

<b>TITLE</b>	<b>SCREENING AIR SANITIZATION TEST USING STERIL AIR PRO IN AN AIRLOCK CHAMBER AGAINST AN AEROSOL OF <i>E. coli</i> K12</b>		
<b>SPONSOR</b>	TECNO-GAZ S.p.A.		
	STRADA CAVALLI, 4		
	43038 SALA BAGANZA (PR)		
	ITALY		
<b>TEST ITEM</b>			
DEVICE IDENTIFICATION	Steril Air Pro		
DESCRIPTION	Indoor air purification device		
BATCH	OEZSA5529	CODE	Not Provided
MANUFACTURING DATE	Not Provided	EXPIRY DATE	Not Provided
ACTIVE INGREDIENT	Not Provided		
PARCEL REGISTRATION N.	IP-LV-2020099-ANY	RECEIVING DATE	08-Apr-2020
MATERIAL ITEM ALIQUOT	LV-MAT-F5PH-20-111-0502:a		
<b>ANALYSIS STARTING DATE</b>	05-May-2020	<b>ANALYSIS ENDING DATE</b>	14-May-2020
<b>METHOD SET-UP</b>			
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 m<sup>3</sup> 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 duplicate.</p>		
TEST STRAIN	<i>Escherichia coli</i> K12	DSM 11250	
INOCULUM CONCENTRATION	1.5 – 5.0 x10 <sup>7</sup> cfu/ml		
NEBULIZATION TIME	30 minutes		
INNER CHAMBER VOLUME	1 m <sup>3</sup>		
CONTACT TIME (AFTER NEBULIZATION)	Immediately after nebulization (time 0)		

<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°C ± 5°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<sub>2</sub>O<sub>2</sub> solution before and after each run, then dried with sterile wipes after 30 minutes exposure to H<sub>2</sub>O<sub>2</sub>. 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 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°-35°C for 2 days and then at 20-25°C for 5 days.</p>
<p>EXPERIMENTAL PHASE</p>	<p>A bacterial suspension of <i>E. coli K12</i> showing a concentration of 1.5 – 5.0 x10<sup>7</sup> cfu/ml has been diluted up to the decimal dilutions 10<sup>-5</sup> and 10<sup>-6</sup>. Each dilution was pour plated in duplicate.</p> <p>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 <b>N</b> 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 hours at 37°C±1°C and the number of CFU/plate (<b>Nc</b>) was determined. This procedure has been performed in duplicate, in order to confirm the reproducibility of the adopted experimental conditions and the homogeneous dispersion of the microbial aerosol.</p>
<p>RESULTS</p>	<p><b>See Addendum N. 1</b></p>
<p>CONCLUSIONS OF METHOD SET-UP</p>	<p>Since the suspension dispersed in the air was not stable enough to allow the measurement of surviving microorganisms through the use of SAS, 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.</p> <p>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>


PRELIMINARY TEST	
NOTE	A preliminary test has been conducted to verify a contact time of 30 minutes, in order to decide the final contact time of the screening phase. The test has been performed in duplicate.
TEST STRAIN	<i>Escherichia coli K12</i> DSM 11250
INOCULUM CONCENTRATION	1.5 – 5.0 x10 <sup>7</sup> cfu/ml
NEBULIZATION TIME	30 minutes
CONTACT TIME (AFTER NEBULIZATION)	30 minutes
PREPARATION AND COUNT OF THE BACTERIAL TEST SUSPENSION	The bacterial suspension with a concentration of 1.5 – 5.0 x10 <sup>7</sup> cfu/ml has been diluted up to the decimal dilutions 10 <sup>-5</sup> and 10 <sup>-6</sup> . 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 <b>N</b> value, was calculated.
PREPARATION OF THE TEST CHAMBER (FOR EACH NEBULIZATION RUN)	<p>The test chamber surfaces were sanitized with wipes imbibed with 6% H<sub>2</sub>O<sub>2</sub> solution before and after each run, then dried with sterile wipes after 30 minutes exposure to H<sub>2</sub>O<sub>2</sub>. 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	<p>The device has been placed inside the test chamber with the filter near the nebulization delivery tube. 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). After 30 minutes the nebulization was stopped and the device has been left on for a contact time of 30 minutes. 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 (<b>Na</b>) was determined.</p>

UNTREATED CONTROL	<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 (<b>Nc</b>) 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        Nc = number of cfu/plate in the untreated control at time 0        Na = number of cfu/plate in the test assay at the set contact time</p>												
RESULTS	<table border="1" style="width: 100%; border-collapse: collapse;"> <thead> <tr> <th colspan="3" style="text-align: center;">% of Reduction after 30 minutes of contact time</th> </tr> <tr> <th style="width: 30%;">Microorganism</th> <th style="width: 35%;">Replica 1</th> <th style="width: 35%;">Replica 2</th> </tr> </thead> <tbody> <tr> <td><i>Escherichia coli K12</i> DSM 11250</td> <td style="text-align: center;">99.35</td> <td style="text-align: center;">98.81</td> </tr> <tr> <td style="text-align: center;"><b>% R Average</b></td> <td colspan="2" style="text-align: center;">99.08</td> </tr> </tbody> </table>	% of Reduction after 30 minutes of contact time			Microorganism	Replica 1	Replica 2	<i>Escherichia coli K12</i> DSM 11250	99.35	98.81	<b>% R Average</b>	99.08	
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<b>% R Average</b>	99.08												
	<p><b>See Addendum N. 2</b></p>												
CONCLUSIONS OF PRELIMINARY TEST	<p>The air treatment with STERIL AIR PRO resulted <b>EFFECTIVE</b> against <i>E. coli K12</i> after 30 minutes of contact time, in the adopted test conditions.        It has been decided to maintain 30 minutes of contact time for the Screening phase.</p>												

<b>EXPERIMENTAL PROCEDURE - SCREENING AIR SANITIZATION TEST</b>	
NOTE	On the basis of the results obtained in the preliminary test, it has been decided to maintain 30 minutes as contact time for the screening test.
TEST STRAIN	<i>Escherichia coli K12</i> DSM 11250
INOCULUM CONCENTRATION	1.5 – 5.0 x10 <sup>7</sup> cfu/ml
NEBULIZATION TIME	30 minutes
CONTACT TIME (AFTER NEBULIZATION)	30 minutes
PREPARATION AND COUNT OF THE BACTERIAL TEST SUSPENSION	The bacterial suspension with a concentration of 1.5 – 5.0 x10 <sup>7</sup> cfu/ml has been diluted up to the decimal dilutions 10 <sup>-5</sup> and 10 <sup>-6</sup> . 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 <b>N</b> value, was calculated.
PREPARATION OF THE TEST CHAMBER (FOR EACH NEBULIZATION RUN)	<p>The test chamber surfaces were sanitized with wipes imbibed with 6% H<sub>2</sub>O<sub>2</sub> solution before and after each run, then dried with sterile wipes after 30 minutes exposure to H<sub>2</sub>O<sub>2</sub>. 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. 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). After 30 minutes the nebulization was stopped and the device has been left on for a contact time of 30 minutes. 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 (<b>Na</b>) 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 (<b>Nc</b>) was determined.</p>																
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<i>Escherichia coli K12</i> DSM 11250	99.44	99.02	99.21														
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<b>CONCLUSIONS</b>	<p>The air treatment with STERIL AIR PRO resulted <b>EFFECTIVE</b> against <i>E. coli K12</i> after 30 minutes of contact time, in the adopted test conditions.</p> <p>In particular, the treatment determined an average reduction of 99.22% in viability of the test organism.</p>																
<b>ADDENDA</b>	<p>N. 1: RAW DATA ELABORATION – SET-UP PHASE (3 pages)          N. 2: RAW DATA ELABORATION – PRELIMINARY TEST (4 pages)          N. 3: RAW DATA ELABORATION – SCREENING TEST (6 pages)</p>																

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

	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): 05/05/2020

ID. studio (ID. Study): STULV20AA1791-1

ID. campione (ID. sample): LV-MAT-F5PH-20-111-0502:a

**Bacterial Suspension Concentration**

Microorganism test	N (count test suspension)		
	Dil.	x (cfu/plate)	x' (cfu/plate)
Escherichia coli K12 DSM 11250	10 <sup>-5</sup>	267	284
	10 <sup>-6</sup>	25	29
	Count (CFU/ml)	2.8E+07	VALID

**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	0	7	PASS
plate 3	8	0	8	PASS
plate 4	5	0	5	PASS
plate 5	0	0	0	PASS
plate 6	6	2	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)	0	0	0	PASS
plate 2 (near collision)	0	1	1	PASS
plate 3 (near collision)	1	0	1	PASS
plate 4 (work bench)	0	0	0	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	12	0	12	PASS
plate 2	13	1	14	PASS
plate 3	8	0	8	PASS
plate 4	16	1	17	PASS
plate 5	9	0	9	PASS
plate 6	5	0	5	PASS

**Nc - Sedimental plates into the test chamber**


Sedimental plates	Nc - control at time 0
	(cfu/plate)
plate 1	316
plate 2	284
plate 3	296
plate 4	322
plate 5	330
plate 6	298
plate 7	275
plate 8	288
cfu/plate average	301
Log	2.48

Sigla tecnico (Technician signature): 

Data fine (Finished on): 07/05/2020

Sigla Approvazione (Approval signature): 

Data (Date): 07/05/2020

	Prova per la valutazione dell'integrità di chiusura di contenitori verso spore nebulizzate
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	Dil.	x (cfu/plate)	x' (cfu/plate)
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	Count (CFU/ml)	2.8E+07	VALID

**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	9	1	10	PASS
plate 3	12	2	14	PASS
plate 4	8	1	9	PASS
plate 5	6	0	6	PASS
plate 6	2	0	2	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	0	0	PASS
plate 5 (work bench)	1	0	1	PASS
plate 6 (work bench)	1	0	1	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	3	2	5	PASS
plate 2	5	2	7	PASS
plate 3	13	0	13	PASS
plate 4	7	0	7	PASS
plate 5	6	1	7	PASS
plate 6	4	0	4	PASS

**Nc - Sedimental plates into the test chamber**

Sedimental plates	Nc - control at time 0
	(cfu/plate)
plate 1	300
plate 2	274
plate 3	252
plate 4	269
plate 5	276
plate 6	292
plate 7	304
plate 8	268
cfu/plate average	279
Log	2.45


Sigla tecnico (Technician signature): 

Data fine (Finished on): 07/05/2020

Sigla Approvazione (Approval signature): 

Data (Date): 07/05/2020



	Prova per la valutazione dell'integrità di chiusura di contenitori verso spore nebulizzate
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**Bacterial Suspension Concentration**

Microorganism test	N (count test suspension)		
	Dil.	x (cfu/plate)	x' (cfu/plate)
Escherichia coli K12 DSM 11250	10 <sup>-5</sup>	267	284
	10 <sup>-6</sup>	25	29
	Count (CFU/ml)	2.8E+07	VALID

**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	3	0	3	PASS
plate 2	7	2	9	PASS
plate 3	10	1	11	PASS
plate 4	5	0	5	PASS
plate 5	9	1	10	PASS
plate 6	4	0	4	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)	1	0	1	PASS
plate 3 (near collision)	1	0	1	PASS
plate 4 (work bench)	0	0	0	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	12	2	14	PASS
plate 2	11	0	11	PASS
plate 3	9	2	11	PASS
plate 4	17	0	17	PASS
plate 5	5	1	6	PASS
plate 6	6	0	6	PASS

**Nc - Sedimental plates into the test chamber**


Sedimental plates	Nc - control at time 0
	(cfu/plate)
plate 1	266
plate 2	242
plate 3	258
plate 4	279
plate 5	282
plate 6	255
plate 7	242
plate 8	267
cfu/plate average	261
Log	2.42

Sigla tecnico (Technician signature): 

Data fine (Finished on): 07/05/2020

Sigla Approvazione (Approval signature): 

Data (Date): 07/05/2020

	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): 06/05/2020

ID. studio (ID. Study): STULV20AA1791-1 ID. campione (ID. sample): LV-MAT-F5PH-20-111-0502:a

**Bacterial Suspension Concentration**

Microorganism test	N (count test suspension)		
	Dil.	x (cfu/plate)	x' (cfu/plate)
Escherichia coli K12 DSM 11250	10 <sup>-5</sup>	276	251
	10 <sup>-6</sup>	29	27
	Count (CFU/ml)	2.7E+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	12	0	12	PASS
plate 2	4	2	6	PASS
plate 3	9	1	10	PASS
plate 4	5	0	5	PASS
plate 5	10	0	10	PASS
plate 6	7	1	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)	0	0	0	PASS
plate 2 (near collision)	0	0	0	PASS
plate 3 (near collision)	1	1	2	PASS
plate 4 (work bench)	2	0	2	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	11	0	11	PASS
plate 2	8	0	8	PASS
plate 3	7	3	10	PASS
plate 4	13	0	13	PASS
plate 5	16	2	18	PASS
plate 6	9	1	10	PASS

Sigla tecnico (Technician signature): 

Data fine (Finished on): 08/05/2020

Sigla Approvazione (Approval signature): 

Data (Date): 08/05/2020

	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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ID. studio (ID. Study): STULV20AA1791-1

ID. campione (ID. sample): LV-MAT-F5PH-20-111-0502: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	0	5	PASS
plate 2	9	1	10	PASS
plate 3	10	0	10	PASS
plate 4	8	0	8	PASS
plate 5	6	1	7	PASS
plate 6	7	1	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)	0	0	0	PASS
plate 2 (near collision)	0	0	0	PASS
plate 3 (near collision)	3	1	4	PASS
plate 4 (work bench)	0	0	0	PASS
plate 5 (work bench)	0	0	0	PASS
plate 6 (work bench)	1	0	1	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	2	15	PASS
plate 2	15	2	17	PASS
plate 3	12	0	12	PASS
plate 4	9	1	10	PASS
plate 5	11	0	11	PASS
plate 6	8	0	8	PASS

**Assay - Sedimental plates into the test chamber**

Sedimental plates	Nc - control at time 0	Na - test at 30 minutes
	(cfu/plate)	(cfu/plate)
plate 1	288	1
plate 2	271	1
plate 3	256	0
plate 4	269	3
plate 5	274	5
plate 6	243	0
plate 7	259	1
plate 8	290	3
cfu/plate average	269	2
Log	2.43	0.24
Log R		2.19
% of Reduction in viability		99.35

Sigla tecnico (Technician signature): 

Data fine (Finished on): 08/05/2020

Sigla Approvazione (Approval signature): 

Data (Date): 08/05/2020



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): 06/05/2020

ID. studio (ID. Study): STULV20AA1791-1

ID. campione (ID. sample): LV-MAT-F5PH-20-111-0502:a

**Bacterial Suspension Concentration**

Microorganism test	N (count test suspension)		
	Dil.	x (cfu/plate)	x' (cfu/plate)
Escherichia coli K12 DSM 11250	10 <sup>-5</sup>	254	271
	10 <sup>-6</sup>	21	25
	Count (CFU/ml)	2.6E+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	9	1	10	PASS
plate 2	5	1	6	PASS
plate 3	6	1	7	PASS
plate 4	10	0	10	PASS
plate 5	12	0	12	PASS
plate 6	8	1	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)	0	0	0	PASS
plate 2 (near collision)	0	0	0	PASS
plate 3 (near collision)	2	1	3	PASS
plate 4 (work bench)	0	0	0	PASS
plate 5 (work bench)	3	0	3	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	9	0	9	PASS
plate 2	12	0	12	PASS
plate 3	18	3	21	PASS
plate 4	16	0	16	PASS
plate 5	7	0	7	PASS
plate 6	20	2	22	PASS

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

Data fine (Finished on): 08/05/2020

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

Data (Date): 08/05/2020

	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): 06/05/2020

ID. studio (ID. Study): STULV20AA1791-1

ID. campione (ID. sample): LV-MAT-F5PH-20-111-0502: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	0	5	PASS
plate 2	9	0	9	PASS
plate 3	10	4	14	PASS
plate 4	8	1	9	PASS
plate 5	13	1	14	PASS
plate 6	6	1	7	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	1	1	PASS
plate 3 (near collision)	2	0	2	PASS
plate 4 (work bench)	3	2	5	PASS
plate 5 (work bench)	0	0	0	PASS
plate 6 (work bench)	1	0	1	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	0	12	PASS
plate 2	15	1	16	PASS
plate 3	9	0	9	PASS
plate 4	8	0	8	PASS
plate 5	16	2	18	PASS
plate 6	21	3	24	PASS

**Assay - Sedimental plates into the test chamber**


Sedimental plates	Nc - control at time 0	Na - test at 30 minutes
	(cfu/plate)	(cfu/plate)
plate 1	302	5
plate 2	274	3
plate 3	252	6
plate 4	296	3
plate 5	283	4
plate 6	255	2
plate 7	249	0
plate 8	267	3
cfu/plate average	272	3
Log	2.43	0.51
Log R	1.92	
% of Reduction in viability	98.81	

Sigla tecnico (Technician signature): 

Data fine (Finished on): 08/05/2020

Sigla Approvazione (Approval signature): 

Data (Date): 08/05/2020

	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): 12/05/2020

ID. studio (ID. Study): STULV20AA1791-1

ID. campione (ID. sample): LV-MAT-F5PH-20-111-0502:a

**Bacterial Suspension Concentration**

Microorganism test	N (count test suspension)		
	Dil.	x (cfu/plate)	x' (cfu/plate)
Escherichia coli K12 DSM 11250	10 <sup>-5</sup>	300	279
	10 <sup>-6</sup>	33	28
	Count (CFU/ml)	2.9E+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	10	1	11	PASS
plate 2	12	0	12	PASS
plate 3	8	0	8	PASS
plate 4	14	3	17	PASS
plate 5	11	1	12	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)	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	1	1	PASS
plate 5 (work bench)	2	0	2	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	11	1	12	PASS
plate 2	8	1	9	PASS
plate 3	9	0	9	PASS
plate 4	5	0	5	PASS
plate 5	12	2	14	PASS
plate 6	14	2	16	PASS

Sigla tecnico (Technician signature): 

Data fine (Finished on): 14/05/2020

Sigla Approvazione (Approval signature): 

Data (Date): 14/05/2020

	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): 12/05/2020

ID. studio (ID. Study): STULV20AA1791-1

ID. campione (ID. sample): LV-MAT-F5PH-20-111-0502: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	2	7	PASS
plate 2	7	0	7	PASS
plate 3	10	1	11	PASS
plate 4	12	0	12	PASS
plate 5	9	0	9	PASS
plate 6	6	1	7	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)	1	0	1	PASS
plate 3 (near collision)	2	0	2	PASS
plate 4 (work bench)	0	1	1	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)**

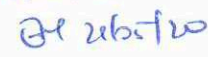
Contact plates	Growth observed after 2 days @30-35°C	Growth observed after 5 days @20-25°C	Results (CFU/plate)	Pass/Fail
plate 1	8	0	8	PASS
plate 2	7	2	9	PASS
plate 3	9	0	9	PASS
plate 4	11	1	12	PASS
plate 5	12	0	12	PASS
plate 6	6	0	6	PASS

**Assay - Sedimental plates into the test chamber**


Sedimental plates	Nc - control at time 0	Na - test at 30 minutes
	(cfu/plate)	(cfu/plate)
plate 1	302	0
plate 2	324	4
plate 3	297	0
plate 4	286	2
plate 5	300	0
plate 6	281	2
plate 7	269	4
plate 8	283	1
cfu/plate average	293	2
Log	2.47	0.21
Log R	2.26	
% of Reduction in viability	99.44	

Sigla tecnico (Technician signature): 

Data fine (Finished on): 14/05/2020

Sigla Approvazione (Approval signature): 

Data (Date): 14/05/2020

	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): 12/05/2020

ID. studio (ID. Study): STULV20AA1791-1

ID. campione (ID. sample): LV-MAT-F5PH-20-111-0502:a

**Bacterial Suspension Concentration**

Microorganism test	N (count test suspension)		
	Dil.	x (cfu/plate)	x' (cfu/plate)
Escherichia coli K12 DSM 11250	10 <sup>-5</sup>	292	284
	10 <sup>-6</sup>	29	31
	Count (CFU/ml)	2.9E+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	5	0	5	PASS
plate 2	9	0	9	PASS
plate 3	6	2	8	PASS
plate 4	11	0	11	PASS
plate 5	12	1	13	PASS
plate 6	8	1	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)	0	0	0	PASS
plate 2 (near collision)	0	0	0	PASS
plate 3 (near collision)	0	0	0	PASS
plate 4 (work bench)	1	0	1	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	9	0	9	PASS
plate 2	6	0	6	PASS
plate 3	8	1	9	PASS
plate 4	14	3	17	PASS
plate 5	16	2	18	PASS
plate 6	7	0	7	PASS


Sigla tecnico (Technician signature): 

Data fine (Finished on): 14/05/2020

Sigla Approvazione (Approval signature): 

Data (Date): 14/05/2020



	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): 12/05/2020

ID. studio (ID. Study): STULV20AA1791-1

ID. campione (ID. sample): LV-MAT-F5PH-20-111-0502: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	11	1	12	PASS
plate 2	5	0	5	PASS
plate 3	7	0	7	PASS
plate 4	12	1	13	PASS
plate 5	9	1	10	PASS
plate 6	8	1	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)	0	0	0	PASS
plate 4 (work bench)	2	0	2	PASS
plate 5 (work bench)	0	0	0	PASS
plate 6 (work bench)	3	1	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	8	2	10	PASS
plate 2	11	0	11	PASS
plate 3	15	3	18	PASS
plate 4	9	1	10	PASS
plate 5	13	0	13	PASS
plate 6	5	0	5	PASS

**Assay - Sedimental plates into the test chamber**


Sedimental plates	Nc - control at time 0	Na - test at 30 minutes
	(cfu/plate)	(cfu/plate)
plate 1	297	4
plate 2	258	3
plate 3	292	6
plate 4	316	3
plate 5	279	2
plate 6	314	1
plate 7	288	0
plate 8	305	4
cfu/plate average	294	3
Log	2.47	0.46
Log R	2.01	
% of Reduction in viability	99.02	

Sigla tecnico (Technician signature): 

Data fine (Finished on): 14/05/2020

Sigla Approvazione (Approval signature): 

Data (Date): 14/05/2020

	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): 12/05/2020

ID. studio (ID. Study): STULV20AA1791-1

ID. campione (ID. sample): LV-MAT-F5PH-20-111-0502:a

**Bacterial Suspension Concentration**

Microorganism test	N (count test suspension)		
	Dil.	x (cfu/plate)	x' (cfu/plate)
Escherichia coli K12 DSM 11250	10 <sup>-5</sup>	316	308
	10 <sup>-6</sup>	32	33
	Count (CFU/ml)	3.1E+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	13	3	16	PASS
plate 2	5	0	5	PASS
plate 3	16	0	16	PASS
plate 4	8	2	10	PASS
plate 5	9	1	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)	1	1	2	PASS
plate 2 (near collision)	1	0	1	PASS
plate 3 (near collision)	2	0	2	PASS
plate 4 (work bench)	1	0	1	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	9	0	9	PASS
plate 2	8	1	9	PASS
plate 3	16	0	16	PASS
plate 4	8	0	8	PASS
plate 5	17	2	19	PASS
plate 6	5	0	5	PASS

Sigla tecnico (Technician signature): 

Data fine (Finished on): 14/05/2020

Sigla Approvazione (Approval signature): 

Data (Date): 14/05/2020

	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): 12/05/2020

ID. studio (ID. Study): STULV20AA1791-1

ID. campione (ID. sample): LV-MAT-F5PH-20-111-0502: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	8	0	8	PASS
plate 2	6	0	6	PASS
plate 3	12	2	14	PASS
plate 4	9	0	9	PASS
plate 5	14	2	16	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)	1	1	2	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)	0	0	0	PASS
plate 6 (work bench)	1	0	1	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	5	0	5	PASS
plate 3	8	2	10	PASS
plate 4	6	1	7	PASS
plate 5	13	0	13	PASS
plate 6	8	1	9	PASS

**Assay - Sedimental plates into the test chamber**

Sedimental plates	Nc - control at time 0	Na - test at 30 minutes
	(cfu/plate)	(cfu/plate)
plate 1	316	0
plate 2	314	3
plate 3	279	5
plate 4	298	0
plate 5	311	0
plate 6	275	4
plate 7	286	5
plate 8	319	2
cfu/plate average	300	2
Log	2.48	0.38
Log R	2.10	
% of Reduction in viability	99.21	

Sigla tecnico (Technician signature): 

Data fine (Finished on): 14/05/2020

Sigla Approvazione (Approval signature): 

Data (Date): 14/05/2020