1°C.
COUNT OF THE MICROBIAL SUSPENSIONS	The microbial suspensions diluted 20/1 v/v with the interfering substance were counted both with pour-plating method and filtration method. The bacterial suspensions have been diluted up to 10^{-6}, 10^{-7} and 10^{-8}. Pour-plating method: 1 ml for each dilution was pour-plated in duplicate. After the incubation period the number of colony-forming units per ml was determined and **N1** value was calculated. Filtration method: 1 ml for each dilution was filtered in duplicate and the membranes were washed 3 times using 50 ml of rinsing fluid; after the complete filtration the membranes were laid down on agar plates. After the incubation period the number of colony-forming units per ml was determined and **N2** value was calculated.
COUNT OF MICROORGANISMS ON THE CARRIER	At the end of the drying step each carrier was left in the lab in their lidded plates for the scheduled test duration under the provided temperature and relative humidity conditions and then was put into a flask containing 100 ml of diluent and glass beads, and was strongly stirred for 1 minute. The mixture was diluted in diluent using a decimal dilution and a duplicate plating (1 ml) for each dilution was performed by agar inclusion. After the incubation period the number of cfu/ml for each carrier was performed and the mean value (**T**) was calculated for each strain.
VALIDATION OF THE ABSENCE OF RESIDUAL EFFECT	One carrier for each tested strain was contaminated with 0.05 ml of WFI diluted 20/1 v/v with the interfering substance and left to dry as above described; the, it was exposed to the test item with the provided test conditions. At the end of the contact time each carrier was transferred into 100 ml of diluent in order to recover the residual of disinfectant. The recovery solution (**S**) was so obtained and the following evaluations were performed. *Screening for an inhibitory effect in the agar medium* 1ml of the dilution 10^{-7} (for bacterial strain) of the suspension N1 was put separately into a Petri plate with 1 ml of the correspondent solution S previously obtained, which was mixed after adding of the agar medium. After the incubation period the number of cfu/ml for each carrier was performed and the mean value (**n1**) was calculated. *Screening for an inhibitory effect in filter membranes* For each test strain, 98 ml of the correspondent solution S previously obtained were filtered and the membranes were washed 3 times using 50 ml of rinsing fluid. Another 50 ml of the rinsing fluid were added to the filter apparatus and they were inoculated with 1 ml of the dilution 10^{-7} (for bacterial strain) of the suspension N1; after complete filtration the membranes were laid down on agar plates. After the incubation period the number of cfu/ml for each carrier was performed and the mean value (**n2**) was calculated. *Screening for a carrier-vectored inhibitory effect in the agar medium* For each test strain, 1 ml of the dilution 10^{-7} (for bacterial strain) of the suspension N1 was put separately into a Petri plate with the carrier recovered from the correspondent solution S and was included into agar medium. After the incubation period the number of cfu/ml for each carrier was determined and the mean value (**n3**) was calculated.
TEST	For each tested strain, 3 carriers previously contaminated were exposed to the test item with the planned test conditions. After the nebulizer time the carriers were left in contact with the airborne disinfectant for the contact time

	and then transferred into a container with 100 ml of rinsing liquid with glass beads. After having accurately suspended the residual microorganisms by means of stirring for 1 minute, 1 ml of this test mixture was diluted in 9 ml of diluent and serial decimal dilutions were performed up 10^{-3}; 1 ml twice of the mother solution and of each dilution was sending into Petri plate and a count by inclusion method was performed into agar medium. Then, 10 ml and the residual 87 ml of the test mixture was separately filtered on a membranes and washed 3 times using 50 ml of rinsing fluid. The membranes were then laid down on agar plates. After the incubation period the number of the survived microorganisms (cfu/ml) was determined and the mean value (**n'1**) was calculated. The carriers were taken in aseptic way and laid down on a Petri dish and covered with agar medium. The microorganisms still present on the 3 carriers were counted and the mean value (**n'2**) was calculated. Microorganisms surviving on the membranes were counted and the average **n'1** was calculated.
INCUBATION PARAMETERS	For bacterial strain the plates are incubated with TSA (as Agar medium) at 37°C ±1°C within 2 days.
ASSAY VALIDITY CRITERIA	N (bacteria): shall be in the range 5×10^7 to 2×10^9 cfu/ml T (bacteria) ≥ 10^6 (on the carrier) n1, n3 > 0.5 N1 n2 > 0.5 N2
	The test item is considered effective (bactericidal) when d ≥ 5 Log against the strains provided by the reference standard at the temperature of 20±2°C and relative humidity between 40% and 80%, if used the stainless steel carrier and a test room with a volume included between 30 m^3 and 150 m^3.

RESULTS	**Log reductions (d)**
Staphylococcus aureus ATCC 6538	>5.89
Pseudomonas aeruginosa ATCC 15442	>5.15
Escherichia coli ATCC 10536	>5.43
Enterococcus hirae ATCC 10541	>5.05
See Attachment N.1	

CONCLUSIONS	**BACTERICIDAL** in the adopted test conditions, in compliance with the requirements of the method AFNOR NF T 72-281:2014 and according to the Sponsor request.
ATTACHMENT	N. 1: RAW DATA ELABORATION *(3 pages)*

STUDY DIRECTOR (CAMILLA CARLONI)	Camilla Carloni	ISSUED ON:	5-Sep-17

eurofins	Metodo di disinfezione di superfici per vie aeree - Determinazione dell'attività battericida, fungicida, levuricida, micobattericida, tubercolicida e sporicida) *(Methods of airbone disinfection of surfaces - Determination of bacterial, fungicidal, yeasticidal mycobactericidal, tuberculicidal and sporicidal activity)* Norma *(Standard)*: AFNOR NF T 72-281:2014	EDR: 1-P-PR-TEM-9005778

Data inizio *(Started on)*: 03/08/17

ID. studio *(ID. Study)*: S-2017-02179 SAM

ID. campione (ID. sample): ACE-2017-00081432
ACE-2017-00081445

Saggi preliminari *(Preliminary assay)*

Microrganismi test *(Test Microorganisms)*		N		N1 Inclusione *(Inclusion)*		N2 Filtrazione *(Filtration)*			T	
	Dil	ufc *(cfu)* /ml	ufc *(cfu)* /ml	ufc *(cfu)* /ml	ufc *(cfu)* /ml	ufc *(cfu)* /ml	ufc *(cfu)* /ml	*Dil*	ufc *(cfu)* /ml	ufc *(cfu)* /ml
Staphylococcus aureus ATCC6538	-6	>330	>330	>330	>330	>165	>165	-2	>330	>330
	-7	31	49	33	30	22	29	-3	139	90
	-8	2	3	2	2	2	2	-4	10	6
	Mean values	4.0E+08		3.2E+08		2.6E+08		cfu/carrier	1.1E+07	VALIDO (VALID)
		VALIDO (VALID)		VALIDO (VALID)		VALIDO (VALID)		Log/carrier	7.04	
Pseudomonas aeruginosa ATCC15442	-6	>330	>330	209	244	>165	>165	-2	186	204
	-7	105	81	16	20	42	39	-3	21	24
	-8	9	6	2	3	4	4	-4	2	1
	Mean values	9.3E+08		2.2E+08		4.1E+08		cfu/carrier	2.0E+06	VALIDO (VALID)
		VALIDO (VALID)		VALIDO (VALID)		VALIDO (VALID)		Log/carrier	6.30	
Escherichia coli ATCC10536	-6	>330	>330	>330	>330	>165	>165	-2	>330	>330
	-7	58	33	46	31	41	55	-3	42	33
	-8	5	4	3	2	5	6	-4	2	2
	Mean values	4.6E+08		3.9E+08		4.8E+08		cfu/carrier	3.8E+06	VALIDO (VALID)
		VALIDO (VALID)		VALIDO (VALID)		VALIDO (VALID)		Log/carrier	6.58	
Enterococcus hirae ATCC10541	-6	121	156	182	168	142	>165	-2	148	162
	-7	10	14	18	12	12	21	-3	15	18
	-8	1	1	1	1	1	2	-4	1	0
	Mean values	1.4E+08		1.8E+08		1.5E+08		cfu/carrier	1.6E+06	VALIDO (VALID)
		VALIDO (VALID)		VALIDO (VALID)		VALIDO (VALID)		Log/carrier	6.20	

N: conteggio sospensione batterica (N: count of the bacterial suspension)
T: conteggio microrganismi sul supporto (T: count of the microrganisms on the carrier)
N1: conteggio sospensione microbica/soluzione latte 10% 1/20 v/v per inclusione (N1: count of microbial suspension/lait solution 10% 1/20 v/v by inclusion)
N2: conteggio sospensione microbica/soluzione latte 10% 1/20 v/v per filtrazione (N2: count of microbial suspension/lait solution 10% 1/20 v/v by filtration)

Sigla tecnico *(Technician signature)*:

Sigla Approvazione *(Approval signature)*:

Data fine *(Finished on)*: 05/08/2017

Data *(Date)*: 29/08/2017

Data inizio *(Started on)*: 03/08/17

ID. studio *(ID. Study)*: S-2017-02179 SAM

ID. campione (ID. sample): ACE-2017-00081432
ACE-2017-00081445

Validazione assenza dell'effetto residuo del disinfettante *(Evaluation of disinfectant residual effect)*

Microrganismi test *(Test Microorganisms)*		S					
		n_1 Inclusione *(Inclusion)*		n_2 Filtrazione *(Filtration)*		n_3	
	Dil	ufc *(cfu)* /ml		ufc *(cfu)* /98ml		ufc *(cfu)* /carrier	
Staphylococcus aureus ATCC6538	-7	39		30		48	
		3.9E+08	VALIDO (VALID)	3.0E+08	VALIDO (VALID)	4.8E+08	VALIDO (VALID)
Pseudomonas aeruginosa ATCC15442	-7	73		82		88	
		7.3E+08	VALIDO (VALID)	8.2E+08	VALIDO (VALID)	8.8E+08	VALIDO (VALID)
Escherichia coli ATCC10536	-7	61		78		83	
		6.1E+08	VALIDO (VALID)	7.8E+08	VALIDO (VALID)	8.3E+08	VALIDO (VALID)
Enterococcus hirae ATCC10541	-7	40		62		42	
		4.0E+08	VALIDO (VALID)	6.2E+08	VALIDO (VALID)	4.2E+08	VALIDO (VALID)

S: Validazione assenza effetto residuo del disinfettante (Evaluation of disinfectant residual effect)
n1: Ricerca di un effetto inibitore su agar (Research of inibitor effect on agar)
n2: Ricerca di un effetto inibitore sulla membrana filtrante (Research of inibitor effect on membrane)
n3: Ricerca di un effetto inibitore del supporto in agar (Research of inibitor effect on the support into agar)

Sigla tecnico *(Technician signature)*:

Sigla Approvazione *(Approval signature)*:

Data fine *(Finished on)*: 05/08/2017

Data *(Date)*: 29/08/2017

eurofins	Metodo di disinfezione di superfici per vie aeree - Determinazione dell'attività battericida, fungicida, levuricida, micobattericida, tubercolicida e sporicida) *(Methods of airbone disinfection of surfaces - Determination of bacterial, fungicidal, yeasticidal mycobactericidal, tuberculicidal and sporicidal activity)* Norma *(Standard)*: AFNOR NF T 72-281:2014	EDR: 1-P-PR-TEM-9005778

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ACE-2017-00081445

Saggio *(Assay)*

Microrganismi test *(Test Microorganisms)*	Dil	n'1							Filtrazione *(Filtration)*		n'2
		Inclusione *(Inclusion)*									
		Carrier 1		Carrier 2		Carrier 3					
		ufc *(cfu)* /ml	ufc *(cfu)* /ml	ufc *(cfu)* /ml	ufc *(cfu)* /ml	ufc *(cfu)* /ml	ufc *(cfu)* /ml		ufc *(cfu)* /10ml	ufc *(cfu)* /87ml	ufc *(cfu)* /carrier
Staphylococcus aureus ATCC6538	F	0	0	0	0	0	0	carrier 1	0	0	0
	-1	0	0	0	0	0	0	carrier 2	0	0	0
	-2	0	0	0	0	0	0	carrier 3	0	0	0
	-3	0	0	0	0	0	0				
	mean values cfu/ml	< 14		< 14		< 14		mean values	< 1.40E+01	< 1.40E+01	< 1.40E+01
	Log values	< 1.15						Log values	< 1.15	< 1.15	< 1.15
	Mean (Log)	< 1.15									
	d	> 5.89									
Pseudomonas aeruginosa ATCC15442	F	0	0	0	0	0	0		0	0	0
	-1	0	0	0	0	0	0		0	0	0
	-2	0	0	0	0	0	0		0	0	0
	-3	0	0	0	0	0	0				
	mean values cfu/ml	< 14		< 14		< 14		mean values	< 1.40E+01	< 1.40E+01	< 1.40E+01
	Log values	< 1.15						Log values	< 1.15	< 1.15	< 1.15
	Mean (Log)	< 1.15									
	d	> 5.15									
Escherichia coli ATCC10536	F	0	0	0	0	0	0		0	0	0
	-1	0	0	0	0	0	0		0	0	0
	-2	0	0	0	0	0	0		0	0	0
	-3	0	0	0	0	0	0				
	mean values cfu/ml	< 14		< 14		< 14		mean values	< 1.40E+01	< 1.40E+01	< 1.40E+01
	Log values	< 1.15						Log values	< 1.15	< 1.15	< 1.15
	Mean (Log)	< 1.15									
	d	> 5.43									
Enterococcus hirae ATCC10541	F	0	0	0	0	0	0	carrier 1	0	0	0
	-1	0	0	0	0	0	0	carrier 2	0	0	0
	-2	0	0	0	0	0	0	carrier 3	0	0	0
	-3	0	0	0	0	0	0				
	mean values cfu/ml	< 14		< 14		< 14		mean values	< 1.40E+01	< 1.40E+01	< 1.40E+01
	Log values	< 1.15						Log values	< 1.15	< 1.15	< 1.15
	Mean (Log)	< 1.15									
	d	> 5.05									

n'1: Conteggio per filtrazione dei microrganismi sopravvissuti *(Filtration count of surviver microrganisms)*
n'2: Conteggio dei microrganismi sopravvissuti sul carrier *(Surviver microrganisms on the carrier)*

Sigla tecnico *(Technician signature)*:

Sigla Approvazione *(Approval signature)*:

Data fine *(Finished on)*: 05/08/2017

Data *(Date)*: 29/08/17