

TITLE	SCREENING AIR SANITIZATION TEST USING P.h.o.e.b.e Technology for BRID IN AN AIRLOCK CHAMBER AGAINST AN AEROSOL OF <i>E. coli</i> K12		
SPONSOR	COLOROBIA CONSULTING SRL		
	VIA PIETRAMARINA 53		
	50059 SOVIGLIANA, VINCI (FI)		
	ITALY		
TEST ITEM			
DEVICE IDENTIFICATION	P.h.o.e.b.e Technology for BRID		
DESCRIPTION	Indoor air purification device		
BATCH	0366C2	Code	HC25022019
MANUFACTURING DATE	July 2018	EXPIRY DATE	Not Provided
ACTIVE INGREDIENT	Colorobbia Photocatalyst (as per Sponsor's declaration)		
PARCEL REGISTRATION N.	IP-LV-2020014-AHA	RECEIVING DATE	14-Feb-2020
MATERIAL ITEM ALIQUOT	LV-MAT-FOV7-20-042-0745:a		
ANALYSIS STARTING DATE	12-Mar-2020	ANALYSIS ENDING DATE	07-May-2020
METHOD SETUP			
NOTE	<p>A set up phase has been conducted in order to verify the recovery of a nebulization of <i>E. coli</i> K12 inside a 1 m³ volume air lock chamber.</p> <p>The aim of the set up phase is to determine the starting inoculum, the nebulization time and the experimental conditions that allow to a significant recovery of microorganisms in the air after nebulization and verify their reproducibility.</p> <p>Test has been performed in triplicate.</p>		
TEST STRAIN	<i>Escherichia coli</i> K12	DSM 11250	
NEBULIZATION TIME	30 minutes		
AIR SAMPLING VOLUME	1 m ³		
CONTACT TIME (AFTER NEBULIZATION)	Immediately after nebulization (time 0) 30 minutes after nebulization 60 minutes after nebulization		


<p>PREPARATION OF THE TEST CHAMBER (FOR EACH NEBULIZATION RUN)</p>	<p>The sterilized Collison nebulizer - filled with bacterial suspension - was connected to the test chamber via a sterilized glass aerosol delivery tube surrounded by thermostatic water, in order to obtain a temperature in the aerosol of $20^{\circ}\text{C} \pm 5^{\circ}\text{C}$. The Collison nebulizer was connected to the air-flow system. The test chamber and its content were exposed to the spore bacterial aerosol for 30 minutes.</p> <p>The test chamber surfaces were sanitized with wipes imbibed with 6% H_2O_2 solution before and after each run, then dried with sterile wipes after 30 minutes exposure to H_2O_2. 6 contact plates were used to verify the microbial contamination after the sanitizing treatment. The contact plates were incubated at $30^{\circ}\text{-}35^{\circ}\text{C}$ for 2 days and then at $20\text{-}25^{\circ}\text{C}$ for 5 days.</p> <p>The level of the environmental contamination after test chamber opening and sanitization were monitored during the experimental phase in order to validate the sanitizing procedure using 6 witness plates placed outside the test chamber. Plates were incubated at $30^{\circ}\text{-}35^{\circ}\text{C}$ for 2 days and then at $20\text{-}25^{\circ}\text{C}$ for 5 days.</p>
<p>PHASE I</p>	<p>A bacterial suspension of <i>E. coli K12</i> showing a concentration of $1.5 - 5.0 \times 10^6$ cfu/ml has been diluted up to the decimal dilutions 10^{-4} and 10^{-5}. Each dilution was pour plated in duplicate. The number of colony-forming units per ml has been determined following incubation for 48 hours at $37^{\circ}\text{C} \pm 1^{\circ}\text{C}$ and the actual count of the microbial test suspension, expressed as N value, was calculated.</p> <p>The suspension has been nebulized inside the test chamber for 30 minutes. The aerosol dispersion inside the chamber has been maintained with using two internal fans. 4 TSA sterile plates were inserted into the test chamber as witness plates and opened just before closure of the chamber in order to sample and record bacteria touching the lower chamber surface during the exposure time (considering a sufficiently homogeneous dispersion of the aerosolized inoculum). After 30 minutes the nebulization was stopped and an air volume of 1 m^3 has been immediately sampled using SAS. Plates were incubated for 48 hours at $37^{\circ}\text{C} \pm 1^{\circ}\text{C}$ and the number of CFU/plate (Nc) was determined. This procedure has been performed in triplicate, in order to confirm the reproducibility of the adopted experimental conditions.</p> <p>The same procedure has been repeated in order to verify the recovery of the microorganism at different contact times (i.e. 30 minutes and 60 minutes) after nebulization.</p>
<p>PHASE II</p>	<p>A bacterial suspension of <i>E. coli K12</i> showing a concentration of $1.5 - 5.0 \times 10^7$ cfu/ml has been diluted up to the decimal dilutions 10^{-5} and 10^{-6}. Each dilution was pour plated in duplicate. The number of colony-forming units per ml has been determined following incubation for 48 hours at $37^{\circ}\text{C} \pm 1^{\circ}\text{C}$ and the actual count of the microbial test suspension, expressed as N value, was calculated.</p> <p>The suspension has been nebulized inside the test chamber for 30 minutes. 8 TSA sterile plates were inserted into the test chamber as sedimental plates and distributed in order to cover the entire surface base area. Plates were opened just before closure of the chamber in order to sample and record bacteria touching the lower chamber surface during the exposure time (considering a sufficiently homogeneous dispersion of the aerosolized inoculum). After 30 minutes the nebulization was stopped and the 8 sedimental plates recovered in order to measure the microorganism contamination. Plates were incubated for 48</p>

	<p>hours at 37°C±1°C and the number of CFU/plate (Nc) was determined. This procedure has been performed in triplicate, in order to confirm the reproducibility of the adopted experimental conditions and the homogeneous dispersion of the microbial aerosol.</p>	
RESULTS	See Addendum N. 1	
CONCLUSIONS OF METHOD SET-UP	<p>The use of fans inside the test chamber was not sufficient to keep the suspension circulating in the air for long contact times. For this reason, it has been decided to consider the number of surviving microorganisms recovered from the surface base area after nebulization, that ensure a better and reproducible recovery of <i>E. coli K12</i> in the adopted test conditions. Since recovery is not stable for longer contact times after nebulization, the reduction in viable count of bacteria after the use of the device is calculated in comparison to the recovery at time 0.</p>	
EXPERIMENTAL PROCEDURE - SCREENING AIR SANITIZATION TEST		
TEST STRAIN	<i>Escherichia coli K12</i>	DSM 11250
INOCULUM CONCENTRATION	1.5 – 5.0 x10 ⁷ cfu/ml	
NEBULIZATION TIME	30 minutes	
CONTACT TIME (AFTER NEBULIZATION)	4 hours	
PREPARATION AND COUNT OF THE BACTERIAL TEST SUSPENSION	<p>The bacterial suspension with a concentration of 1.5 – 5.0 x10⁷ cfu/ml has been diluted up to the decimal dilutions 10⁻⁵ and 10⁻⁶. Each dilution was pour plated in duplicate. The number of colony-forming units per ml has been determined following incubation for 48 hours at 37°C±1°C and the actual count of the microbial test suspension, expressed as N value, was calculated.</p>	
PREPARATION OF THE TEST CHAMBER (FOR EACH NEBULIZATION RUN)	<p>The test chamber surfaces were sanitized with wipes imbibed with 6% H₂O₂ solution before and after each run, then dried with sterile wipes after 30 minutes exposure to H₂O₂. 6 contact plates were used to verify the microbial contamination after the sanitizing treatment. The contact plates were incubated at 30°-35°C for 2 days and then at 20-25°C for 5 days.</p> <p>The sterilized Collison nebulizer - filled with bacterial suspension - was connected to the test chamber via a sterilized glass aerosol delivery tube surrounded by thermostatic water, in order to obtain a temperature in the aerosol of 20°C ± 5°C. The Collison nebulizer was connected to the air-flow system. The test chamber and its content were exposed to the bacterial aerosol for 30 minutes.</p> <p>The level of the environmental contamination after test chamber opening and sanitization were monitored during the experimental phase in order to validate the sanitizing procedure using 6 witness plates placed outside near the test chamber. Plates were incubated at 30°-35°c for 2 days and then at 20-25°C for 5 days.</p>	

ASSAY (TO BE PERFORMED IN TRIPLICATE)	<p>The device has been placed inside the test chamber with the filter near the nebulization delivery tube and it has been switched on for at least one hour before the beginning of the test.</p> <p>Then, a bacterial suspension of <i>E. coli K12</i> has been nebulized inside the test chamber for 30 minutes.</p> <p>8 TSA sterile plates were inserted into the test chamber as sedimental plates and distributed in order to cover the entire surface base area. Plates were opened just before closure of the chamber in order to sample and record bacteria touching the lower chamber surface during the exposure time (considering a sufficiently homogeneous dispersion of the aerosolized inoculum).</p> <p>After 30 minutes the nebulization was stopped and the device has been left on for a contact time of 4 hours. At the end of the set contact time, the 8 sedimental plates were recovered and incubated for at least 48 hours at 37°C±1°C, in order to measure the microorganism contamination. The number of CFU/plate (Na) was determined.</p>								
UNTREATED CONTROL (TO BE PERFORMED IN TRIPLICATE)	<p>An <i>untreated control (Nc)</i> has been performed, without the device, in order to measure the initial microbial contamination inside the test chamber.</p> <p>A bacterial suspension of <i>E. coli K12</i> has been nebulized inside the test chamber for 30 minutes.</p> <p>8 TSA sterile plates were inserted into the test chamber as sedimental plates and distributed in order to cover the entire surface base area. Plates were opened just before closure of the chamber in order to sample and record bacteria touching the lower chamber surface during the exposure time (considering a sufficiently homogeneous dispersion of the aerosolized inoculum).</p> <p>After 30 minutes the nebulization was stopped and the 8 sedimental plates were recovered and incubated for at least 48 hours at 37°C±1°C, in order to measure the microorganism contamination. The number of CFU/plate (Nc) was determined.</p>								
INTERPRETATION OF RESULTS	<p>Vitality reduction has been calculated at the end of the process as follows:</p> $R = Nc - Na$ <p>where:</p> <p>R = % Reduction of vitality</p> <p>Nc = number of cfu/plate in the untreated control at time 0</p> <p>Na = number of cfu/plate in the test assay at the set contact time</p>								
RESULTS	% of Reduction after 4 hours of contact time								
	<table border="1" style="width: 100%; border-collapse: collapse;"> <thead> <tr> <th style="width: 30%;">Microorganism</th> <th style="width: 20%;">Replica 1</th> <th style="width: 20%;">Replica 2</th> <th style="width: 30%;">Replica 3</th> </tr> </thead> <tbody> <tr> <td><i>Escherichia coli K12</i> DSM 11250</td> <td style="text-align: center;">99.34%</td> <td style="text-align: center;">98.81%</td> <td style="text-align: center;">99.22%</td> </tr> </tbody> </table>	Microorganism	Replica 1	Replica 2	Replica 3	<i>Escherichia coli K12</i> DSM 11250	99.34%	98.81%	99.22%
	Microorganism	Replica 1	Replica 2	Replica 3					
<i>Escherichia coli K12</i> DSM 11250	99.34%	98.81%	99.22%						
<table border="1" style="width: 100%; border-collapse: collapse;"> <tbody> <tr> <td style="width: 30%;">% R Average</td> <td colspan="3" style="text-align: center;">99.14%</td> </tr> </tbody> </table>	% R Average	99.14%							
% R Average	99.14%								
See Addendum N. 2									

CONCLUSIONS	The air treatment with P.h.o.e.b.e Technology for BRID resulted EFFECTIVE against <i>E. coli K12</i> after 4 hours of contact time, in the adopted test conditions. In particular, the treatment determined an average reduction of 99.14% in viability of the test organism.
ADDENDA	N. 1: RAW DATA ELABORATION – SETUP PHASE (11 <i>pages</i>) N. 2: RAW DATA ELABORATION – SCREENING PHASE (6 <i>pages</i>)

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	Prova per la valutazione dell'integrità di chiusura di contenitori verso spore nebulizzate
	(Validation of container closure integrity vs aerosolised spore)

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Data inizio (Started on): 12/03/2020

REPLICA 1

ID. studio (ID. Study): STULV20AA0485-1

ID. campione (ID. sample): LV-MAT-FOV7-20-042-0745:a

Replica 1 - Microbial control of test chamber after sanitizing treatment (before starting the assay)

Contact plates	Growth observed after 2 days @30-35°C	Growth observed after 5 days @20-25°C	Results (CFU/plate)	Pass/Fail
plate 1	6	0	6	PASS
plate 2	9	1	10	PASS
plate 3	2	1	3	PASS
plate 4	5	0	5	PASS
plate 5	8	0	8	PASS
plate 6	12	0	12	PASS

Replica 1 - Bacterial Suspension Concentration

Microorganism test	N (count test suspension)		
	Dil.	x (cfu/plate)	x' (cfu/plate)
Bacillus atrophaeus ATCC9372	10 ⁻⁴	260	252
	10 ⁻⁵	26	28
	Count (CFU/ml)	2.6E+06	VALID

Replica 1 - Witness plates (into the test chamber during the assay)

Sedimental plates	Growth observed after 5 days @30-35°C
plate 1	>330
plate 2	>330
plate 3	>330
plate 4	>330

Replica 1 - Microbial control of the room during the assay

Sedimental plates	Growth observed after 2 days @30-35°C	Growth observed after 5 days @20-25°C	Results (CFU/plate)	Pass/Fail
plate 1 (near collision)	21	0	21	PASS
plate 2 (near collision)	15	0	15	PASS
plate 3 (near collision)	13	1	14	PASS
plate 4 (work bench)	10	1	11	PASS
plate 5 (work bench)	17	0	17	PASS
plate 6 (work bench)	6	0	6	PASS

Replica 1 - Microbial control of test chamber after sanitizing treatment (after ending the assay)


Contact plates	Growth observed after 2 days @30-35°C	Growth observed after 5 days @20-25°C	Results (CFU/plate)	Pass/Fail
plate 1	25	1	26	PASS
plate 2	29	0	29	PASS
plate 3	31	2	33	PASS
plate 4	15	0	15	PASS
plate 5	22	0	22	PASS
plate 6	43	1	44	PASS

Sigla tecnico (Technician signature): 

Data fine (Finished on): 14/03/2020

Sigla Approvazione (Approval signature): 

Data (Date): 25/03/2020

	Prova per la valutazione dell'integrità di chiusura di contenitori verso spore nebulizzate
	(Validation of container closure integrity vs aerosolised spore)

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Data inizio (Started on): 12/03/2020

ID. studio (ID. Study): STULV20AA0485-1

ID. campione (ID. sample): LV-MAT-FOV7-20-042-0745:a

Replica 2 - Microbial control of test chamber after sanitizing treatment (before starting the assay)

Contact plates	Growth observed after 2 days @30-35°C	Growth observed after 5 days @20-25°C	Results (CFU/plate)	Pass/Fail
plate 1	7	0	7	PASS
plate 2	4	0	4	PASS
plate 3	5	2	7	PASS
plate 4	10	1	11	PASS
plate 5	9	1	10	PASS
plate 6	11	0	11	PASS

Replica 2 - Bacterial Suspension Concentration

Microorganism test	N (count test suspension)		
	Dil.	x (cfu/plate)	x' (cfu/plate)
Escherichia coli K12 DSM 11250	10 ⁻⁴	273	261
	10 ⁻⁵	30	28
	Count (CFU/ml)	2.7E+06	VALID

Replica 2 - Witness plates (into the test chamber during the assay)

Sedimental plates	Growth observed after 5 days @30-35°C
plate 1	>330
plate 2	>330
plate 3	>330
plate 4	>330

Replica 2 - Microbial control of the room during the assay

Sedimental plates	Growth observed after 2 days @30-35°C	Growth observed after 5 days @20-25°C	Results (CFU/plate)	Pass/Fail
plate 1 (near collision)	18	1	19	PASS
plate 2 (near collision)	17	0	17	PASS
plate 3 (near collision)	13	2	15	PASS
plate 4 (work bench)	9	3	12	PASS
plate 5 (work bench)	11	0	11	PASS
plate 6 (work bench)	3	0	3	PASS

Replica 2 - Microbial control of test chamber after sanitizing treatment (after ending the assay)


Contact plates	Growth observed after 2 days @30-35°C	Growth observed after 5 days @20-25°C	Results (CFU/plate)	Pass/Fail
plate 1	13	0	13	PASS
plate 2	11	3	14	PASS
plate 3	16	0	16	PASS
plate 4	15	1	16	PASS
plate 5	21	1	22	PASS
plate 6	20	0	20	PASS

Sigla tecnico (Technician signature): SD 27/03/20

Data fine (Finished on): 14/03/2020

Sigla Approvazione (Approval signature): JM 26/03/20

Data (Date): 25/03/2020

	Prova per la valutazione dell'integrità di chiusura di contenitori verso spore nebulizzate
	(Validation of container closure integrity vs aerosolised spore)

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Data inizio (Started on): 12/03/2020

REPLICA 3

ID. studio (ID. Study): STULV20AA0485-1

ID. campione (ID. sample): LV-MAT-FOV7-20-042-0745:a

Replica 3 - Microbial control of test chamber after sanitizing treatment (before starting the assay)

Contact plates	Growth observed after 2 days @30-35°C	Growth observed after 5 days @20-25°C	Results (CFU/plate)	Pass/Fail
plate 1	2	0	2	PASS
plate 2	5	0	5	PASS
plate 3	6	0	6	PASS
plate 4	4	2	6	PASS
plate 5	3	0	3	PASS
plate 6	7	1	8	PASS

Replica 3 - Bacterial Suspension Concentration

Microorganism test	N (count test suspension)		
	Dil.	x (cfu/plate)	x' (cfu/plate)
Escherichia coli K12 DSM 11250	10 ⁻⁴	288	293
	10 ⁻⁵	27	31
	Count (CFU/ml)	2.9E+06	VALID

Replica 3 - Witness plates (into the test chamber during the assay)

Sedimental plates	Growth observed after 5 days @30-35°C
plate 1	>330
plate 2	>330
plate 3	>330
plate 4	>330

Replica 3 - Microbial control of the room during the assay

Sedimental plates	Growth observed after 2 days @30-35°C	Growth observed after 5 days @20-25°C	Results (CFU/plate)	Pass/Fail
plate 1 (near collision)	20	1	21	PASS
plate 2 (near collision)	11	2	13	PASS
plate 3 (near collision)	18	0	18	PASS
plate 4 (work bench)	9	0	9	PASS
plate 5 (work bench)	7	0	7	PASS
plate 6 (work bench)	13	0	13	PASS

Replica 3 - Microbial control of test chamber after sanitizing treatment (after ending the assay)


Contact plates	Growth observed after 2 days @30-35°C	Growth observed after 5 days @20-25°C	Results (CFU/plate)	Pass/Fail
plate 1	6	0	6	PASS
plate 2	7	0	7	PASS
plate 3	3	0	3	PASS
plate 4	11	1	12	PASS
plate 5	9	0	9	PASS
plate 6	10	1	11	PASS

Sigla tecnico (Technician signature): SD 2763/20

Data fine (Finished on): 14/03/2020

Sigla Approvazione (Approval signature): ST 2663/20

Data (Date): 25/03/2020

	Prova per la valutazione dell'integrità di chiusura di contenitori verso spore nebulizzate
	(Validation of container closure integrity vs aerosolised spore)

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Data inizio (Started on): 12/03/2020

ID. studio (ID. Study): STULV20AA0485-1

ID. campione (ID. sample): LV-MAT-FOV7-20-042-0745:a

Set up results**Untreated control (Nc)**


Test microorganism	0 min after nebulization		
	Replica 1 (cfu/plate)	Replica 2 (cfu/plate)	Replica 3 (cfu/plate)
Escherichia coli K12 DSM 11250	221	169	193
Log value	2.34	2.23	2.29

Sigla tecnico (Technician signature): 

Data fine (Finished on): 14/03/2020

Sigla Approvazione (Approval signature): 

Data (Date): 25/03/2020

	Prova per la valutazione dell'integrità di chiusura di contenitori verso spore nebulizzate
	(Validation of container closure integrity vs aerosolised spore)

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Data inizio (Started on): 19/03/2020

REPLICA 1

ID. studio (ID. Study): STULV20AA0485-1

ID. campione (ID. sample): LV-MAT-FOV7-20-042-0745:a

Replica 1 - Microbial control of test chamber after sanitizing treatment (before starting the assay)

Contact plates	Growth observed after 2 days @30-35°C	Growth observed after 5 days @20-25°C	Results (CFU/plate)	Pass/Fail
plate 1	5	0	5	PASS
plate 2	7	0	7	PASS
plate 3	2	0	2	PASS
plate 4	11	2	13	PASS
plate 5	9	0	9	PASS
plate 6	9	1	10	PASS

Replica 1 - Bacterial Suspension Concentration

Microorganism test	N (count test suspension)		
	Dil.	x (cfu/plate)	x' (cfu/plate)
Bacillus atrophaeus ATCC9372	10 ⁻⁴	290	277
	10 ⁻⁵	28	31
	Count (CFU/ml)	2.8E+06	VALID

Replica 1 - Witness plates (into the test chamber during the assay)


Sedimental plates	Growth observed after 5 days @30-35°C
plate 1	>330
plate 2	>330
plate 3	>330
plate 4	>330

Replica 1 - Microbial control of the room during the assay

Sedimental plates	Growth observed after 2 days @30-35°C	Growth observed after 5 days @20-25°C	Results (CFU/plate)	Pass/Fail
plate 1 (near collision)	12	0	12	PASS
plate 2 (near collision)	23	2	25	PASS
plate 3 (near collision)	17	0	17	PASS
plate 4 (work bench)	11	0	11	PASS
plate 5 (work bench)	19	2	21	PASS
plate 6 (work bench)	10	1	11	PASS

Replica 1 - Microbial control of test chamber after sanitizing treatment (after ending the assay)


Contact plates	Growth observed after 2 days @30-35°C	Growth observed after 5 days @20-25°C	Results (CFU/plate)	Pass/Fail
plate 1	10	0	10	PASS
plate 2	17	1	18	PASS
plate 3	16	0	16	PASS
plate 4	20	3	23	PASS
plate 5	11	3	14	PASS
plate 6	10	0	10	PASS

Sigla tecnico (Technician signature): 

Data fine (Finished on): 21/03/2020

Sigla Approvazione (Approval signature): 

Data (Date): 25/03/2020

	Prova per la valutazione dell'integrità di chiusura di contenitori verso spore nebulizzate
	(Validation of container closure integrity vs aerosolised spore)

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Data inizio (Started on): 19/03/2020

REPLICA 2

ID. studio (ID. Study): STULV20AA0485-1

ID. campione (ID. sample): LV-MAT-FOV7-20-042-0745:a

Replica 2 - Microbial control of test chamber after sanitizing treatment (before starting the assay)

Contact plates	Growth observed after 2 days @30-35°C	Growth observed after 5 days @20-25°C	Results (CFU/plate)	Pass/Fail
plate 1	11	0	11	PASS
plate 2	6	1	7	PASS
plate 3	12	2	14	PASS
plate 4	7	0	7	PASS
plate 5	5	0	5	PASS
plate 6	9	1	10	PASS

Replica 2- Bacterial Suspension Concentration

Microorganism test	N (count test suspension)		
	Dil.	x (cfu/plate)	x' (cfu/plate)
Escherichia coli K12 DSM 11250	10 ⁻⁴	279	294
	10 ⁻⁵	26	29
	Count (CFU/ml)	2.9E+06	VALID

Replica 2 - Witness plates (into the test chamber during the assay)

Sedimental plates	Growth observed after 5 days @30-35°C
plate 1	>330
plate 2	>330
plate 3	>330
plate 4	>330

Replica 2- Microbial control of the room during the assay


Sedimental plates	Growth observed after 2 days @30-35°C	Growth observed after 5 days @20-25°C	Results (CFU/plate)	Pass/Fail
plate 1 (near collision)	12	0	12	PASS
plate 2 (near collision)	13	1	14	PASS
plate 3 (near collision)	19	0	19	PASS
plate 4 (work bench)	7	0	7	PASS
plate 5 (work bench)	10	1	11	PASS
plate 6 (work bench)	9	1	10	PASS

Replica 2 - Microbial control of test chamber after sanitizing treatment (after ending the assay)


Contact plates	Growth observed after 2 days @30-35°C	Growth observed after 5 days @20-25°C	Results (CFU/plate)	Pass/Fail
plate 1	21	0	21	PASS
plate 2	22	0	22	PASS
plate 3	15	0	15	PASS
plate 4	17	4	21	PASS
plate 5	10	3	13	PASS
plate 6	16	1	17	PASS

Sigla tecnico (Technician signature): 

Data fine (Finished on): 21/03/2020

Sigla Approvazione (Approval signature): 

Data (Date): 25/03/2020

	Prova per la valutazione dell'integrità di chiusura di contenitori verso spore nebulizzate
	(Validation of container closure integrity vs aerosolised spore)

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Data inizio (Started on): 19/03/2020

ID. studio (ID. Study): STULV20AA0485-1

ID. campione (ID. sample): LV-MAT-FOV7-20-042-0745:a

Replica 3

Replica 3 - Microbial control of test chamber after sanitizing treatment (before starting the assay)

Contact plates	Growth observed after 2 days @30-35°C	Growth observed after 5 days @20-25°C	Results (CFU/plate)	Pass/Fail
plate 1	16	2	18	PASS
plate 2	16	1	17	PASS
plate 3	7	0	7	PASS
plate 4	12	0	12	PASS
plate 5	15	3	18	PASS
plate 6	9	0	9	PASS

Replica 3 - Bacterial Suspension Concentration

Microorganism test	N (count test suspension)		
	Dil.	x (cfu/plate)	x' (cfu/plate)
Escherichia coli K12 DSM 11250	10 ⁻⁴	301	288
	10 ⁻⁵	31	29
	Count (CFU/ml)	3.0E+06	VALID

Replica 3 - Witness plates (into the test chamber during the assay)

Sedimental plates	Growth observed after 5 days @30-35°C
plate 1	>330
plate 2	>330
plate 3	>330
plate 4	>330

Replica 3 - Microbial control of the room during the assay


Sedimental plates	Growth observed after 2 days @30-35°C	Growth observed after 5 days @20-25°C	Results (CFU/plate)	Pass/Fail
plate 1 (near collision)	16	2	18	PASS
plate 2 (near collision)	12	0	12	PASS
plate 3 (near collision)	19	1	20	PASS
plate 4 (work bench)	11	0	11	PASS
plate 5 (work bench)	15	1	16	PASS
plate 6 (work bench)	17	1	18	PASS

Replica 3 - Microbial control of test chamber after sanitizing treatment (after ending the assay)


Contact plates	Growth observed after 2 days @30-35°C	Growth observed after 5 days @20-25°C	Results (CFU/plate)	Pass/Fail
plate 1	5	0	5	PASS
plate 2	9	2	11	PASS
plate 3	10	0	10	PASS
plate 4	6	0	6	PASS
plate 5	13	2	15	PASS
plate 6	8	2	10	PASS

Sigla tecnico (Technician signature): 

Data fine (Finished on): 21/03/2020

Sigla Approvazione (Approval signature): 

Data (Date): 25/03/2020

	Prova per la valutazione dell'integrità di chiusura di contenitori verso spore nebulizzate
	<i>(Validation of container closure integrity vs aerosolised spore)</i>

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Data inizio (Started on): 19/03/2020

ID. studio (ID. Study): STULV20AA0485-1

ID. campione (ID. sample): LV-MAT-FOV7-20-042-0745:a

Set up results**Untreated control (Nc)**


Test microorganism	0 min	30 min	60 min
	Replica 1 (cfu/plate)	Replica 2 (cfu/plate)	Replica 3 (cfu/plate)
Escherichia coli K12 DSM 11250	187	22	3
Log value	2.27	1.34	0.48

Sigla tecnico (Technician signature): *SD 27/03/20*

Data fine (Finished on): 21/03/2020

Sigla Approvazione (Approval signature): *SD 26/03/20*

Data (Date): 25/03/2020

	Prova per la valutazione dell'integrità di chiusura di contenitori verso spore nebulizzate
	(Validation of container closure integrity vs aerosolised spore)

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Data inizio (Started on): 22/04/2020

REPLICA

ID. studio (ID. Study): STULV20AA0485-1

ID. campione (ID. sample): LV-MAT-FOV7-20-042-0745:a

Microbial control of test chamber after sanitizing treatment (before starting the assay)

Contact plates	Growth observed after 2 days @30-35°C	Growth observed after 5 days @20-25°C	Results (CFU/plate)	Pass/Fail
plate 1	25	1	26	PASS
plate 2	14	1	15	PASS
plate 3	17	0	17	PASS
plate 4	23	0	23	PASS
plate 5	20	0	20	PASS
plate 6	12	0	12	PASS

Bacterial Suspension Concentration

Microorganism test	N (count test suspension)		
	Dil.	x (cfu/plate)	x' (cfu/plate)
Escherichia coli K12 DSM 11250	10 ⁻⁵	214	199
	10 ⁻⁶	20	18
	Count (CFU/ml)	2.1E+07	VALID

Microbial control of the room during the assay

Sedimental plates	Growth observed after 2 days @30-35°C	Growth observed after 5 days @20-25°C	Results (CFU/plate)	Pass/Fail
plate 1 (near collision)	2	0	2	PASS
plate 2 (near collision)	6	0	6	PASS
plate 3 (near collision)	0	0	0	PASS
plate 4 (work bench)	1	1	2	PASS
plate 5 (work bench)	4	0	4	PASS
plate 6 (work bench)	7	2	9	PASS

Microbial control of test chamber after sanitizing treatment (after ending the assay)

Contact plates	Growth observed after 2 days @30-35°C	Growth observed after 5 days @20-25°C	Results (CFU/plate)	Pass/Fail
plate 1	31	0	31	PASS
plate 2	26	0	26	PASS
plate 3	28	1	29	PASS
plate 4	19	0	19	PASS
plate 5	26	2	28	PASS
plate 6	20	2	22	PASS

Nc - Untreated control


Sedimental plates	Nc - control at time 0
	(cfu/plate)
plate 1	205
plate 2	150
plate 3	109
plate 4	129
plate 5	128
plate 6	124
plate 7	137
plate 8	159
cfu/plate average	143
Log	2.15

Sigla tecnico (Technician signature): CD 19/05/20

Data fine (Finished on): 24/04/2020

Sigla Approvazione (Approval signature): [Signature]

Data (Date): 27/04/2020

	Prova per la valutazione dell'integrità di chiusura di contenitori verso spore nebulizzate
	(Validation of container closure integrity vs aerosolised spore)

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Data inizio (Started on): 22/04/2020

REPLICA 2

ID. studio (ID. Study): STULV20AA0485-1

ID. campione (ID. sample): LV-MAT-FOV7-20-042-0745:a

Microbial control of test chamber after sanitizing treatment (before starting the assay)

Contact plates	Growth observed after 2 days @30-35°C	Growth observed after 5 days @20-25°C	Results (CFU/plate)	Pass/Fail
plate 1	11	0	11	PASS
plate 2	17	0	17	PASS
plate 3	16	2	18	PASS
plate 4	14	1	15	PASS
plate 5	22	0	22	PASS
plate 6	18	0	18	PASS

Bacterial Suspension Concentration

Microorganism test	N (count test suspension)		
	Dil.	x (cfu/plate)	x' (cfu/plate)
Escherichia coli K12 DSM 11250	10 ⁻⁵	214	199
	10 ⁻⁶	20	18
	Count (CFU/ml)	2.1E+07	VALID

Microbial control of the room during the assay

Sedimental plates	Growth observed after 2 days @30-35°C	Growth observed after 5 days @20-25°C	Results (CFU/plate)	Pass/Fail
plate 1 (near collision)	0	0	0	PASS
plate 2 (near collision)	1	1	2	PASS
plate 3 (near collision)	0	0	0	PASS
plate 4 (work bench)	3	0	3	PASS
plate 5 (work bench)	1	0	1	PASS
plate 6 (work bench)	4	0	4	PASS

Microbial control of test chamber after sanitizing treatment (after ending the assay)

Contact plates	Growth observed after 2 days @30-35°C	Growth observed after 5 days @20-25°C	Results (CFU/plate)	Pass/Fail
plate 1	29	1	30	PASS
plate 2	25	0	25	PASS
plate 3	13	3	16	PASS
plate 4	16	0	16	PASS
plate 5	24	0	24	PASS
plate 6	18	1	19	PASS

Nc - Untreated control


Sedimental plates	Nc - control at time 0
	(cfu/plate)
plate 1	133
plate 2	155
plate 3	110
plate 4	180
plate 5	118
plate 6	132
plate 7	166
plate 8	169
cfu/plate average	145
Log	2.16

Sigla tecnico (Technician signature): CO 19105120

Data fine (Finished on): 24/04/2020

Sigla Approvazione (Approval signature): GH 1965120

Data (Date): 27/04/2020

	Prova per la valutazione dell'integrità di chiusura di contenitori verso spore nebulizzate
	(Validation of container closure integrity vs aerosolised spore)

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Data inizio (Started on): 22/04/2020

REPLICA 3

ID. studio (ID. Study): STULV20AA0485-1

ID. campione (ID. sample): LV-MAT-FOV7-20-042-0745:a

Microbial control of test chamber after sanitizing treatment (before starting the assay)

Contact plates	Growth observed after 2 days @30-35°C	Growth observed after 5 days @20-25°C	Results (CFU/plate)	Pass/Fail
plate 1	15	0	15	PASS
plate 2	19	1	20	PASS
plate 3	22	1	23	PASS
plate 4	17	3	20	PASS
plate 5	12	0	12	PASS
plate 6	10	0	10	PASS

Bacterial Suspension Concentration

Microorganism test	N (count test suspension)		
	Dil.	x (cfu/plate)	x' (cfu/plate)
Escherichia coli K12 DSM 11250	10 ⁻⁵	214	199
	10 ⁻⁶	20	18
	Count (CFU/ml)	2.1E+07	VALID

Microbial control of the room during the assay

Sedimental plates	Growth observed after 2 days @30-35°C	Growth observed after 5 days @20-25°C	Results (CFU/plate)	Pass/Fail
plate 1 (near collision)	0	0	0	PASS
plate 2 (near collision)	0	0	0	PASS
plate 3 (near collision)	0	1	1	PASS
plate 4 (work bench)	3	0	3	PASS
plate 5 (work bench)	7	0	7	PASS
plate 6 (work bench)	0	2	2	PASS

Microbial control of test chamber after sanitizing treatment (after ending the assay)

Contact plates	Growth observed after 2 days @30-35°C	Growth observed after 5 days @20-25°C	Results (CFU/plate)	Pass/Fail
plate 1	33	2	35	PASS
plate 2	24	0	24	PASS
plate 3	18	0	18	PASS
plate 4	25	1	26	PASS
plate 5	22	0	22	PASS
plate 6	19	2	21	PASS

Nc - Untreated control


Sedimental plates	Nc - control at time 0
	(cfu/plate)
plate 1	178
plate 2	160
plate 3	122
plate 4	156
plate 5	179
plate 6	158
plate 7	140
plate 8	142
cfu/plate average	154
Log	2.19

Sigla tecnico (Technician signature): CD 19/05/20

Data fine (Finished on): 24/04/2020

Sigla Approvazione (Approval signature): AT 20/05/20

Data (Date): 27/04/2020

	Prova per la valutazione dell'integrità di chiusura di contenitori verso spore nebulizzate
	(Validation of container closure integrity vs aerosolised spore)

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Data inizio (Started on): 24/04/2020

REPLIC 1

ID. studio (ID. Study): STULV20AA0485-1

ID. campione (ID. sample): LV-MAT-FOV7-20-042-0745:a

Bacterial Suspension Concentration

Microorganism test	N (count test suspension)		
	Dil.	x (cfu/plate)	x' (cfu/plate)
Escherichia coli K12 DSM 11250	10 ⁻⁵	236	241
	10 ⁻⁶	22	25
	Count (CFU/ml)	2.4E+07	VALID

Preparation of the test chamber - Nc**Microbial control of test chamber after sanitizing treatment (before starting the assay)**

Contact plates	Growth observed after 2 days @30-35°C	Growth observed after 5 days @20-25°C	Results (CFU/plate)	Pass/Fail
plate 1	11	1	12	PASS
plate 2	16	0	16	PASS
plate 3	20	4	24	PASS
plate 4	14	1	15	PASS
plate 5	10	0	10	PASS
plate 6	8	0	8	PASS

Microbial control of the room during the assay

Sedimental plates	Growth observed after 2 days @30-35°C	Growth observed after 5 days @20-25°C	Results (CFU/plate)	Pass/Fail
plate 1 (near collision)	5	0	5	PASS
plate 2 (near collision)	0	2	2	PASS
plate 3 (near collision)	1	0	1	PASS
plate 4 (work bench)	2	1	3	PASS
plate 5 (work bench)	0	0	0	PASS
plate 6 (work bench)	0	0	0	PASS

Microbial control of test chamber after sanitizing treatment (after ending the assay)


Contact plates	Growth observed after 2 days @30-35°C	Growth observed after 5 days @20-25°C	Results (CFU/plate)	Pass/Fail
plate 1	22	1	23	PASS
plate 2	15	1	16	PASS
plate 3	18	0	18	PASS
plate 4	26	0	26	PASS
plate 5	16	0	16	PASS
plate 6	19	0	19	PASS

Sigla tecnico (Technician signature): 

Data fine (Finished on): 27/04/2020

Sigla Approvazione (Approval signature): 

Data (Date): 30/04/2020

	Prova per la valutazione dell'integrità di chiusura di contenitori verso spore nebulizzate
	(Validation of container closure integrity vs aerosolised spore)

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Data inizio (Started on): 24/04/2020

REPLICA 1

ID. studio (ID. Study): STULV20AA0485-1

ID. campione (ID. sample): LV-MAT-FOV7-20-042-0745:a

Preparation of the test chamber - Na**Microbial control of test chamber after sanitizing treatment (before starting the assay)**

Contact plates	Growth observed after 2 days @30-35°C	Growth observed after 5 days @20-25°C	Results (CFU/plate)	Pass/Fail
plate 1	9	0	9	PASS
plate 2	12	0	12	PASS
plate 3	8	2	10	PASS
plate 4	17	3	20	PASS
plate 5	13	0	13	PASS
plate 6	5	4	9	PASS

Microbial control of the room during the assay

Sedimental plates	Growth observed after 2 days @30-35°C	Growth observed after 5 days @20-25°C	Results (CFU/plate)	Pass/Fail
plate 1 (near collision)	3	0	3	PASS
plate 2 (near collision)	1	0	1	PASS
plate 3 (near collision)	0	1	1	PASS
plate 4 (work bench)	0	0	0	PASS
plate 5 (work bench)	1	0	1	PASS
plate 6 (work bench)	0	0	0	PASS

Microbial control of test chamber after sanitizing treatment (after ending the assay)

Contact plates	Growth observed after 2 days @30-35°C	Growth observed after 5 days @20-25°C	Results (CFU/plate)	Pass/Fail
plate 1	25	0	25	PASS
plate 2	16	1	17	PASS
plate 3	18	0	18	PASS
plate 4	12	1	13	PASS
plate 5	9	1	10	PASS
plate 6	14	0	14	PASS

Assay - Sedimental plates into the test chamber


Sedimental plates	Nc - control at time 0	Na - test at 4 hours
	(cfu/plate)	(cfu/plate)
plate 1	159	2
plate 2	166	2
plate 3	152	1
plate 4	133	0
plate 5	123	2
plate 6	160	0
plate 7	185	1
plate 8	142	0
cfu/plate average	153	1
Log	2.18	0.00
Log R	2.18	
% of Reduction in viability	99.34	

Sigla tecnico (Technician signature): 

Data fine (Finished on): 27/04/2020

Sigla Approvazione (Approval signature): 

Data (Date): 30/04/2020

	Prova per la valutazione dell'integrità di chiusura di contenitori verso spore nebulizzate
	(Validation of container closure integrity vs aerosolised spore)

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Data inizio (Started on): 28/04/2020

REPLICA 2

ID. studio (ID. Study): STULV20AA0485-1

ID. campione (ID. sample): LV-MAT-FOV7-20-042-0745:a

Bacterial Suspension Concentration

Microorganism test	N (count test suspension)		
	Dil.	x (cfu/plate)	x' (cfu/plate)
Escherichia coli K12 DSM 11250	10 ⁻⁵	161	172
	10 ⁻⁶	16	20
	Count (CFU/ml)	1.7E+07	VALID

Preparation of the test chamber - Nc**Microbial control of test chamber after sanitizing treatment (before starting the assay)**

Contact plates	Growth observed after 2 days @30-35°C	Growth observed after 5 days @20-25°C	Results (CFU/plate)	Pass/Fail
plate 1	15	1	16	PASS
plate 2	12	3	15	PASS
plate 3	18	0	18	PASS
plate 4	20	0	20	PASS
plate 5	24	2	26	PASS
plate 6	12	0	12	PASS

Microbial control of the room during the assay

Sedimental plates	Growth observed after 2 days @30-35°C	Growth observed after 5 days @20-25°C	Results (CFU/plate)	Pass/Fail
plate 1 (near collision)	0	0	0	PASS
plate 2 (near collision)	0	0	0	PASS
plate 3 (near collision)	1	0	1	PASS
plate 4 (work bench)	0	0	0	PASS
plate 5 (work bench)	4	0	4	PASS
plate 6 (work bench)	0	0	0	PASS

Microbial control of test chamber after sanitizing treatment (after ending the assay)

Contact plates	Growth observed after 2 days @30-35°C	Growth observed after 5 days @20-25°C	Results (CFU/plate)	Pass/Fail
plate 1	23	0	23	PASS
plate 2	21	0	21	PASS
plate 3	18	1	19	PASS
plate 4	26	2	28	PASS
plate 5	14	0	14	PASS
plate 6	16	1	17	PASS

Sigla tecnico (Technician signature):


SD 19/05/20

Data fine (Finished on): 30/04/2020

Sigla Approvazione (Approval signature):

JE 19/05/20

Data (Date): 04/05/2020

	Prova per la valutazione dell'integrità di chiusura di contenitori verso spore nebulizzate
	(Validation of container closure integrity vs aerosolised spore)

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Data inizio (Started on): 28/04/2020

ID. studio (ID. Study): STULV20AA0485-1

ID. campione (ID. sample): LV-MAT-FOV7-20-042-0745:a

REPLICAZ

Preparation of the test chamber - Na**Microbial control of test chamber after sanitizing treatment (before starting the assay)**

Contact plates	Growth observed after 2 days @30-35°C	Growth observed after 5 days @20-25°C	Results (CFU/plate)	Pass/Fail
plate 1	11	1	12	PASS
plate 2	7	1	8	PASS
plate 3	3	0	3	PASS
plate 4	5	0	5	PASS
plate 5	13	2	15	PASS
plate 6	9	0	9	PASS

Microbial control of the room during the assay

Sedimental plates	Growth observed after 2 days @30-35°C	Growth observed after 5 days @20-25°C	Results (CFU/plate)	Pass/Fail
plate 1 (near collision)	1	0	1	PASS
plate 2 (near collision)	0	0	0	PASS
plate 3 (near collision)	2	0	2	PASS
plate 4 (work bench)	2	1	3	PASS
plate 5 (work bench)	0	1	1	PASS
plate 6 (work bench)	0	0	0	PASS

Microbial control of test chamber after sanitizing treatment (after ending the assay)

Contact plates	Growth observed after 2 days @30-35°C	Growth observed after 5 days @20-25°C	Results (CFU/plate)	Pass/Fail
plate 1	13	1	14	PASS
plate 2	12	0	12	PASS
plate 3	19	0	19	PASS
plate 4	15	2	17	PASS
plate 5	22	0	22	PASS
plate 6	8	1	9	PASS

Assay - Sedimental plates into the test chamber


Sedimental plates	Nc - control at time 0	Na - test at 4 hours
	(cfu/plate)	(cfu/plate)
plate 1	110	3
plate 2	172	1
plate 3	185	1
plate 4	116	2
plate 5	131	0
plate 6	168	3
plate 7	185	1
plate 8	198	4
cfu/plate average	158	2
Log	2.20	0.27
Log R	1.93	
% of Reduction in viability	98.81	

Sigla tecnico (Technician signature): SD 19105120

Data fine (Finished on): 30/04/2020

Sigla Approvazione (Approval signature): AT 19105120

Data (Date): 04/05/2020

	Prova per la valutazione dell'integrità di chiusura di contenitori verso spore nebulizzate
	(Validation of container closure integrity vs aerosolised spore)

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Data inizio (Started on): 29/04/2020

REPLICA 3

ID. studio (ID. Study): STULV20AA0485-1

ID. campione (ID. sample): LV-MAT-FOV7-20-042-0745:a

Bacterial Suspension Concentration

Microorganism test	N (count test suspension)		
	Dil.	x (cfu/plate)	x' (cfu/plate)
Escherichia coli K12 DSM 11250	10 ⁻⁵	219	237
	10 ⁻⁶	24	25
	Count (CFU/ml)	2.3E+07	VALID

Preparation of the test chamber - Nc**Microbial control of test chamber after sanitizing treatment (before starting the assay)**

Contact plates	Growth observed after 2 days @30-35°C	Growth observed after 5 days @20-25°C	Results (CFU/plate)	Pass/Fail
plate 1	16	1	17	PASS
plate 2	21	1	22	PASS
plate 3	14	1	15	PASS
plate 4	10	0	10	PASS
plate 5	13	0	13	PASS
plate 6	20	2	22	PASS

Microbial control of the room during the assay


Sedimental plates	Growth observed after 2 days @30-35°C	Growth observed after 5 days @20-25°C	Results (CFU/plate)	Pass/Fail
plate 1 (near collision)	1	0	1	PASS
plate 2 (near collision)	0	0	0	PASS
plate 3 (near collision)	0	0	0	PASS
plate 4 (work bench)	0	1	1	PASS
plate 5 (work bench)	2	0	2	PASS
plate 6 (work bench)	3	0	3	PASS

Microbial control of test chamber after sanitizing treatment (after ending the assay)


Contact plates	Growth observed after 2 days @30-35°C	Growth observed after 5 days @20-25°C	Results (CFU/plate)	Pass/Fail
plate 1	27	1	28	PASS
plate 2	19	0	19	PASS
plate 3	15	0	15	PASS
plate 4	23	1	24	PASS
plate 5	16	0	16	PASS
plate 6	11	0	11	PASS

Sigla tecnico (Technician signature):  19/05/20

Data fine (Finished on): 04/05/2020

Sigla Approvazione (Approval signature):  19/05/20

Data (Date): 05/05/2020

	Prova per la valutazione dell'integrità di chiusura di contenitori verso spore nebulizzate
	(Validation of container closure integrity vs aerosolised spore)

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Data inizio (Started on): 29/04/2020

REPLICA 3

ID. studio (ID. Study): STULV20AA0485-1

ID. campione (ID. sample): LV-MAT-FOV7-20-042-0745:a

Preparation of the test chamber - Na**Microbial control of test chamber after sanitizing treatment (before starting the assay)**

Contact plates	Growth observed after 2 days @30-35°C	Growth observed after 5 days @20-25°C	Results (CFU/plate)	Pass/Fail
plate 1	5	1	6	PASS
plate 2	7	0	7	PASS
plate 3	10	3	13	PASS
plate 4	6	0	6	PASS
plate 5	4	1	5	PASS
plate 6	3	0	3	PASS

Microbial control of the room during the assay

Sedimental plates	Growth observed after 2 days @30-35°C	Growth observed after 5 days @20-25°C	Results (CFU/plate)	Pass/Fail
plate 1 (near collision)	2	1	3	PASS
plate 2 (near collision)	0	0	0	PASS
plate 3 (near collision)	1	0	1	PASS
plate 4 (work bench)	1	0	1	PASS
plate 5 (work bench)	0	1	1	PASS
plate 6 (work bench)	0	0	0	PASS

Microbial control of test chamber after sanitizing treatment (after ending the assay)

Contact plates	Growth observed after 2 days @30-35°C	Growth observed after 5 days @20-25°C	Results (CFU/plate)	Pass/Fail
plate 1	12	1	13	PASS
plate 2	16	2	18	PASS
plate 3	8	0	8	PASS
plate 4	17	0	17	PASS
plate 5	6	3	9	PASS
plate 6	12	0	12	PASS

Assay - Sedimental plates into the test chamber

Sedimental plates	Nc - control at time 0	Na - test at 4 hours
	(cfu/plate)	(cfu/plate)
plate 1	154	1
plate 2	173	1
plate 3	182	0
plate 4	161	1
plate 5	139	3
plate 6	142	0
plate 7	166	3
plate 8	161	1
cfu/plate average	160	1
Log	2.20	0.10
Log R	2.11	
% of Reduction in viability	99.22	

Sigla tecnico (Technician signature):

SD 19/05/20

Data fine (Finished on): 04/05/2020

Sigla Approvazione (Approval signature):

AT 19/05/20

Data (Date): 05/05/2020