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Department of Molecular and Developmental Medicine

The study report was written pursuant to a Contract dated 2-10-2018 and 17-10-2018, between the University of Siena and Light Progress

Report (version 1.0)

Siena, 26th June 2020

TEST ON *UV-BOX-E3/40H-NX* BY LIGHT PROGRESS

INDEX

TARGET

To assess the effectiveness of the Light Progress UV-BOX-E3/40H-NX in inactivating selected bacterial isolates at a fixed distance and two exposure times.

OPERATIVE SPHERE

The tests were requested by Light Progress and conducted by qualified staff under the supervision of Prof. Gabriele Messina, affiliated with the Department of Molecular and Developmental Medicine, University of Siena where the tests took place.

EQUIPMENT

- **Light Progress UV-BOX-E3/40H-NX** (Image 1 and 2)
- **50 ml Falcon centrifuge**
- **Plate Count Agar**
- **Sterile 90 mm Ø disposable Petri dishes** for *bacterial* cultures
- **D/E neutralizing broth medium** for recovery phase
- **Microorganisms:** *Pseudomonas aeruginosa ATCC 27853; Escherichia coli ATCC 8739; Staphylococcus aureus ATCC 43300; Salmonella typhimurium ATCC 23853; Klebsiella pneumoniae ATCC BAA-1705*
- **Inoculum carriers:** 20 cm^2 Stainless steel carriers
- **Bio Class thermostat bath, Velp vortex mixer, Kartell hot plate, laminar flow hood with HEPA BIO/4 filter, KW Refrigerator +2 to +8°C, Sartorius precision balance, Nichipet EX micropipette, KW and Isco temperature chambers, Fedegari sterilising autoclave, Sigma phosphate buffered saline (PBS), sterile polypropylene tubes, sterile spatula, sterile pliers, sterile glass bottles, various glassware, centrifuge**
- **Microsoft Excel 2016** for data collection
- **Stata SE/16.0** for statistical analysis

Image 2 Light Progress UV-BOX-E3/40H-NX with light OFF

Image 1 Light Progress UV-BOX-E3/40H-NX with light ON

PARAMETERS ESTABLISHED FOR THE TESTS

Exposure timed: i) in the preliminary stage we tested several exposure times; ii) the main tests were conducted at 2 and 3 minutes
Distance from source: 33 cm (from upper light source)
Repetitions: testing was performed three times in triplicate between May and June 2020
Concentrations: $1.5x10^7$; $1.5x10^6$ CFU/mL

OPERATIVE TECHNIQUE

Propagation of the microorganisms was conducted according to standard operating procedure (ANNEX 1 for details, separate document). The inoculum was re-suspended in test culture at concentrations of approx. $1.5x10^6$ and $1.5x10^7$ CFU/mL.

Set Up
Stainless steel carriers were inoculated with 100 µl of test culture. The inoculum was spread with a sterile spatula over approximately 20 cm^2 of each carrier and left to dry inside the laminar flow hood.

Carriers were placed as follows:
1) Treated sample: 2 horizontal supports (1 per bacterial concentration) were placed in the UV-box, 33 cm from the upper light source of the device.
2) Positive control: 2 supports (1 per bacterial concentration) were left in the lab, out of range of UV radiation.

At the end of exposure, exposed and non-exposed carriers were transferred to 90 mm Petri dishes and 10 mL D/E medium was added. Subsequently, the D/E medium was transferred to a 50 mL Falcon centrifuge and spun for 40 minutes at 4500 rpm. The supernatant was eliminated and the pellet re-suspended in 1 mL D/E medium. Finally, 100 µl was transferred to Plate Count Agar and incubated at 36°C for 48 h.

DATABASE ORGANIZATION

The first tests were conducted in an exploratory way so as to standardize the operative protocol and exposure times. Exposures between 1 and 20 minutes were tested for all microbes (ANNEX 1 for details, separate document). The following variables collected during the study were entered in a database:

- Petri dish ID
- CFUs/mL
- Microorganism species
- Inoculum concentrations

DATA ANALYSIS AND STATISTICS

Data analysis and statistical computations were supervised by Prof. Gabriele Cevenini, Department of Medical Biotechnologies, Laboratory of Applied Bioengineering – IT Engineering in Medicine, University of Siena. Microsoft Excel software (ver. 16) was used for preliminary statistical evaluation of empirical data and to organize the database. Analysis was conducted using Stata software Ver 16. The results of each experiment in triplicate were expressed as mean CFU/mL for each test. The mean logarithmic reduction and the 95% confidence interval were evaluated from the replicate data of each microbe. The tables created show the mean logarithmic reduction in CFU/mL of each microorganism compared to the real positive controls (Tables A) and theoretical positive controls (Tables B), as maximum number of CFU/mL ($1.5x10^7$ CFU/mL and $1.5x10^6$ CFU/mL).

RESULTS

The results for the different microorganisms are shown in Tables 1A to 5B.

Table 1A. *Staphylococcus aureus* ATCC 43300 CFU/mL logarithmic reduction (real positive control reference)

	2 minutes				3 minutes			
	$1.5x10^7$ (CFU/mL)		$1.5x10^6$ (CFU/mL)		$1.5x10^7$ (CFU/mL)		$1.5x10^6$ (CFU/mL)	
	mean	95% IC	mean	95% IC	mean	95% IC	mean	95% IC
Log_{10} reduction	6.68	5.62 - 7.74	6.22	6.04 - 6.39	7.22	7.04 - 7.39	6.22	6.04 - 6.39

Table 1B. *Staphylococcus aureus* ATCC 43300 CFU/mL logarithmic reduction (theoretical positive control reference)

	2 minutes				3 minutes			
	$1.5x10^7$ (CFU/mL)		$1.5x10^6$ (CFU/mL)		$1.5x10^7$ (CFU/mL)		$1.5x10^6$ (CFU/mL)	
	mean	95% IC	mean	95% IC	mean	95% IC	mean	95% IC
Log_{10} reduction	6.94	5.90 - 7.99	6.48	6.48 - 6.48	7.04	6.19 - 7.89	6.48	6.19 - 6.48

Table 2A. *Escherichia coli* ATCC 8739 CFU/mL logarithmic reduction (real positive control reference)

	2 minutes				3 minutes			
	$1.5x10^7$ (CFU/mL)		$1.5x10^6$ (CFU/mL)		$1.5x10^7$ (CFU/mL)		$1.5x10^6$ (CFU/mL)	
	mean	95% IC	mean	95% IC	mean	95% IC	mean	95% IC
Log_{10} reduction	7.45	7.30 - 7.59	6.45	6.30 - 6.59	7.45	3.95 - 7.59	6.45	6.30 - 6.59

Table 2B. *Escherichia coli* ATCC 8739 CFU/mL logarithmic reduction (theoretical positive control reference)

	2 minutes				3 minutes			
	$1.5x10^7$ (CFU/mL)		$1.5x10^6$ (CFU/mL)		$1.5x10^7$ (CFU/mL)		$1.5x10^6$ (CFU/mL)	
	mean	95% IC	mean	95% IC	mean	95% IC	mean	95% IC
Log_{10} reduction	7.48	7.48 - 7.48	6.48	6.48 - 6.48	7.48	7.48 - 7.48	6.48	6.48 - 6.48

Table 3A. *Pseudomonas aeruginosa* ATCC 27853 CFU/mL logarithmic reduction (real positive control reference)

	2 minutes				3 minutes			
	$1.5x10^7$ (CFU/mL)		$1.5x10^6$ (CFU/mL)		$1.5x10^7$ (CFU/mL)		$1.5x10^6$ (CFU/mL)	
	mean	95% IC	mean	95% IC	mean	95% IC	mean	95% IC
Log_{10} reduction	7.05	6.87 - 7.22	6.05	5.87 - 6.22	7.05	6.87 - 7.22	6.05	5.87 - 6.22

Table 3B. *Pseudomonas aeruginosa* ATCC 27853 CFU/mL logarithmic reduction (theoretical positive control reference)

	2 minutes				3 minutes			
	$1.5x10^7$ (CFU/mL)		$1.5x10^6$ (CFU/mL)		$1.5x10^7$ (CFU/mL)		$1.5x10^6$ (CFU/mL)	
	mean	95% IC	mean	95% IC	mean	95% IC	mean	95% IC
Log_{10} reduction	7.48	7.48 - 7.48	6.48	6.48 - 6.48	7.48	7.48 - 7.48	6.48	6.48 - 6.48

Table 4A. *Salmonella typhimurium* ATCC 23853 CFU/mL logarithmic reduction (real positive control reference)

	2 minutes				3 minutes			
	$1.5x10^7$ (CFU/mL)		$1.5x10^6$ (CFU/mL)		$1.5x10^7$ (CFU/mL)		$1.5x10^6$ (CFU/mL)	
	mean	95% IC	mean	95% IC	mean	95% IC	mean	95% IC
Log_{10} reduction	6.03	4.74 - 7.32	5.03	3.74 - 6.32	6.46	4.95 - 7.97	5.66	4.61 - 6.71

Table 4B. *Salmonella typhimurium* ATCC 23853 CFU/mL logarithmic reduction (theoretical positive control reference)

	2 minutes				3 minutes			
	$1.5x10^7$ (CFU/mL)		$1.5x10^6$ (CFU/mL)		$1.5x10^7$ (CFU/mL)		$1.5x10^6$ (CFU/mL)	
	mean	95% IC	mean	95% IC	mean	95% IC	mean	95% IC
Log_{10} reduction	6.31	5.05 - 7.56	5.31	4.05 - 6.56	6.74	5.30 - 8.18	5.94	4.90 - 6.99

Table 5A. *Klebsiella pneumoniae* ATCC BAA-1705 CFU/mL logarithmic reduction (real positive control reference)

	2 minutes				3 minutes			
	$1.5x10^7$ (CFU/mL)		$1.5x10^6$ (CFU/mL)		$1.5x10^7$ (CFU/mL)		$1.5x10^6$ (CFU/mL)	
	mean	95% IC	mean	95% IC	mean	95% IC	mean	95% IC
Log_{10} reduction	6.48	5.85 - 7.11	6.01	5.50 - 6.52	7.01	6.50 - 7.52	6.01	5.50 - 6.52

Table 5B. Klebsiella pneumoniae ATCC BAA-1705 CFU/mL logarithmic reduction (theoretical positive control reference)

	2 minutes				3 minutes			
	$1.5x10^7$ (CFU/mL)		$1.5x10^6$ (CFU/mL)		$1.5x10^7$ (CFU/mL)		$1.5x10^6$ (CFU/mL)	
	mean	95% IC	mean	95% IC	mean	95% IC	mean	95% IC
Log_{10} reduction	6.94	5.90 - 7.99	6.48	6.48 - 6.48	7.48	7.48 - 7.48	6.48	6.48 - 6.48

DISCUSSION AND CONCLUSIONS

The inoculum spread on the steel plates was coherent with those described in the literature and proposed by the manufacturer.

The Light Progress UV-BOX-E3/40H-NX-R had a biocidal effect on all microbes at exposures of 2 and 3 minutes.

The higher bacterial inactivation effect, 6-7 $\log_{10}$, was achieved with 3 minutes of exposure for all five strains.

Different concentrations and dilutions on the carriers could produce different results (see ANNEX 1 for details, separate document).

Different distances and exposure times could produce different results (see ANNEX 1 for details, separate document).

Further tests using different exposure times are underway in order to implement the current CFU/mL logarithmic reduction model for all five strains.

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CONTACTS

Prof. Gabriele MESSINA, University of Siena, Department of Molecular and Developmental Medicine, Via A. Moro 2, 53100 Siena. Phone: +39-(0)577-235-423; Fax: +39-(0)577-234-090; Mobile: + 39-339-6699-422; Email: *gabriele.messina@unisi.it*

Prof. Gabriele Messina

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CERTIFICATO

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EN ISO 9001:2015

La comprova dell'applicazione conforme ai criteri normativi è stata conseguita e viene attestata secondo la procedura TÜV AUSTRIA CERT per

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Dipartimento di Medicina Molecolare e dello Sviluppo
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IT-53100 Siena (SI), Via Banchi di Sotto, 55

Sede operativa:
IT-53100 Siena (SI), Via Aldo Moro, 2

Campo di applicazione

Titolazioni di anticorpi mediante analisi sierologiche; diagnosi microbiologiche; analisi epidemiologiche; determinazioni chimico/biologiche ambientali.

N° registrazione certificato: 20100193005061

Valido fino al 2022-02-05
Prima certificazione: 2019-02-06

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Vienna, 2019-02-06

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ANNEX 1:
TEST ON UV-BOX-E3/40H-NX
BY LIGHT PROGRESS

MICROBES

- *Pseudomonas aeruginosa ATCC 27853*
- *Escherichia coli ATCC 8739*
- *Stafilococco aureus ATCC 43300*
- *Salmonella Typhimurium ATCC 23853*
- *Klebsiella pneumoniae ATCC BAA-1705*

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Operative Protocol

Light Progress Experiments: UV-BOX-E3/40H-NX-R

- Prepare a 0.5 McFarland inoculum of the bacterial strain (use PBS solution to suspend the colonies).
- Prepare two dilution of the inoculum: 1x10^8 and 1X10^7 (use PBS solution for the dilutions).
- Spread 100 µl of each dilutions on the stainless steel carriers.
- Place 1 supports for each strain dilutions inside the UV device (TREATED SAMPLES).
- Place 1 more supports (1 *per* strain dilutions) out of the device range (POSITIVE CONTROLS).
- Set the times in minutes and start the exposure.
- At the end of the exposure, transfer both the exposed and non-exposed supports into 90 mm Petri dishes and add 10 ml of D/E medium.
- After initial shake, let the carrier inside the petri dishes with the medium for 10 minutes.
- Transfer the D/E medium to a 50 ml Falcon and centrifuge for 40 minutes at 4500 rpm.
- Eliminate the supernatant and suspend the pellet in 1 ml of initial D/E medium.
- Transfer 100 µl to a Plate Count Agar and spread until dry (colony count have to be multiplied by 10 considering that we take 1/10 of 1ml).
- Incubate the plates at 36°C for 48h.

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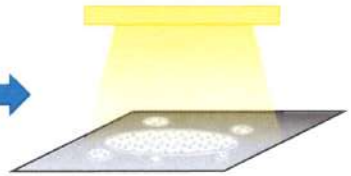
SCHEMATIC PROCEDURE

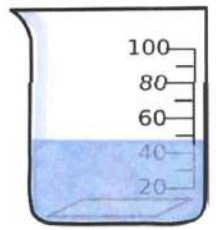
0,5 McF bacteric suspension

10^7, 10^8 diluitions
in PBS

100 µL of bacteric suspension on
steel carrier. Let dry
under the hood



Different exposure times
inside the CABINET



Resuspension in 10 mL of D/E
medium

centrifuged for 40 minutes
at 4500 rpm

100 µl transferred to a Plate Count Agar
and spread until dry

plates incubated at
36°C for 48h

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the following photos represent an example of petri dishes from each experiment

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Stafilococco aureus ATCC 43300:
First test

Concentrations:
- 1x10^7 CFU/mL
- 1x10^6 CFU/mL

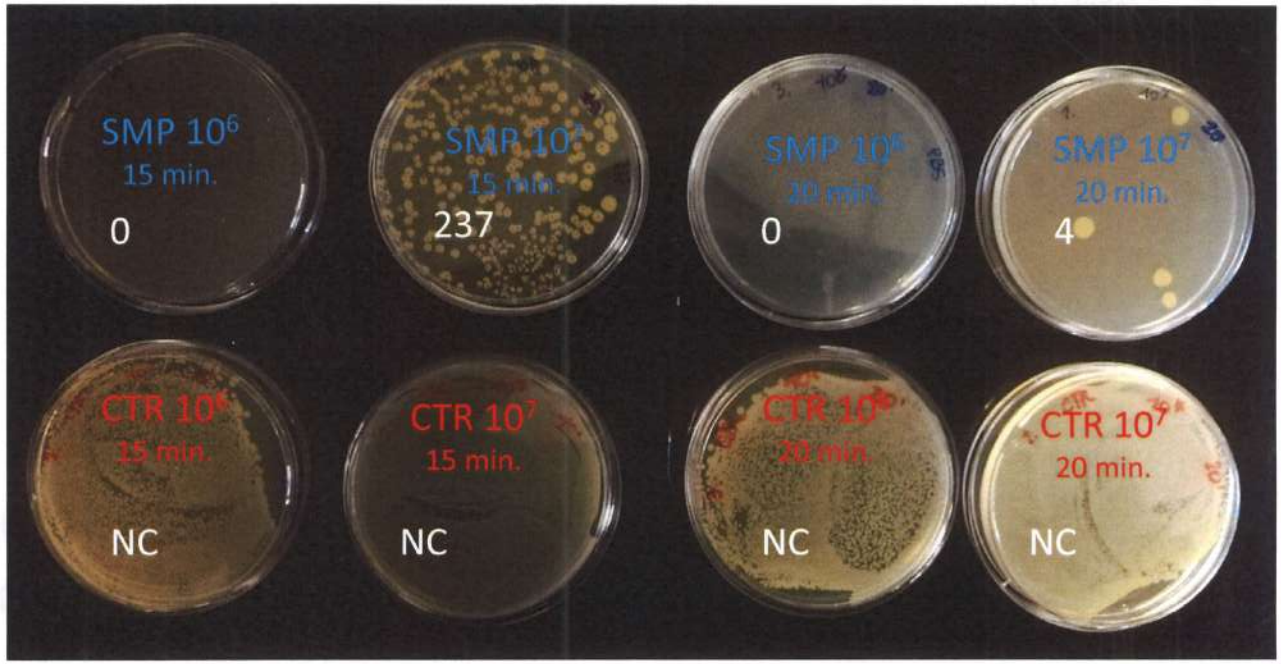
Times:
- 15,20 minutes

Distance:
- 33 cm (from upper light source)

Medium:
- PCA (Plate Count Agar)

Incubation:
- 36 °C for 48h

SMP: Sample
CTR: Positive Control
DIL: Dilution
NC: Not Countable



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Preliminary stage Tests

Stafilococco aureus ATCC 43300:
Second test

Concentrations:
- $1x10^7$ CFU/mL
- $1x10^6$ CFU/mL

Times:
- 5,10,15 minutes

Distance:
- 33 cm (from upper light source)

Medium:
- PCA (Plate Count Agar)

Incubation:
- 36 °C for 48h

SMP: Sample
CTR: Positive Control
DIL: Dilution
NC: Not Countable

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Preliminary stage Tests

Stafilococco aureus ATCC 43300:
Third test

Concentrations:
- $1x10^7$ CFU/mL
- $1x10^6$ CFU/mL

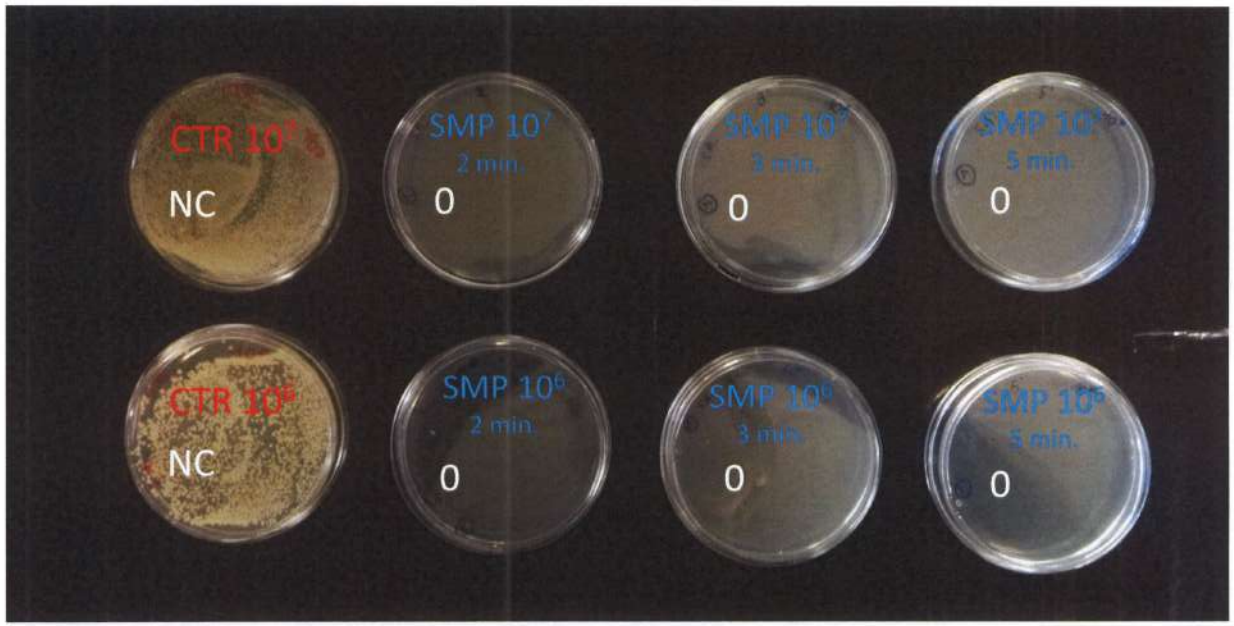
Times:
- 2,3,5 minutes

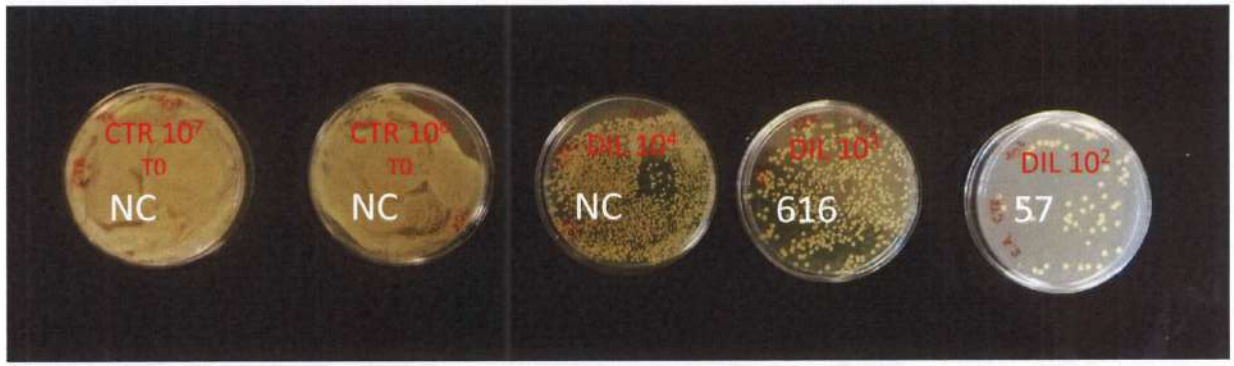
Distance:
- 33 cm (from upper light source)

Medium:
- PCA (Plate Count Agar)

Incubation:
- 36 °C for 48h

SMP: Sample
CTR: Positive Control
DIL: Dilution
NC: Not Countable





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Preliminary and part of main stage Tests

Escherichia coli ATCC 8739:
First test

Concentrations:
- 1x10^7 CFU/mL
- 1x10^6 CFU/mL

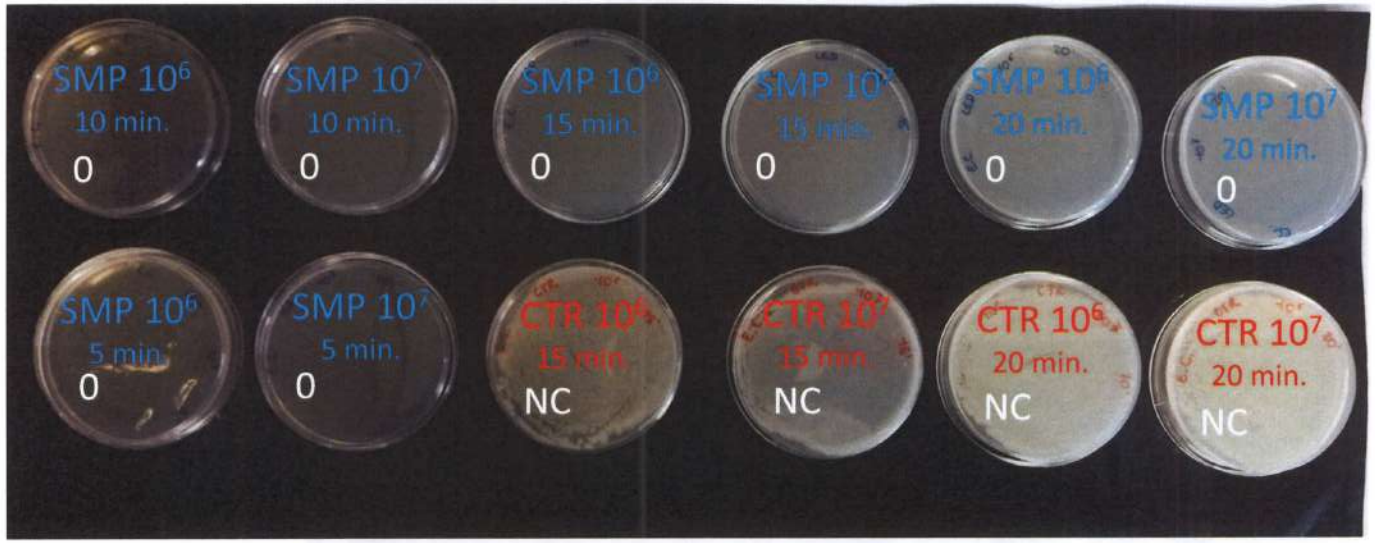
Times:
- 5,10,15,20 minutes

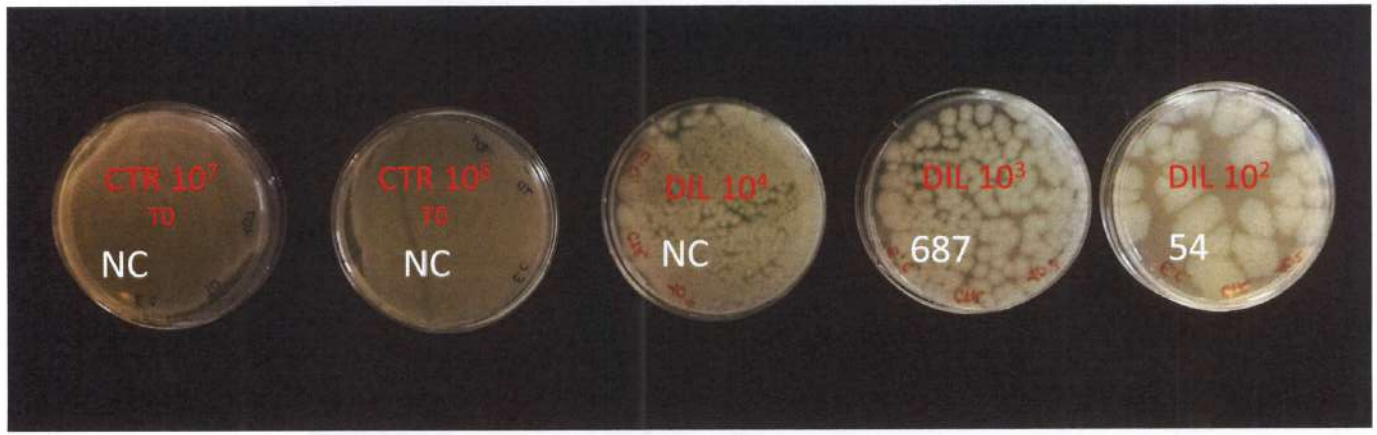
Distance:
- 33 cm (from upper light source)

Medium:
- PCA (Plate Count Agar)

Incubation:
- 36 °C for 48h

SMP: Sample
CTR: Positive Control
DIL: Dilution
NC: Not Countable





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Preliminary stage Tests

Escherichia coli ATCC 8739:
Second test

Concentrations:
- $1x10^7$ CFU/mL
- $1x10^6$ CFU/mL

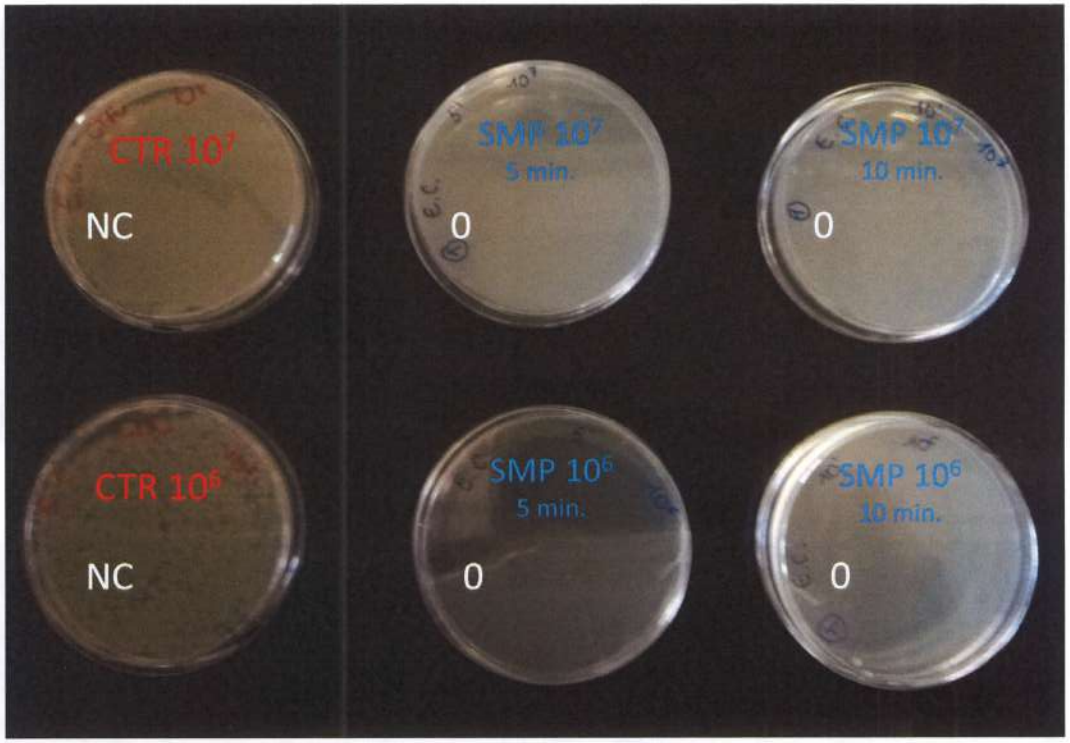
Times:
- 5,10 minutes

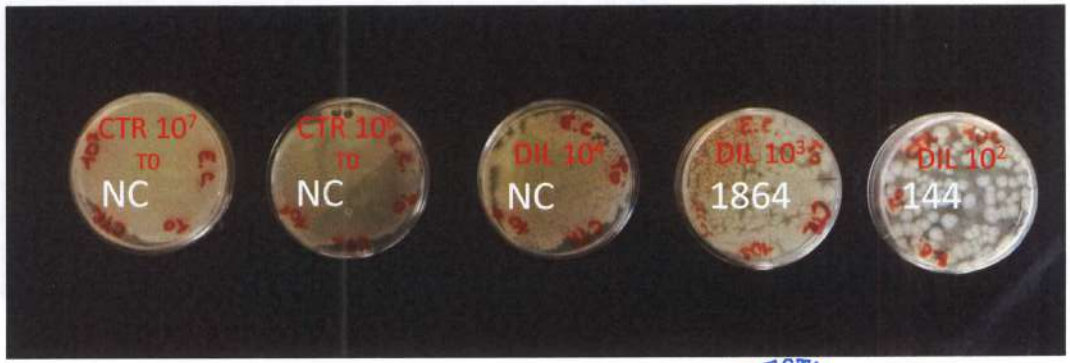
Distance:
-33 cm (from upper light source)

Medium:
- PCA (Plate Count Agar)

Incubation:
- 36 °C for 48h

SMP: Sample
CTR: Positive Control
DIL: Dilution
NC: Not Countable





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Preliminary stage Tests

Escherichia coli ATCC 8739:
Third test

Concentrations:
- 1x10^7 CFU/mL
- 1x10^6 CFU/mL

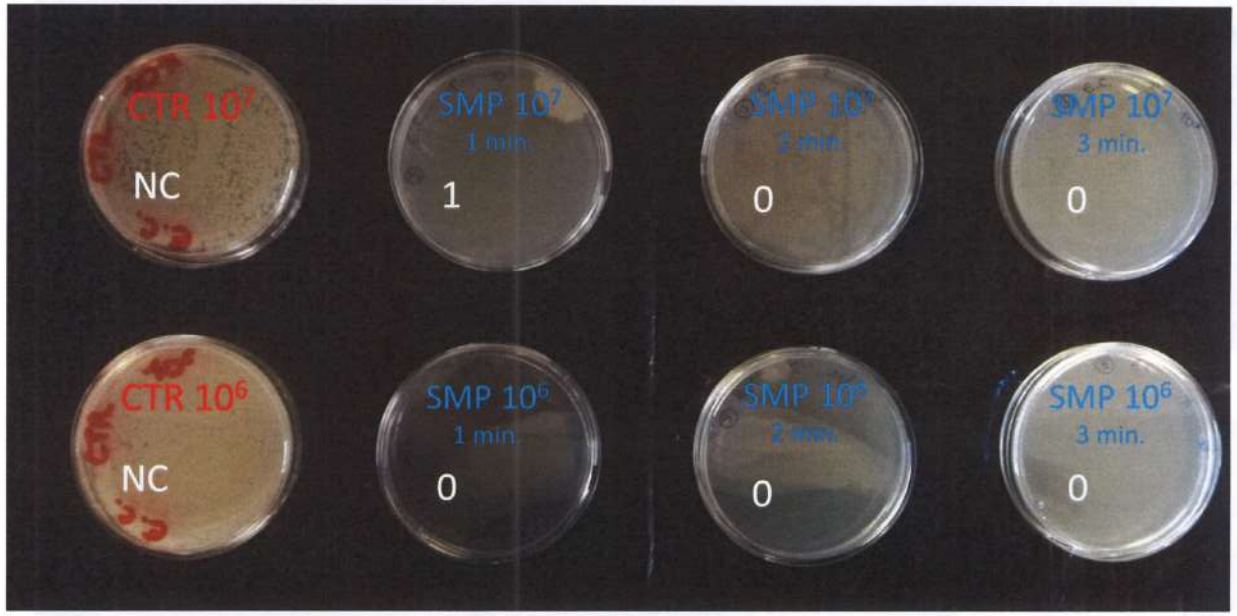
Times:
- 1,2,3 minutes

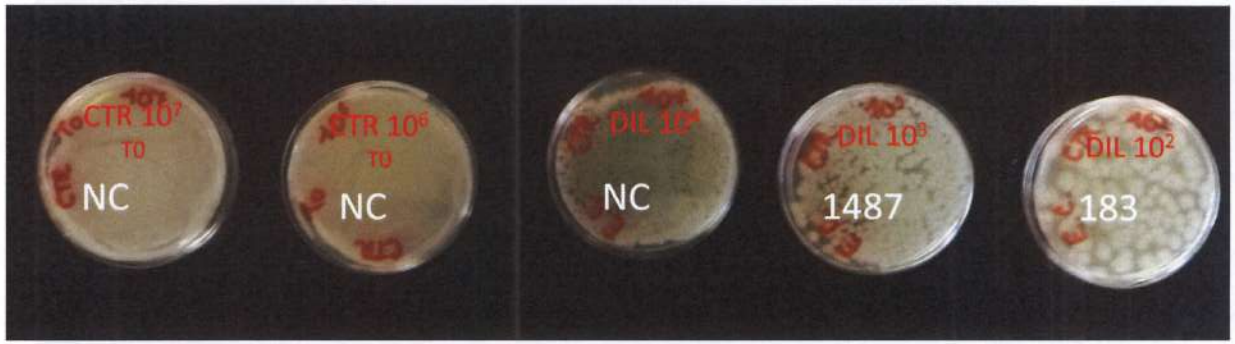
Distance:
- 33 cm (from upper light source)

Medium:
- PCA (Plate Count Agar)

Incubation:
- 36 °C for 48h

SMP: Sample
CTR: Positive Control
DIL: Dilution
NC: Not Countable





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Preliminary and part of main stage Tests

Pseudomonas aeruginosa ATCC 27853:
First test

Concentrations:
- $1x10^7$ CFU/mL
- $1x10^6$ CFU/mL

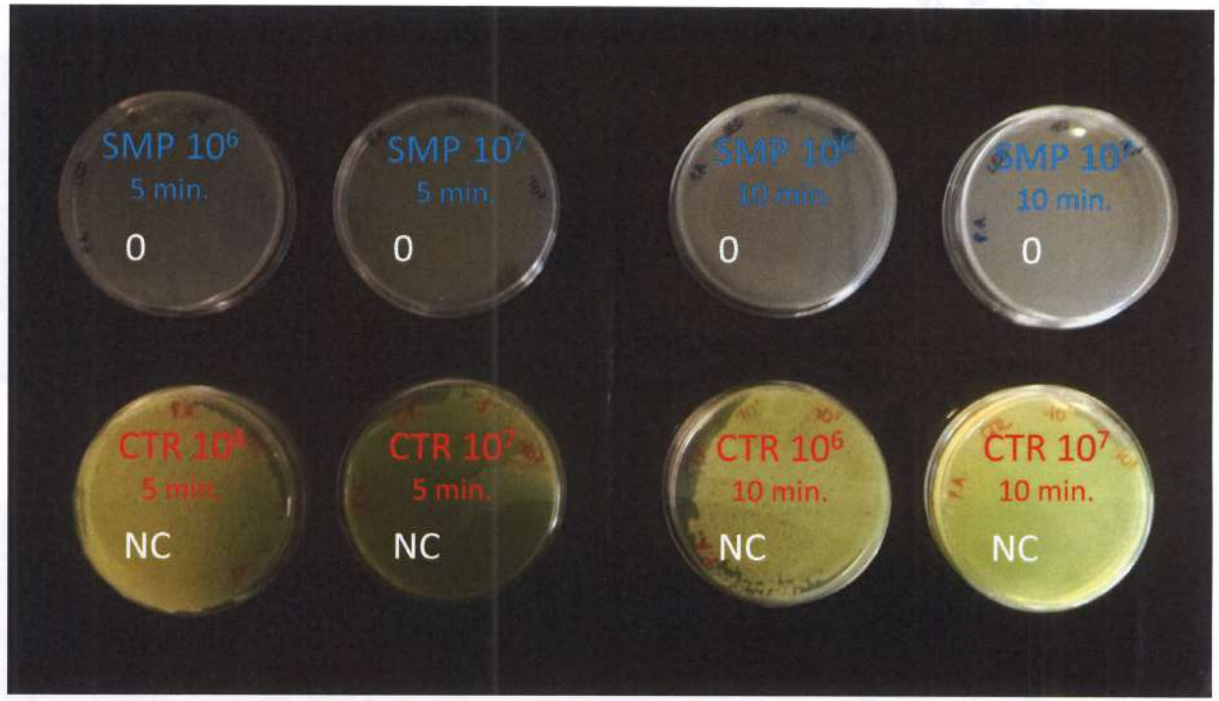
Times:
- 5,10,15,20 minutes

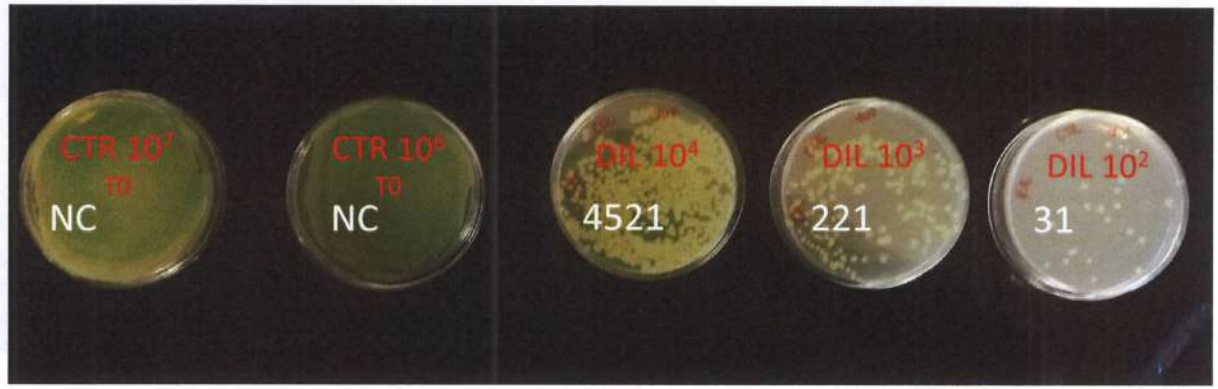
Distance:
- 33 cm (from upper light source)

Medium:
- PCA (Plate Count Agar)

Incubation:
- 36 °C for 48h

SMP: Sample
CTR: Positive Control
DIL: Dilution
NC: Not Countable





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Preliminary stage Tests

Pseudomonas aeruginosa ATCC 27853:
Second test

Concentrations:
- $1x10^7$ CFU/mL
- $1x10^6$ CFU/mL

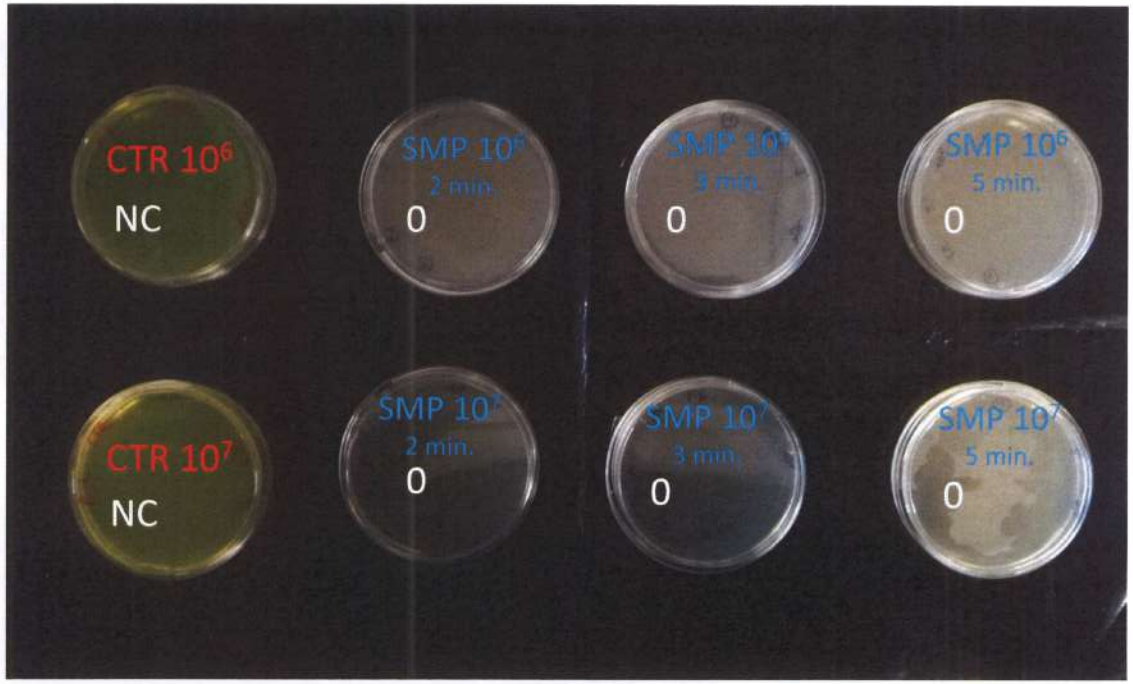
Times:
- 2,3,5 minutes

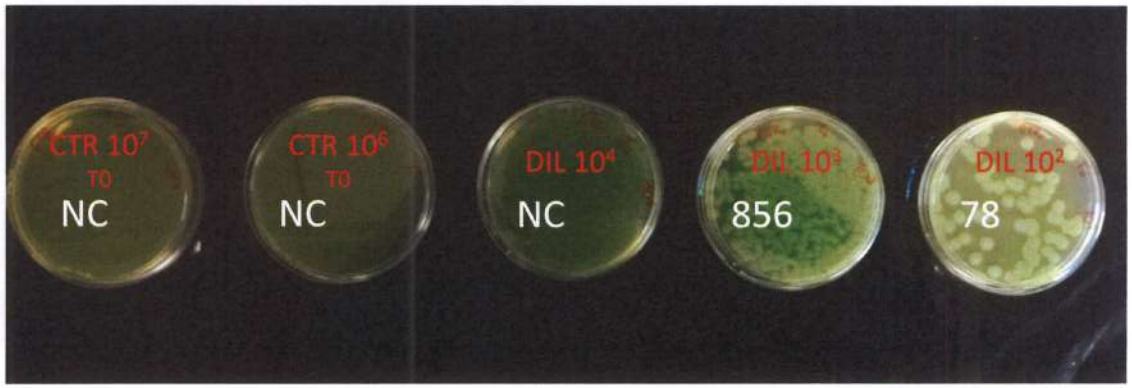
Distance:
- 33 cm (from upper light source)

Medium:
- PCA (Plate Count Agar)

Incubation:
- 36 °C for 48h

SMP: Sample
CTR: Positive Control
DIL: Dilution
NC: Not Countable





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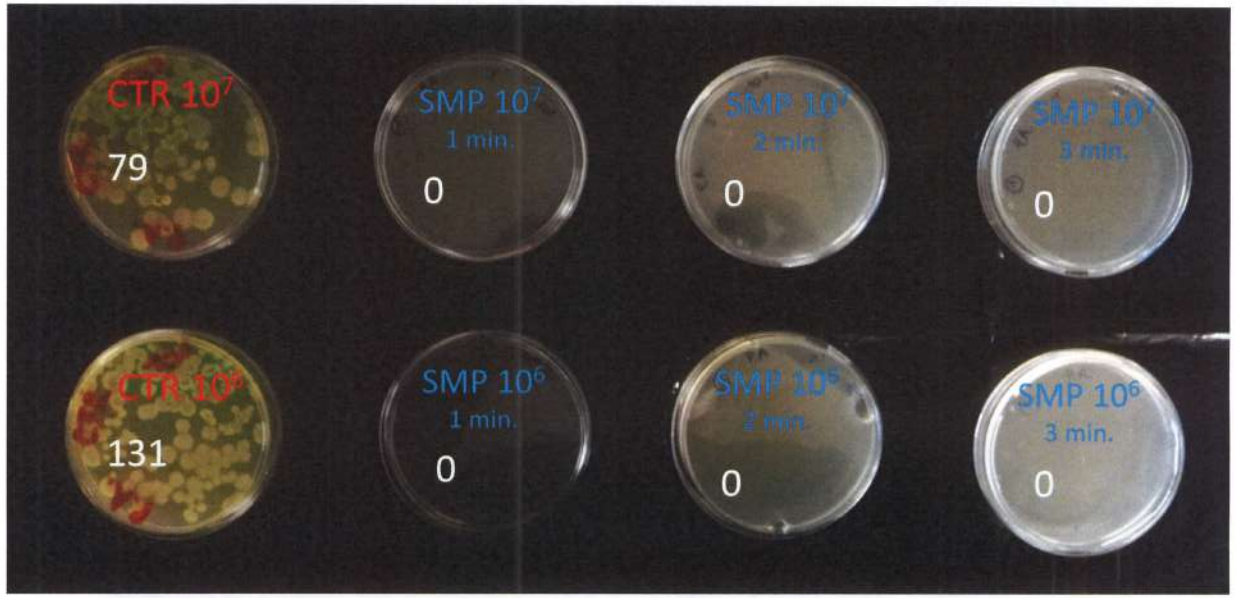
Preliminary and part of main stage Tests

Pseudomonas aeruginosa ATCC 27853:
Third test



Concentrations:
- 1x10^7 CFU/mL
- 1x10^6 CFU/mL

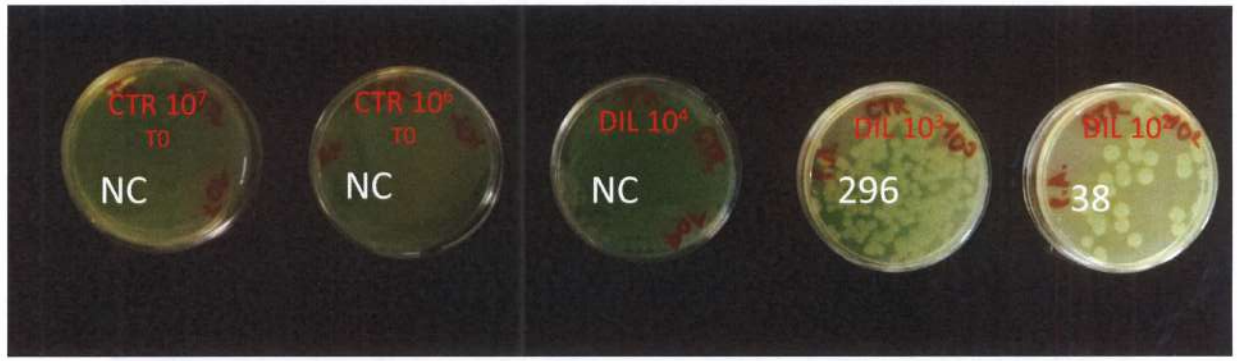
Times:
- 1,2,3 minutes

Distance:
- 33 cm (from upper light source)

Medium:
- PCA (Plate Count Agar)

Incubation:
- 36 °C for 48h

SMP: Sample
CTR: Positive Control
DIL: Dilution
NC: Not Countable



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Preliminary and part of main stage Tests

Salmonella typhimurium ATCC 23853:
First test

Concentrations:
- $1x10^7$ CFU/mL
- $1x10^6$ CFU/mL

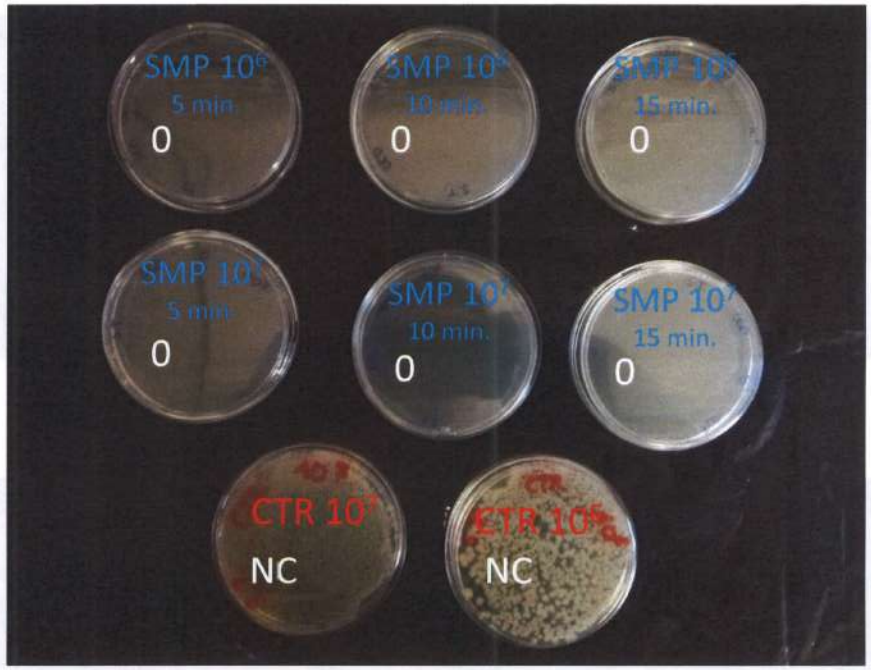
Times:
- 5,10,15 minutes

Distance:
- 33 cm (from upper light source)

Medium:
- PCA (Plate Count Agar)

Incubation:
- 36 °C for 48h

SMP: Sample
CTR: Positive Control
DIL: Dilution
NC: Not Countable



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Preliminary stage Tests

Salmonella typhimurium ATCC 23853:
Second test

Concentrations:
- $1x10^7$ CFU/mL
- $1x10^6$ CFU/mL

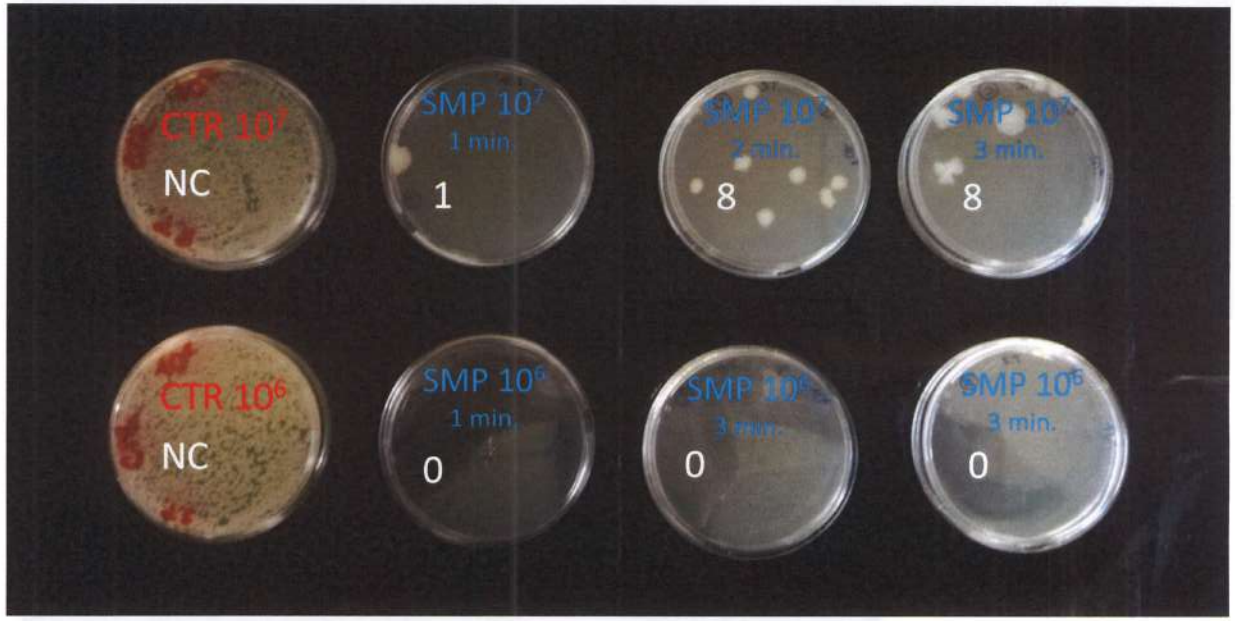
Times:
- 1,2,3 minutes

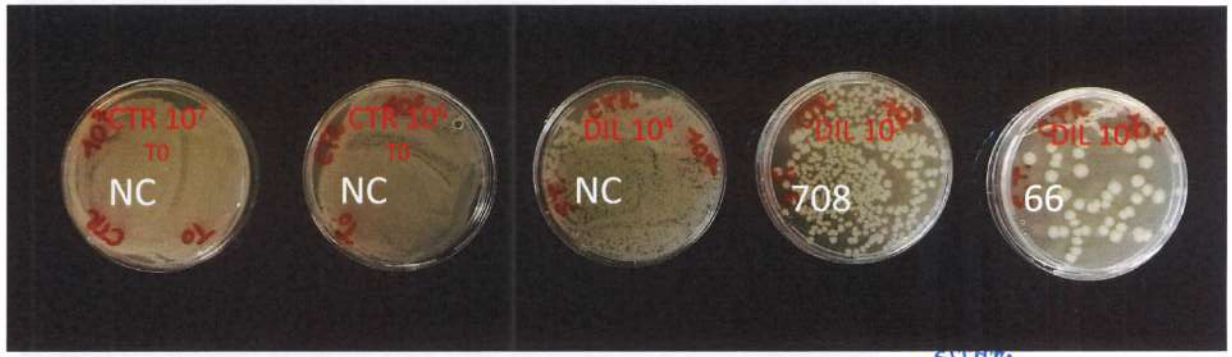
Distance:
- 33 cm (from upper light source)

Medium:
- PCA (Plate Count Agar)

Incubation:
- 36 °C for 48h

SMP: Sample
CTR: Positive Control
DIL: Dilution
NC: Not Countable





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Preliminary and part of main stage Tests

Salmonella typhimurium ATCC 23853:
Third test

Concentrations:
- 1x10^7 CFU/mL
- 1x10^6 CFU/mL

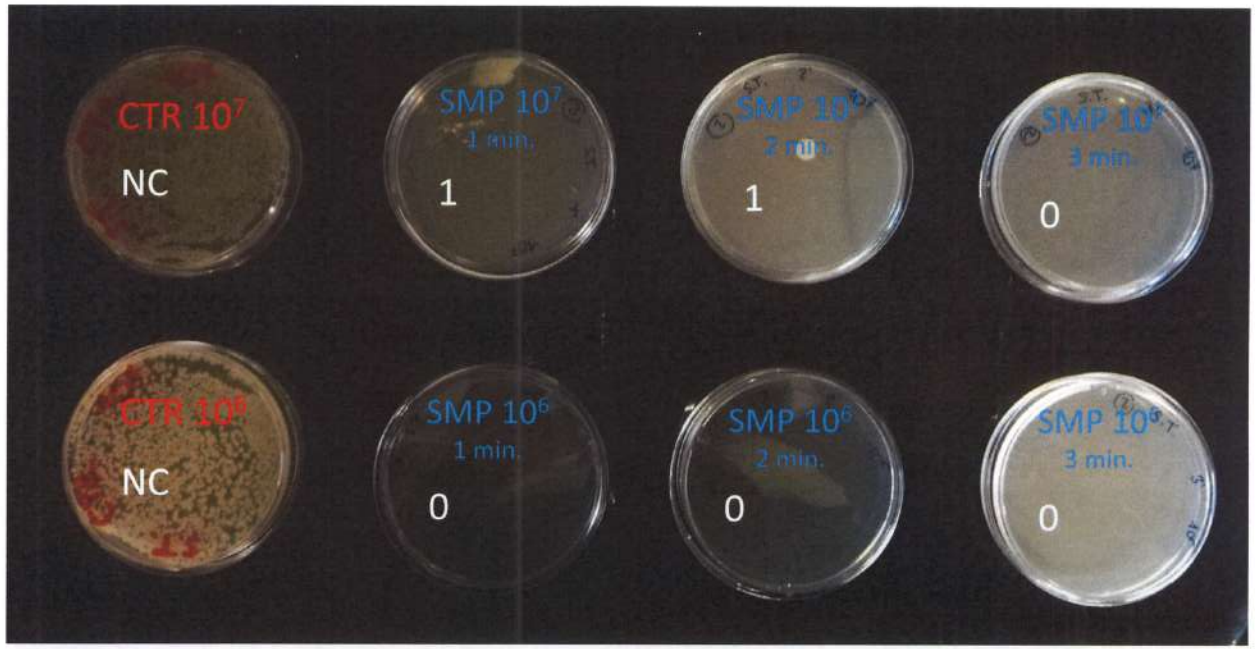
Times:
- 1,2,3 minutes

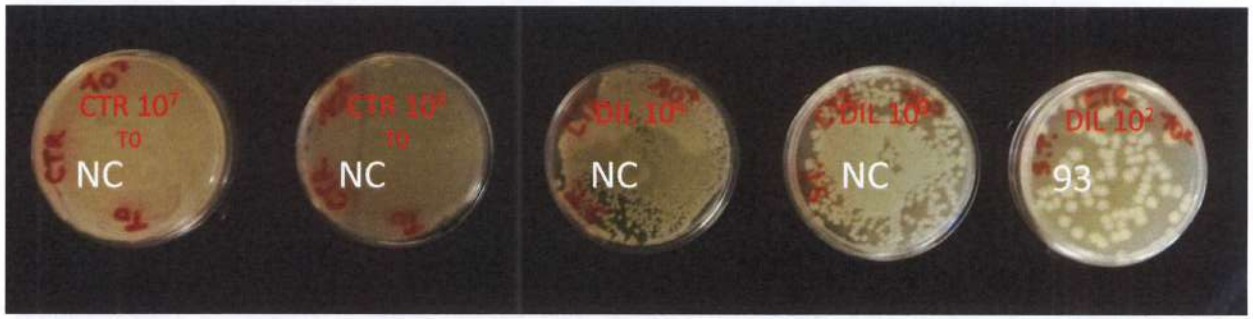
Distance:
- 33 cm (from upper light source)

Medium:
- PCA (Plate Count Agar)

Incubation:
- 36 °C for 48h

SMP: Sample
CTR: Positive Control
DIL: Dilution
NC: Not Countable





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Preliminary and part of main stage Tests

Klebsiella pneumoniae ATCC BAA-1705:
First test

Concentrations:
- $1x10^7$ CFU/mL
- $1x10^6$ CFU/mL

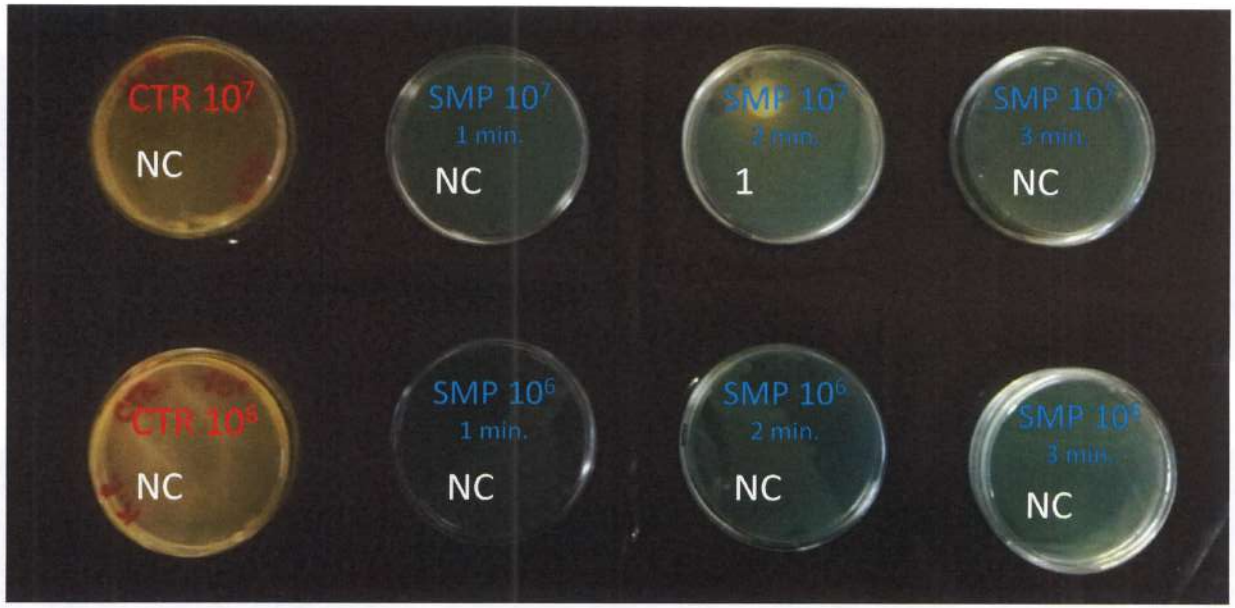
Times:
- 1,2,3 minutes

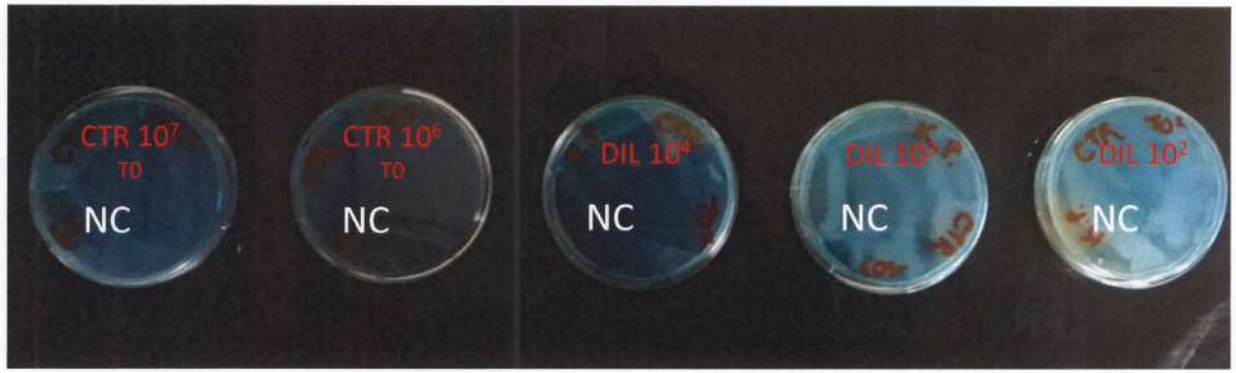
Distance:
- 33 cm (from upper light source)

Medium:
- CLED Agar (Cystine-Lactose-Electrolyte-Deficient)

Incubation:
- 36 °C for 48h

SMP: Sample
CTR: Positive Control
DIL: Dilution
NC: Not Countable





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Preliminary and part of main stage Tests

Klebsiella pneumoniae ATCC BAA-1705:
Second test

Concentrations:
- $1x10^7$ CFU/mL
- $1x10^6$ CFU/mL

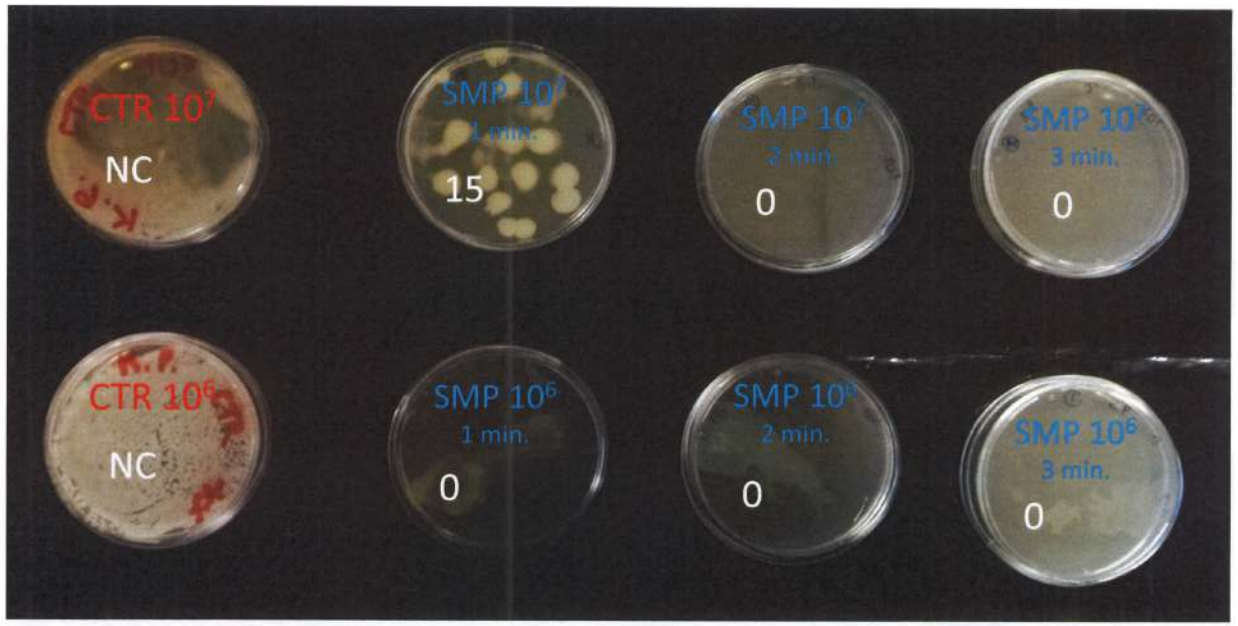
Times:
- 1,2,3 minutes

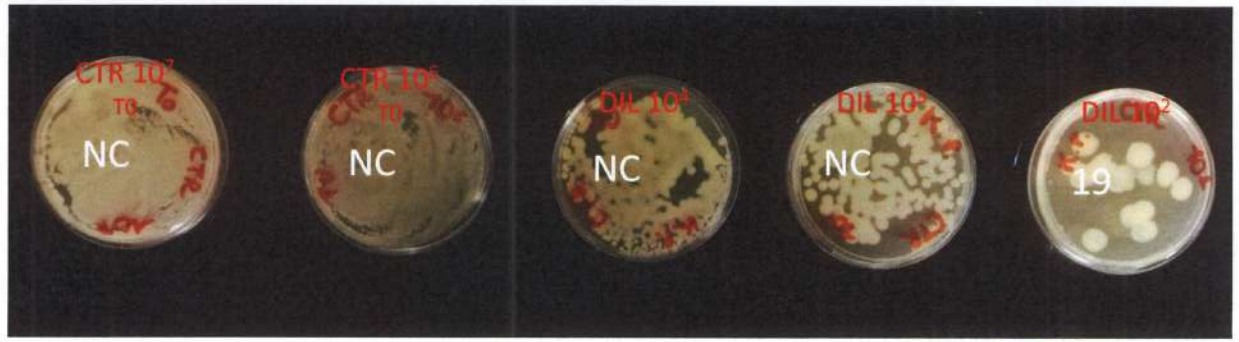
Distance:
- 33 cm (from upper light source)

Medium:
- PCA (Plate Count Agar)

Incubation:
- 36 °C for 48h

SMP: Sample
CTR: Positive Control
DIL: Dilution
NC: Not Countable





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Preliminary and part of main stage Tests

Klebsiella pneumoniae ATCC BAA-1705:
Third test

Concentrations:
- 1x10^7 CFU/mL
- 1x10^6 CFU/mL

Times:
- 1,2,3 minutes

Distance:
- 33 cm (from upper light source)

Medium:
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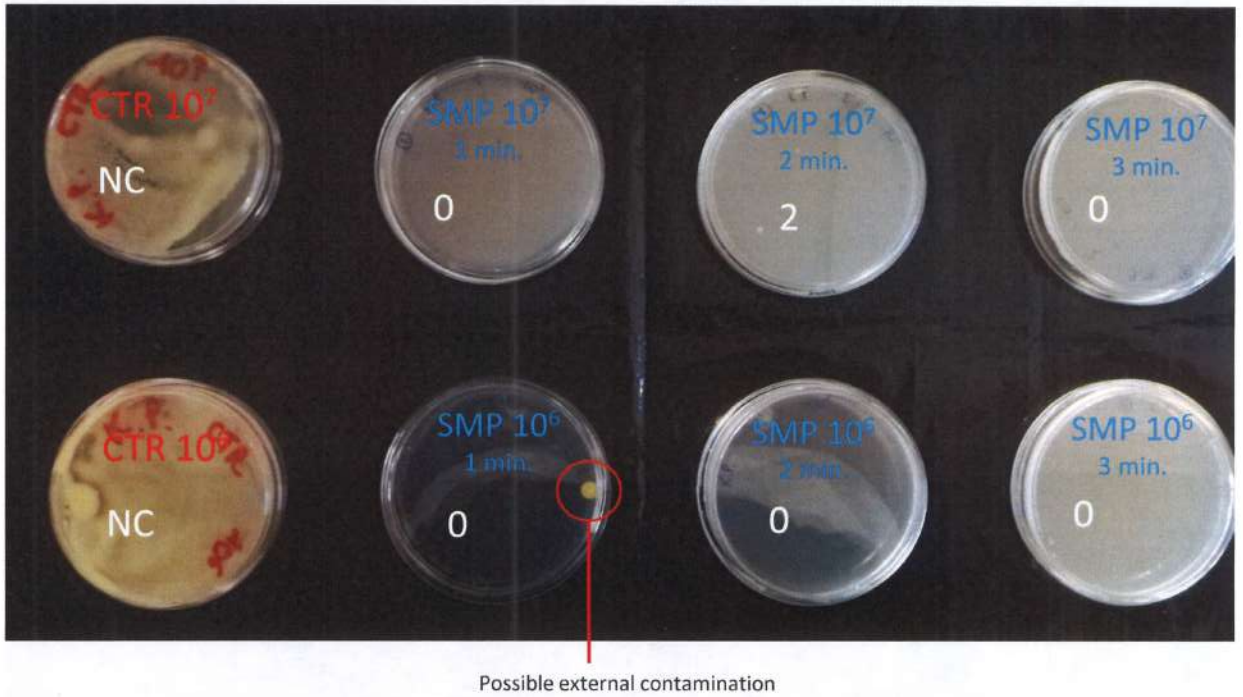

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Preliminary and part of main stage Tests