TEST REPORT S-2017-03397 SAM

REV 1	The present revision supersedes the previous report S-2017-03397 SAM (released on November 06th, 2017) and it is issued in order to correct the conclusion as per Sponsor requirements.
SPONSOR	LABORNUM LTD GRASSALKOVICH ÚT 294 1239 BUDAPEST HUNGARY
TEST METHOD	ASTM F838-15a - Standard test method for determining bacterial 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-2017-00104194 ACE-2017-00110236 (sample integration)		
RECEIVING N.	EUITVI-95609 EUITVI-95802 (sample integration)	RECEIVING DATE	07-Aug-17 28-Aug-17 (sample integration)

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-Oct-17	**ANALYSIS ENDING DATE**	06-Nov-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	*Tryptone water* NaCl 8.5 g Tryptone 1 g Purified Water to 1000 ml
TEST STRAINS	*Pseudomonas aeruginosa ATCC15442*
INCUBATION PERIOD	48 hour and 7 days (for the test) 72 hour and 7 days (for the control)
INCUBATION TEMPERATURE	30°C ±2°C

TEST METHOD (SUMMARY DESCRIPTION)

PREPARATION OF THE BACTERIAL SUSPENSION	The bacterial strains were kept frozen; before they were used, they were transplanted on TSA slants and kept in a refrigerator at 5°C ± 3°C, prepared according to internal Eurofins Biolab procedure SOP/GMB/009. The bacterial strain was transplanted on TSA slants two times and incubated at 37°C for 18-24 hours. Within two hours from the beginning of the test, the final culture was suspended in the diluent using glass beads, and the suspension was diluted to a necessary count using 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 pour plate method by seeding of 1 ml for each dilutions (executed twice) in TSA.
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 inithial 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)**		
	Pseudomonas aeruginosa	REPLICA 1	8.21
		REPLICA 2	8.25
		REPLICA 3	8.28
	See Attachment N.1		

CONCLUSIONS	On the basis of the results obtained, the test sample results effective in the bacterial retention since the number of Log reductions 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:	26-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)*: 25/10/17

ID. studio *(ID. Study)*: S-2017-03397 SAM

ID. campione *(ID. sample)*: ACE-2017-00117477

Test strains	*Dil*	N		Microscopic count		Effective Filtration Area (cm^2)
				cells n1	27	2400
		ufc / plate	ufc / plate	cells n2	19	Inoculum volume (ml)
	-6	>300	>300	cells n3	23	1
	-7	>300	>300	Counted cells	69	Inoculum level/ cm^2
Pseudomonas aeruginosa ATCC15442	-8	263	294	Average/square	1.4	1.2E+07
	Mean values (ufc/ml)	2.8E+10		Dilution factor	100000	VALIDO (VALID)
	Vitality (%)	80.0		Total cells	3.5.E+10	Total Inoculum (2000 ml)
		VALIDO (VALID)				2.8E+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
Pseudomonas aeruginosa ATCC15442	ufc (contaminant)/plate	22	14	3
	ufc/plate	172	156	147
	Na	172	156	147
	R (Log)/filter	8.21	8.25	8.28
		Control		
		REPLICA 1	REPLICA 2	REPLICA 3
	ufc/plate	1	10	6

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)*: 02/11/17

Sigla Approvazione *(Approval signature)*:

Data *(Date)*: 06/11/17