TEST REPORT S-2017-04060 SAM

SPONSOR	LABORNUM LTD		
	GRASSALKOVICH ÚT 294		
	1239 BUDAPEST		
	HUNGARY		
TEST METHOD	ASTM F838-15a - Standard test method for determining microbial retention of membrane filters utilized for liquid filtration.		
TEST SAMPLE			
IDENTIFICATION	*SMART SHOWER FILTER BACTERIA STOP*		
SAMPLE TYPOLOGY	Detergent / household product, i.e. filter cartridges for shower filter		
STERILIZATION	No sterile product		
BATCH N.	Not provided	CODE	Not provided
MANUFACTURING DATE	Not provided	EXPIRY DATE	Not provided
MANUFACTURER	LABORNUM LTD		
MATERIALS	Not provided		
SAMPLE ID	ACE-2017-00117478	RECEIVING N.	EUITVI-95802
		RECEIVING DATE	28-Aug-17
	ACE-2017-00133682	RECEIVING N.	EUITVI-96846
		RECEIVING DATE	24-Oct-17
EQUIPMENT			
Ordinary microbiology laboratory equipment and in particular:			
Filtration system SARTORIUS Cellulose Nitrate (CN) Membrane Filter (0.2µm pore sizes) SARTORIUS Swinnex, Ø47mm (filter holder) MILLIPORE Peristaltic pump MASTERFLEX (Model: 77200-52) GHIARONI			
ANALYSIS STARTING DATE	27-Nov-17	**ANALYSIS ENDING DATE**	12-Dec-17
EXPERIMENTAL CONDITIONS			
NOTE	The filtration validation was performed in triplicate using the standard specifications, i.e. 3×10^{-3} LPM/cm^2.		
TEST TEMPERATURE	Room temperature		
SPORE CONCENTRATION	≥ 10^7 cfu/cm^2 of the filter surface (the container with the microbial suspension was connected to the test device)		
FILTRATION VOLUME	2000 ml		
FILTRATION TIME	About 17 seconds		
TEST SURFACE	2400 cm^2 (EFA, effective filtration area)		
FLOW RATE	Air flow 3 mL/min		

DILUENT/RECOVERY FLUID	*Page's Saline* Sodium Chloride 0.12 g Magnesium Sulphate 0.004 g Calcium Chloride 0.004 g Disodium hydrogen phosphate 0.142 g Potassium dihydrogenphospate 0.136 g Purified water q.s. to 1000 ml
TEST STRAINS	*Legionella pneumophila* ATCC33152
INCUBATION PERIOD	48 hour and 7 days (for the test) 72 hour and 7 days (for the control)
INCUBATION TEMPERATURE	37°C ±1°C

TEST METHOD (SUMMARY DESCRIPTION)

PREPARATION OF THE BACTERIAL SUSPENSION	The bacterial strain was kept freeze dried, according to internal Eurofins Biolab procedure SOP/GMB/009. The bacterial strain was transplanted on BCYE agar once and incubated at 37°C ± 1°C for 7 days. Within two hours from the beginning of the test, the final culture was suspended in Page's Saline using glass beads, and the suspension was centrifuged at 2000 g for 15 minutes. Pellet was suspended in Page's Saline and the bacterial suspension was diluted to a required concentration by means a spectrophotometer.
COUNT OF THE VALIDATION BACTERIAL SUSPENSION	The obtained bacterial strain was then diluted with peptone water to prepare the suspension containing not less than 10^7 CFU/cm^2 of the filter surface. The number of cfu/ml was determined by spreaded method by seeding of 1 ml for each dilutions (executed twice) on BCYE agar.
FLOW RATE VALIDATION	The test solution was filtered through the test sample and the filtered liquid was collected into a volumetric flask of 2000 ml; a stopwatch was started at the begin of the filtration and it was stopped at the finish of the 2000 ml filtration, in order to define the required flow rate. The flow value used in the retention test corresponds to the value obtained by adjusting the power of the peristaltic pump in order to ensure at least the theoretical flow. The flow rate values were expressed as l/min.
TEST EXECUTION	For each replica, the test sample was connected with a Swinnex filter holder containing a CN Membrane Filter (0.45µm pore sizes, previously sterilized), that was used for the control of the final microbial recovery; The volume (2 L) of the test solution, inoculated with a microbial suspension containing not less than 10^7 CFU/cm^2 of the filter surface, was filtered according to the required flow rate by means a container connected at the input with the air flow and at the output to the test filter; a filtration in continuous was so performed through the test sample SMART SHOWER FILTER BACTERIA STOP previously prepared and using a peristaltic pump that was connected with a Swinnex filter holder containing a CN Membrane Filter (0.45µm pore sizes) that was used for the control of the final microbial recovery. At the end of the filtration, the second Cellulose Nitrate Membrane Filter (SARTORIUS) was transferred onto agar plate and after incubation, the number of the residual microbial contamination was determined. The entire procedure was performed on triplicate for each test strain.

CONTROL	In order to verify the initial contamination of the test item and to verify the entire assay system, the same procedure above described for the test was used on the test sample filtering throught this, the equivalent volume of the liquid provided for the test but without microbial inoculum. The control was run immediately prior to the bacterial challenge test, and the control and challenge analysis filters were incubated simultaneously. The entire procedure was performed on triplicate for each test strain.
CALCULATION AND EXPRESSION OF THE RESULTS	The counting was performed using the number of colonies counted on each plate. The plates showing a number of colonies included in a range of 30-300 and 20-200 for the filtration method were used to perform the result calculation. *Microbial Retention* R = No –Na Where: R = Microbial Reduction (filter performance) No = initial inoculum Na = residual microbial counting on the second filter When no colonies are found on the analysis filter, the R is expressed as greater than the total number of the initial inoculum.
ASSAY VALIDITY CRITERIA	*N (inoculum)*: must be not less than 10^7 cfu/cm^2 of the test filter surface area *Control:* no growth/plate The test item is considered effective in the bacterial retention when R ≥ 7 Log.

RESULTS	**Log reductions (R) on the test filter**		
	Legionella pneumophila	REPLICA 1	>9.16
		REPLICA 2	>9.16
		REPLICA 3	>9.16
	See Attachment N.1		

CONCLUSIONS	On the basis of the results obtained, the test sample results effective in the bacterial retention against *Legionella pneumophila* ATCC33152 since the number of Log reductions on the test filter are >7 Log as per ASTM F838-15a and in compliance with the Sponsor requirements.
ATTACHMENT	N. 1: RAW DATA ELABORATION *(1 page)*

STUDY DIRECTOR (LAURA BRAMBILLA)	Laura Brambilla	ISSUED ON:	16-Jan-18

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The test results relate only to the items tested. Sampling, except specific indication on test report, is always intended to be made by the manufacturer. Characterization of the test sample is under Sponsor responsibility.

eurofins	Standard test method for determining bacterial retention of membrane filters utilized for liquid filtration Norma (Standard): ASTM F838 - 15a	EDR: 1-P-QM-TEM-9047099 Pagina *(Page)* 1 / 1

Data inizio *(Started on)*: 27/11/17

ID. studio *(ID. Study)*: S-2017-04060 SAM

ID. campione *(ID. sample)*: ACE-2017-00117478
ACE-2017-00133682

Test strains	*Dil*	N		Microscopic count		Effective Filtration Area (cm^2)
				cells n1	22	2400
		ufc / plate	ufc / plate	cells n2	26	**Inoculum volume (ml)**
	-6	>330	>330	cells n3	31	1
	-7	>330	>330	Counted cells	79	**Inoculum level/ cm^2**
Legionella pneumophila ATCC33152	-8	294	288	Average/square	1.6	1.2E+07
	Mean values (ufc/ml)	2.9E+10		Dilution factor	100000	VALIDO (VALID)
	Vitality (%)	**72.5**		**Total cells**	**4.0.E+10**	**Total Inoculum (2000 ml)**
		NON VALIDO (NOT VALID)				2.9E+10

N: conteggio sospensione batterica (N: count of the bacterial suspension)

Test strains		Retention of membrane filters		
		Test challenge		
		REPLICA 1	REPLICA 2	REPLICA 3
Legionella pneumophila ATCC33152	ufc (contaminant)/plate	0	1	0
	ufc/plate	0	0	0
	Na	< 20	< 20	< 20
	R (Log)/filter	> 9.16	> 9.16	> 9.16
		Control		
		REPLICA 1	REPLICA 2	REPLICA 3
	ufc/plate	20	7	11

Na =microrganismi residui ufc/membrana (Na = residual microrganism cfu/membrane)
R = performance del filtro, riduzione della vitalità (R = filter performance, vitality reduction)

Sigla tecnico *(Technician signature)*:

Data fine *(Finished on)*: 04/12/17

Sigla Approvazione *(Approval signature)*:

Data *(Date)*: 12/12/17