Report on: LOC – Light on Cells
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Requested by: NBioTech Indústria e Comércio Ltda.
FAO: Nilson Cristiano da Cruz

Report issued by: LMA – Laboratory of Applied Microbiology
Responsible: Iolanda Cristina Silveira Duarte
Contact address: iolanda@ufscar.br

LOC – LIGHT ON CELLS

ABSTRACT

This analysis was carried out to evaluate the capacity of the LOC – Light on Cells equipment to detect contamination on surfaces. For this, isolated cultures of *Candida albicans*, *Escherichia coli*, *Staphylococcus aureus*, and *Pseudomonas aeruginosa* were grown on stainless-steel plates until the formation of biofilms. During the incubation period, LOC allowed to observe the gradual formation of biofilms due to the shape, fluorescence, and color of the cultures when exposed to UV light. At the end of the incubation time, biofilms were highlighted as an opaque layer all over the plates. The LOC equipment detected the formation of biofilms from all the strains used in this analysis.

1. METHODS

1.1. Microorganisms and inoculum preparation

From Petri dishes, 10 µL of isolated cultures of *S. aureus*, *C. albicans*, *E. coli*, and *P. aeruginosa* were transferred to 500 mL Erlenmeyer flasks containing 150 mL of Brain Heart Infusion (BHI) culture medium. The cultures were maintained at 150 rpm and 30°C for 48 hours in a Solab SL 223 shaker.

The absorbances of the culture broths were used as a standardization method to quantify the inoculum size in each sample. The culture broths' optical density (OD) was obtained using a Hach DR 5000 UV-Vis spectrophotometer at 446 nm, 530 nm, 600 nm, and 644 nm. The relationship between OD and Colony Forming Units (CFU) in the samples of *S. aureus*, *C. albicans*, *E. coli*, and *P. aeruginosa* were obtained based on the studies by Pimentel-Filho et al. (2014), Rodriguez-Tudela et al., (2001), Lin et al., (2009) and Živković et al., (2018), respectively.

1.2. Growing microorganisms on a sterile stainless-steel surface

In Petri dishes, 15 mL of the culture broth of each strain was added. A sterilized stainless-steel plate was inserted into the Petri dish and submerged into the broth. The procedure was repeated using culture broth dilutions of 1:2 (v/v) and 1:3 (v/v) using fresh BHI medium as diluent. While *S. aureus*, *E. coli*, and *P. aeruginosa* were incubated at 35°C in a Fanem 502 oven, *C. albicans* was kept at 25°C in an Ethik Technology 407 oven. After 24 hours, the stainless-steel plates were transferred to new sterilized Petri dishes without culture broth and were kept at 35ºC for 48 hours. The formation of biofilms over incubating time was assessed every 24 hours using LOC. At the end of the incubation period, a small area of the plates was cleaned with a swab and then lit with LOC to confirm biofilm formation.

2. RESULTS

2.1. Samples optical density

The absorbance results of the samples, quantified in terms of optical density (OD) for each of the cultures, are presented in Table 1. The values of CFU/mL indicate the order of magnitude of the quantity of microorganisms in each sample.

Table 1 – Culture broth optical density: strains, wavelengths, absorbances, and correspondence regarding Colony Forming Units (CFU).

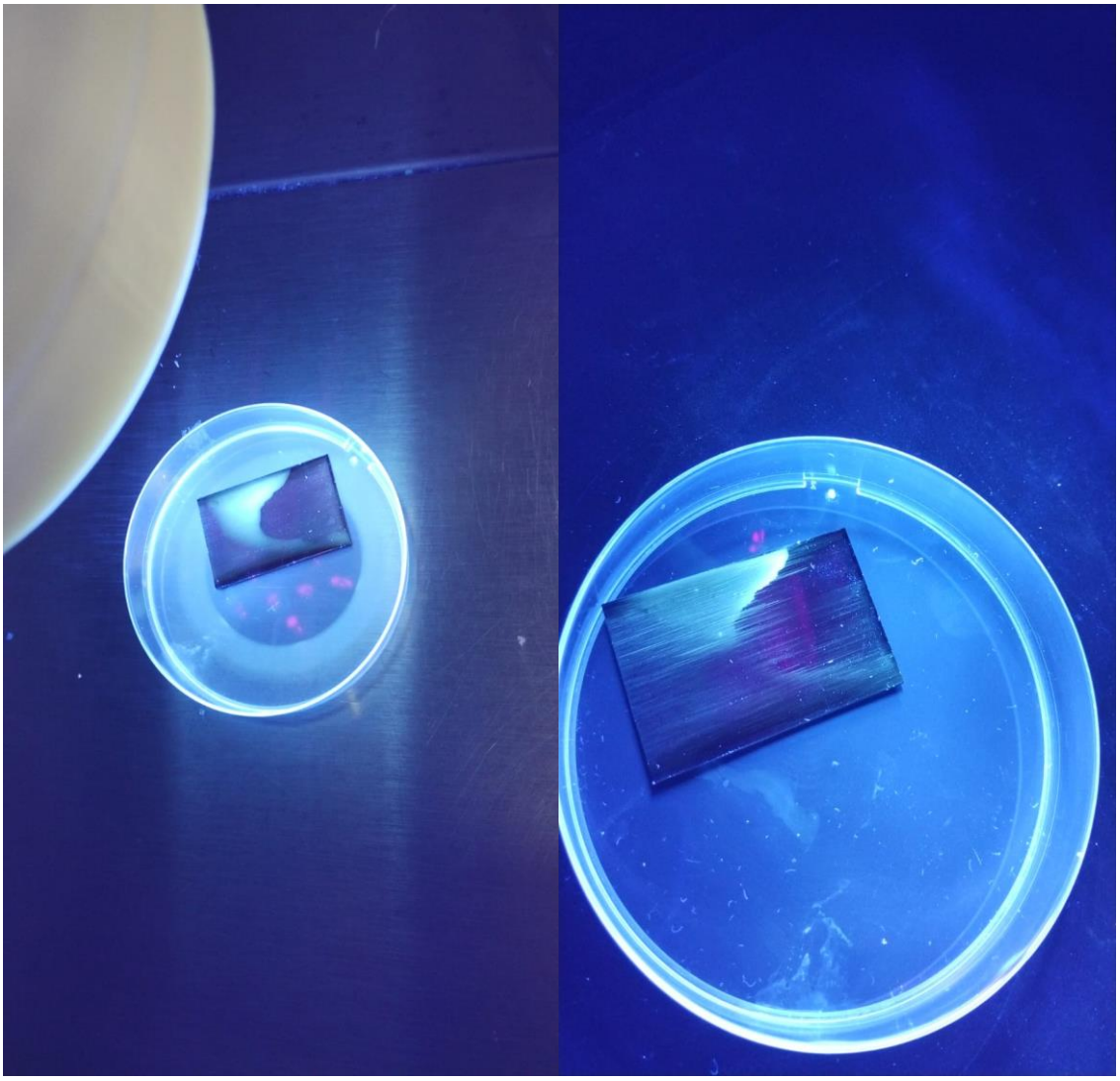
Microorganism	Dilution (v/v)	Wavelength (nm)	Absorbance values (OD)	CFU/mL
S. aureus	1:1	446	1.093	$\sim10^5$
S. aureus	1:2		0.702	$\sim10^5$
S. aureus	1:3		0.476	$\sim10^4$
C. albicans	1:1	530	1.172	$\sim10^8$
C. albicans	1:2		0.670	$\sim10^8$
C. albicans	1:3		0.532	$\sim10^8$
E. coli	1:1	600	1.229	$\sim10^8$
E. coli	1:2		0.915	$\sim10^7$
E. coli	1:3		0.720	$\sim10^7$
P. aeruginosa	1:1	644	1.504	$\sim10^8$
P. aeruginosa	1:2		1.208	$\sim10^8$

OD: Optical Density.

2.2. Biofilm formation on stainless-steel plates

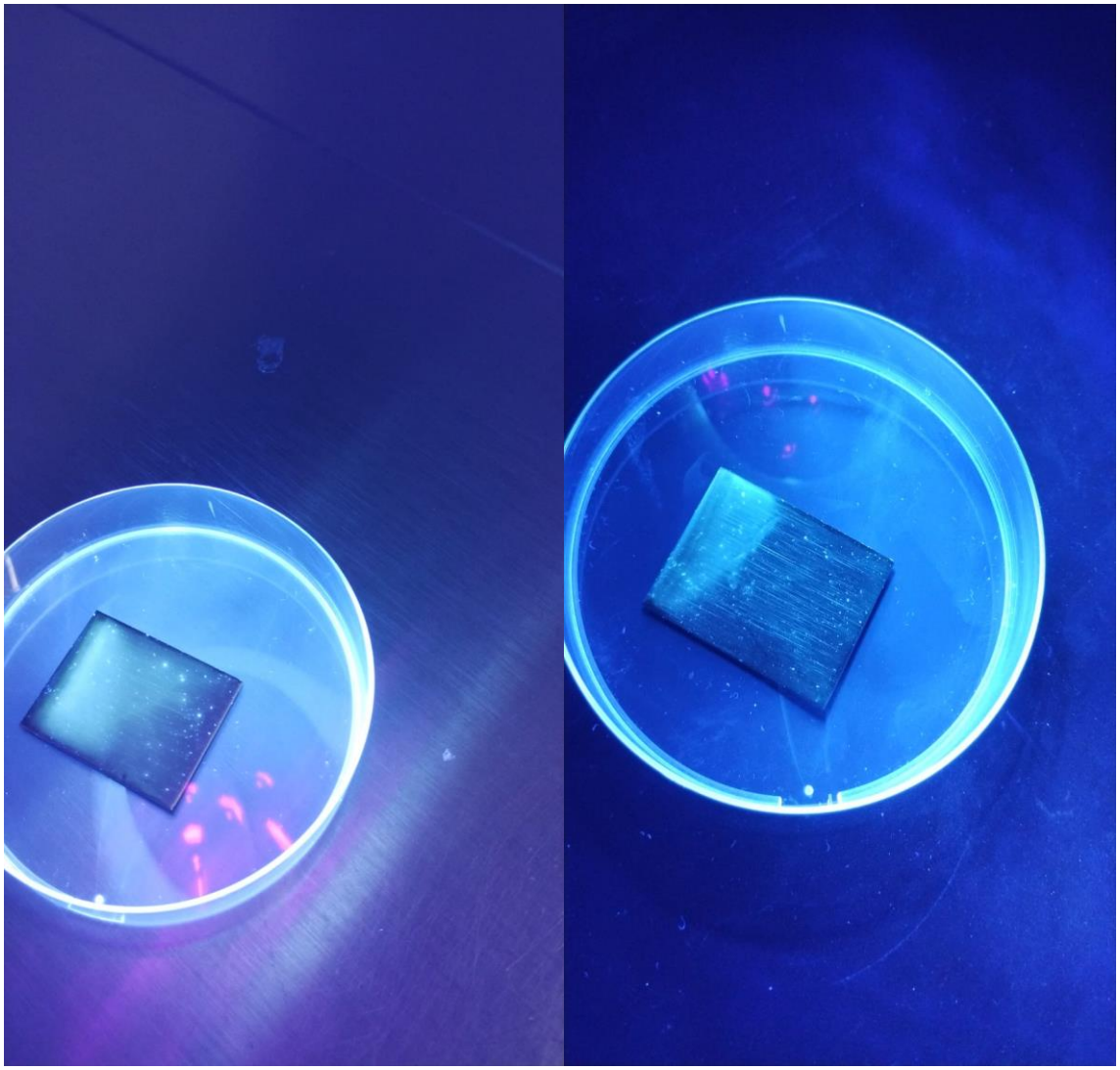
The exposure of the stainless-steel surfaces to LOC UV light stated the presence of continuous, fluorescent layers with a greenish-white color on the plates that were previously submerged in the culture broths of *E. coli* (Figure 1), *S. aureus* (Figure 2) and *P. aeruginosa* (Figure 3). For the plates submerged in *C. albicans* culture broth, small fluorescent clusters with a greenish-white color covered the entire surface (Figure 4).

Figure 1 – *E. coli* biofilm formation on stainless-steel plate*:* a) 24 hours incubation; b) 48 hours incubation.



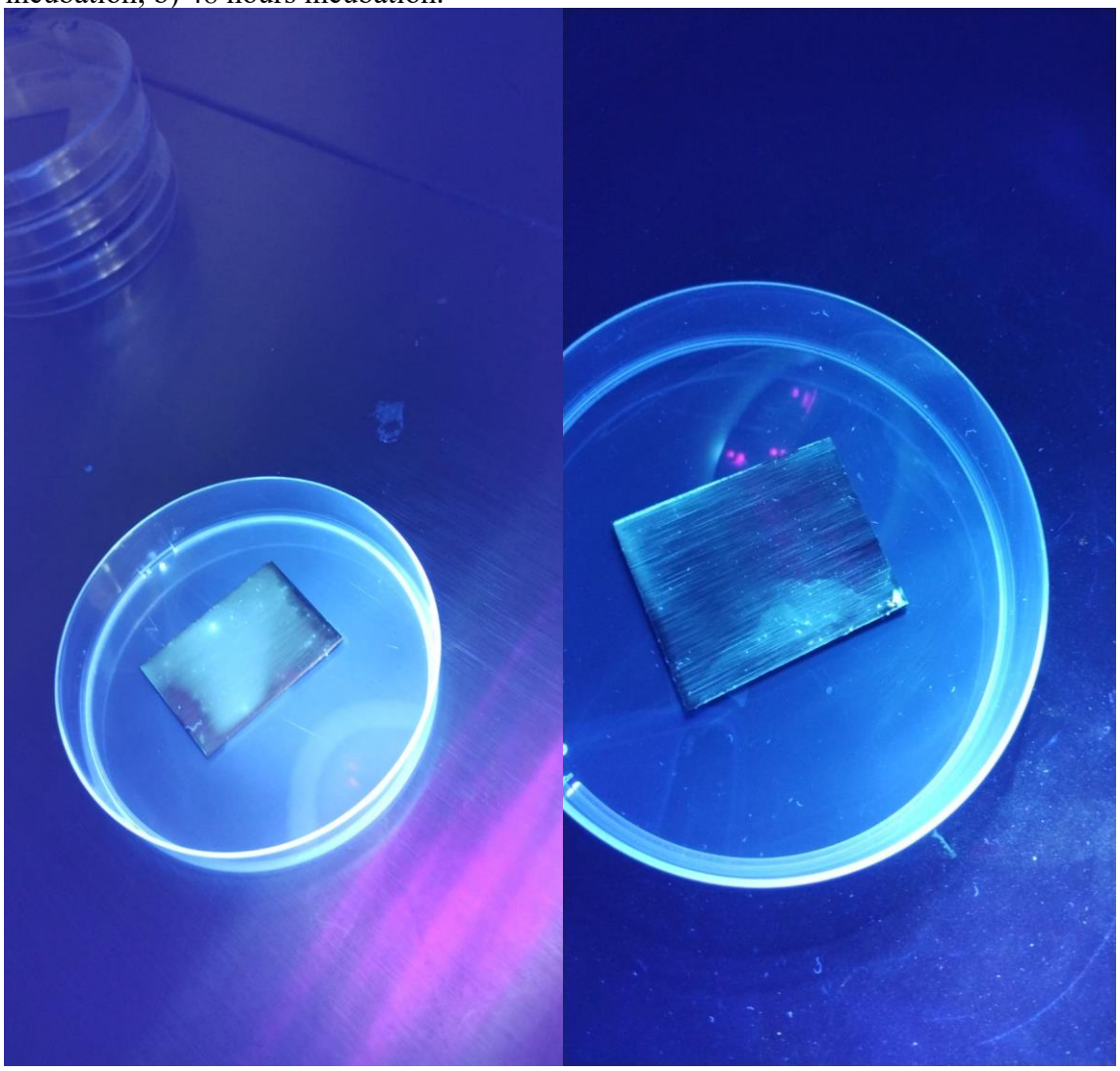
(a) (b)

Figure 2 – *S. aureus* biofilm formation on stainless-steel plate: a) 24 hours incubation; b) 48 hours incubation.



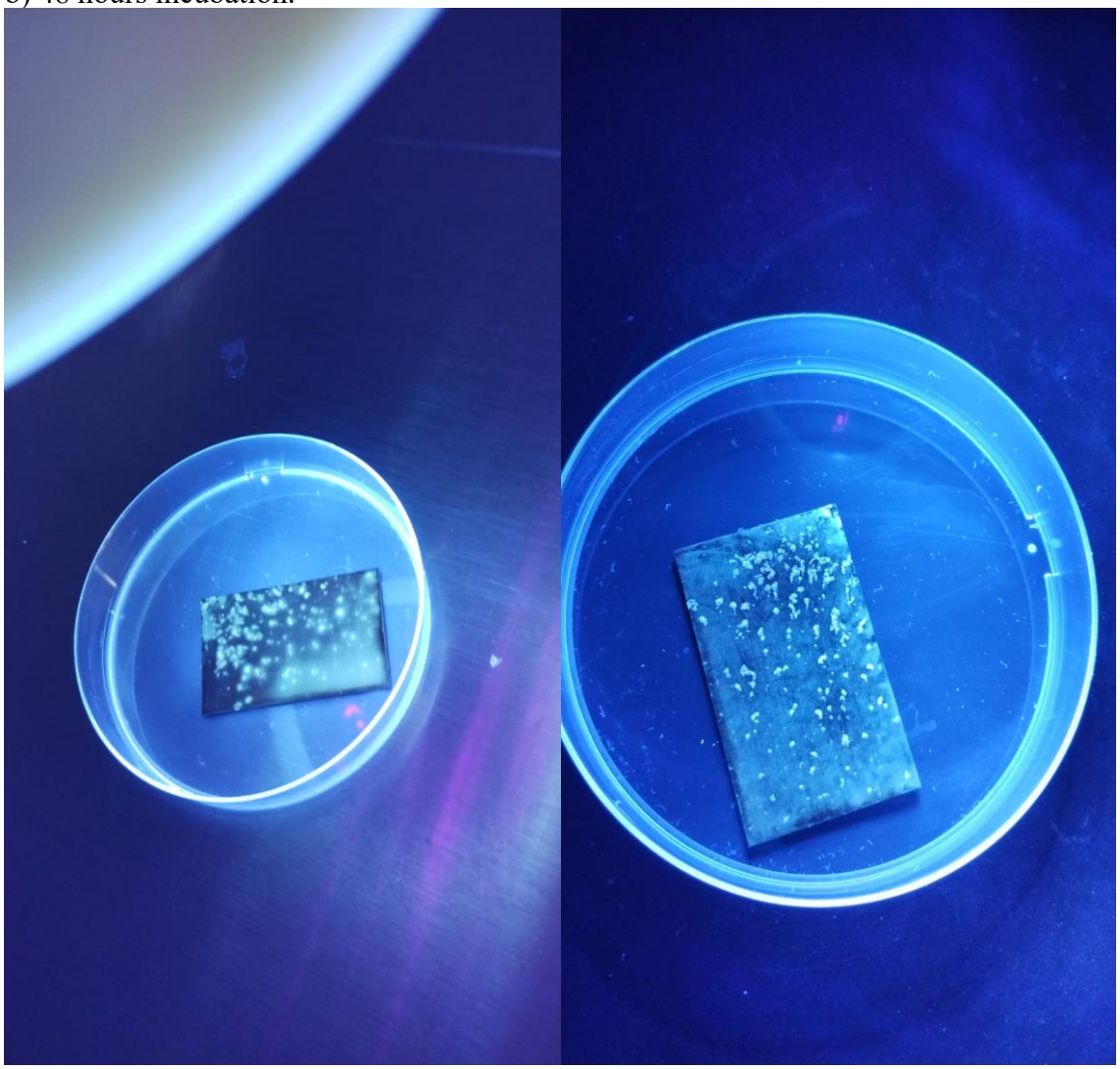
(a) (b)

Figure 3 – *P. aeruginosa* biofilm formation on stainless-steel plate*:* a) 24 hours incubation; b) 48 hours incubation.



(a) (b)

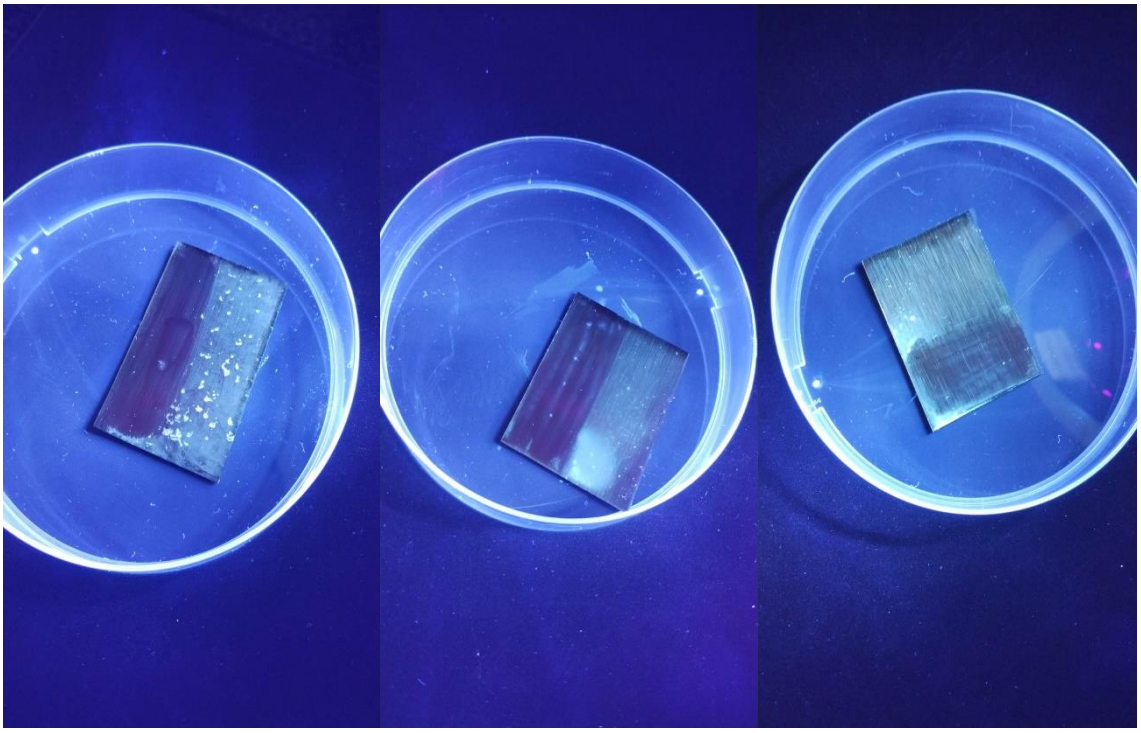
Figure 4 – *C. albicans* biofilm formation on stainless-steel plate*:* a) 24 hours incubation; b) 48 hours incubation.



(a) (b)

After using the swab to clean the stainless-steel plates, a contrast was observed between two surface regions, one opaque, stating the biofilm formation, and the other brighter, indicating the removal of the cells (Figure 5). This distinction was evidenced by the use of the equipment and confirmed the effective formation of biofilm from all the strains used.

Figure 5 – Comparison between surface regions with and without biofilm formation.



C. albicans (1:2) *E. coli (1:3)* *P. aeruginosa (1:2)*

3. CONCLUSION

LOC – Light on Cells equipment allowed to verify the gradual process of biofilm formation of *Staphylococcus aureus*, *Candida albicans*, *Escherichia coli*, and *Pseudomonas aeruginosa* on stainless-steel surfaces. The biofilms were characterized by an opaque layer on the stainless-steel plates and presented a greenish-white fluorescence color when highlighted with LOC UV light. As demonstrated, the equipment may detect biological contamination on surfaces.

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