REPORT

Requested by:
NBioTech Industria e Comércio Ltda
CNPJ: 48.416.479/0001-78
Responsible: Nilson Cristiano da Cruz

Prepared by:
Laboratory of Applied Microbiology
Responsible: Iolanda Cristina Silveira Duarte
E-mail: iolanda@ufscar.br
Date: June 12, 2023

REPORT ON CONTAMINATION VISUALIZATION EQUIPMENT – LOC (LIGHT ON CELLS)

1. SUMMARY

This analysis was conducted to evaluate the capability of the **LOC – Light On Cells** contamination visualization equipment to detect biofilms. To achieve this, isolated cultures of the fungus Candida albicans and the bacteria Escherichia coli, Staphylococcus aureus, and Pseudomonas aeruginosa were used.

To optimize observation conditions, microorganisms were cultivated on a stainless steel surface. The equipment enabled the gradual visualization of biofilm formation during incubation, due to the fluorescence and characteristic coloration of the microorganisms when exposed to UV light.

Biofilm formation was confirmed using the equipment at the end of the incubation period, revealing an opaque layer on the stainless steel surface. The LOC device successfully detected biofilm formation by all microorganisms used in this analysis.

2. MATERIALS AND METHODS

2.1. Microorganism Cultivation

Using a 10 µL inoculation loop, isolates of S. aureus, C. albicans, E. coli, and P. aeruginosa were transferred from Petri dishes to 500 mL Erlenmeyer flasks containing 150 mL of

culture medium (Brain Heart Infusion – BHI broth). The cultures were kept under agitation at 150 rpm and a temperature of 30°C for 48 hours in a Solab SL 223 shaker.

2.2. Inoculum Preparation

Absorbance analysis of the culture broths was used to quantify and standardize microorganisms in the assays. The optical density (OD) values for each microorganism were obtained using a Hach DR 5000 UV-Vis spectrophotometer at wavelengths of 446 nm, 530 nm, 600 nm, and 644 nm, respectively.

The correlation between OD and the number of Colony-Forming Units (CFU) of S. aureus, C. albicans, E. coli, and P. aeruginosa samples was based on the studies of Pimentel-Filho et al. (2014), Rodríguez-Tudela et al. (2001), Lin et al. (2009), and Živković et al. (2018), respectively.

2.3. Microorganism Growth and Biofilm Formation

In Petri dishes, 15 mL of culture broth from each microorganism was added. A previously sterilized rectangular stainless steel plate was placed inside each dish, remaining submerged in the medium.

The procedure was repeated using culture broth dilutions at 1:2 (v/v) and 1:3 (v/v) ratios, with fresh BHI medium used as a diluent. The plates were incubated at 35°C in a Fanem 502 incubator.

After 24 hours, the stainless steel plates were transferred to new sterilized Petri dishes without culture broth and maintained at 35°C until the end of the analysis period. After 48 hours of incubation, the steel plates were swabbed and exposed to UV radiation to verify surface alterations indicative of biofilm removal.

3. RESULTS

3.1. Optical Density of Samples

Microorganism	Dilution (v/v)	Wavelength (nm)	Absorbance (OD)	CFU/mL
S. aureus	1:1	446	1.093	~10^5
S. aureus	1:2		0.702	~10^5
S. aureus	1:3		0.476	~10^4
C. albicans	1:1	530	1.172	~10^8
C. albicans	1:2		0.670	~10^8
C. albicans	1:3		0.532	~10^8
E. coli	1:1	600	1.229	~10^8
E. coli	1:2		0.915	~10^7
E. coli	1:3		0.720	~10^7
P. aeruginosa	1:1	644	1.504	~10^8
P. aeruginosa	1:2		1.208	~10^8

Table 1 presents the absorbance results of the samples, quantified in terms of optical density (OD) for each microorganism culture.
OD: Optical Density.

3.2. Microorganism Growth on Stainless Steel Plates

Exposure of the stainless steel plates to UV radiation immediately after removal from the culture broth revealed the presence of continuous, fluorescent, and white-green colored layers in plates previously submerged in E. coli **(Figure 1)**, S. aureus **(Figure 2)**, and P. aeruginosa **(Figure 3)** cultures. Small fluorescent clusters of the same color pattern covered the entire surface of the plate submerged in the C. albicans culture **(Figure 4)**.

Figure 1: Growth of E. Coli on a stainless steel plate after a) 24 hours of incubation and b) 48 hours of incubation

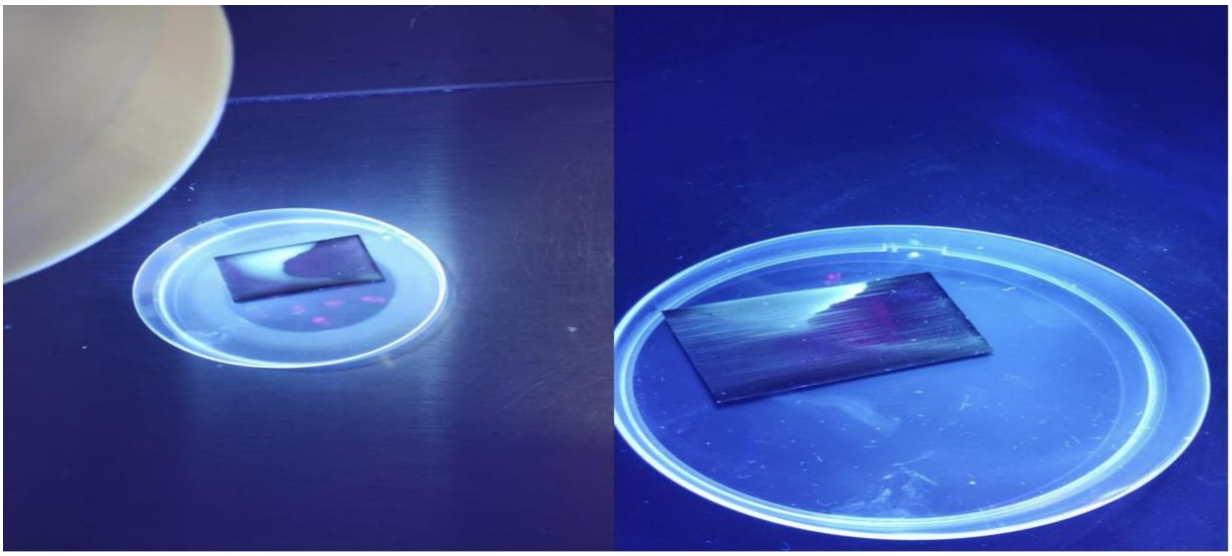


Figure 2: Growth of S. Aureus on a stainless steel plate after a) 24 hours of incubation and b) 48 hours of incubation.

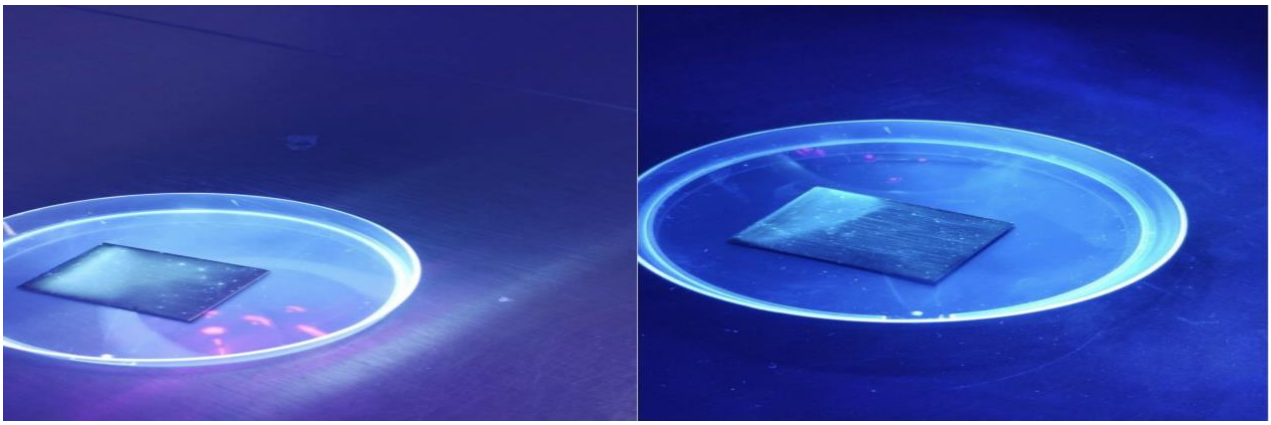


Figure 3: Growth of P. aureginosa on a stainless steel plate after a) 24 hours of incubation and b) 48 hours of incubation.

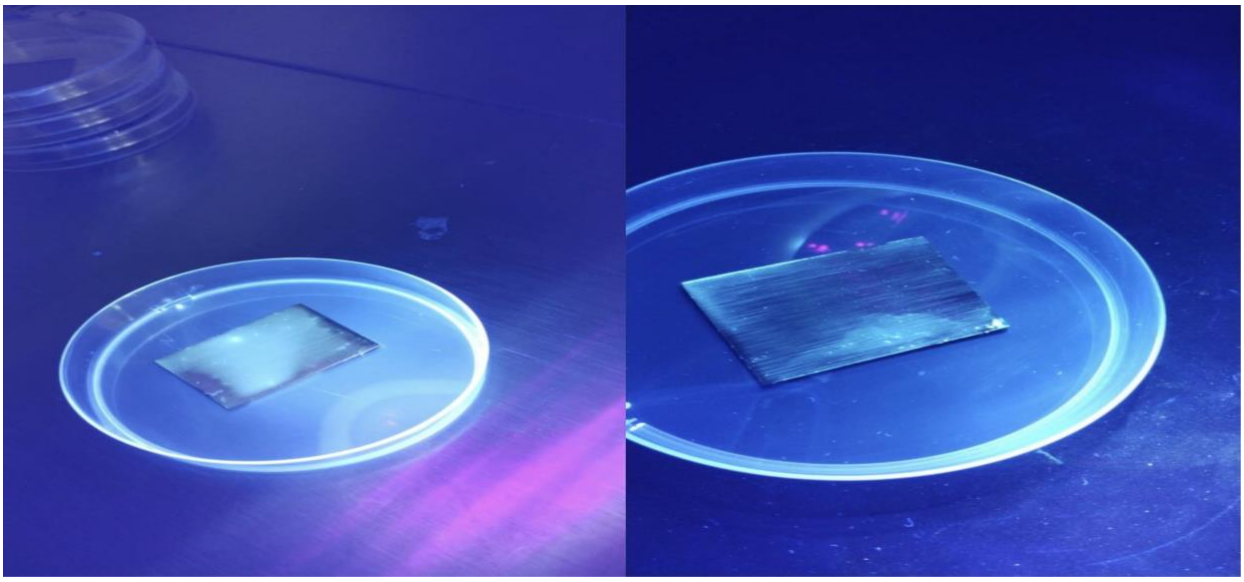
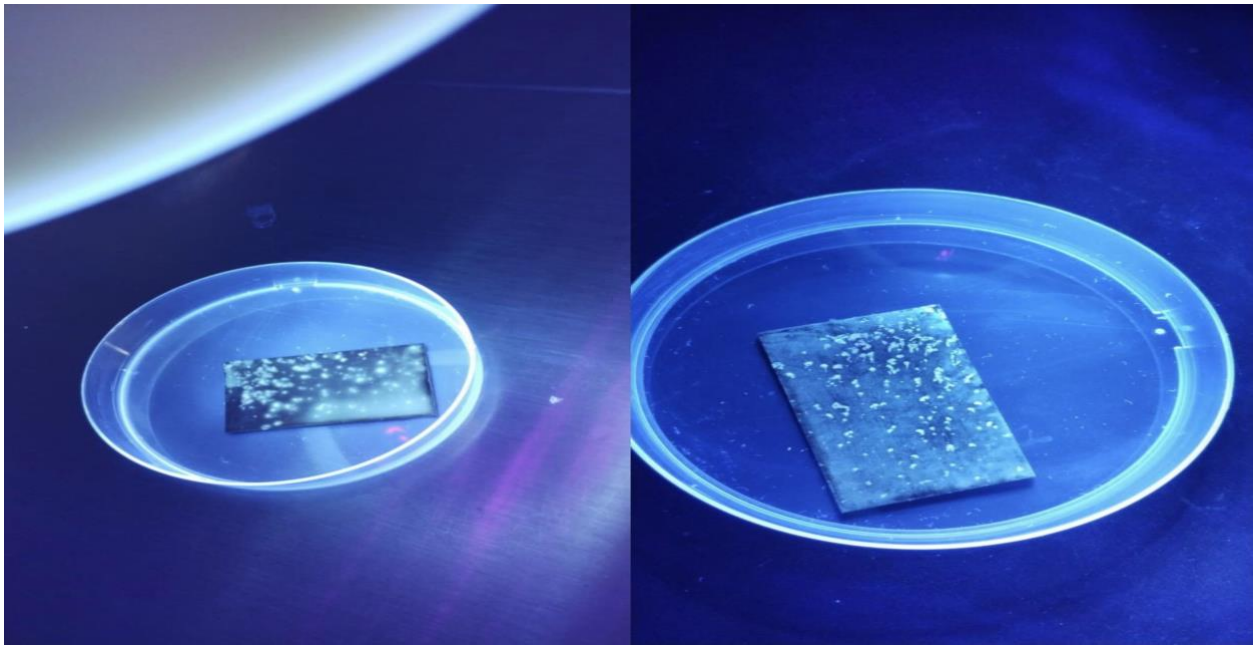
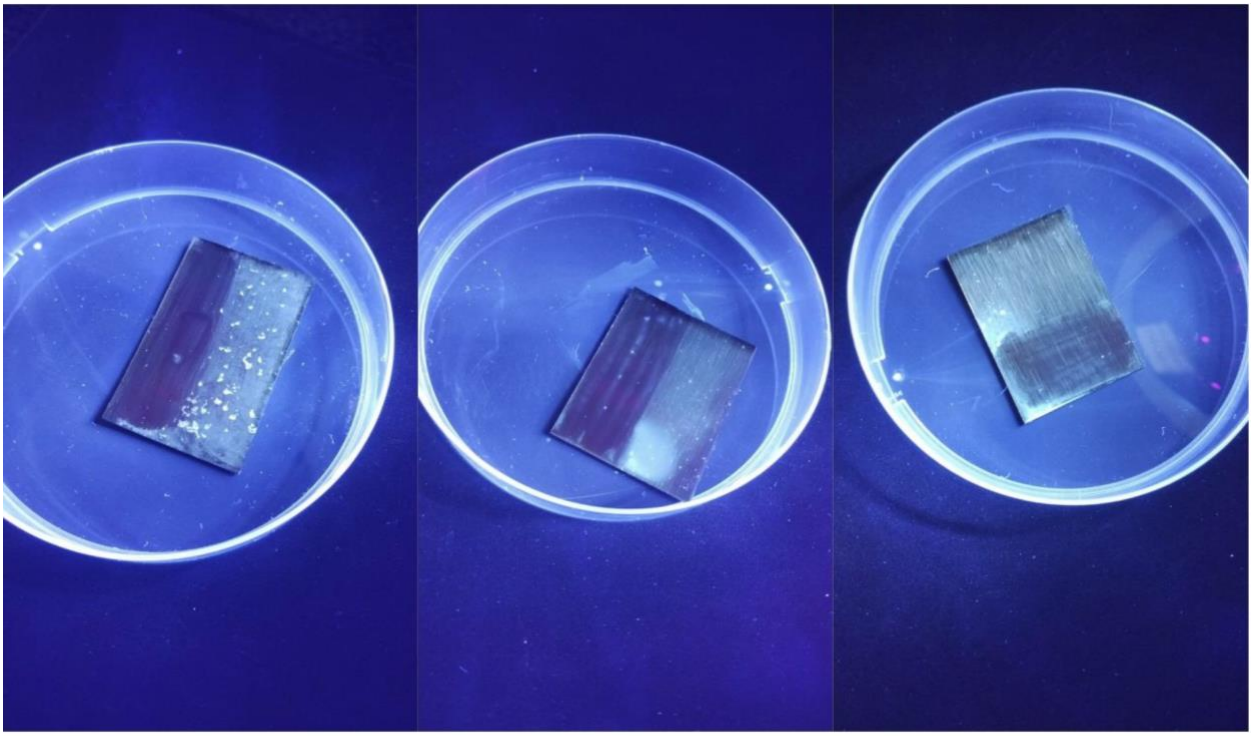


Figure 4: Growth of C. alibicans on a stainless steel plate after a) 24 hours of incubation and b) 48 hours of incubation.



4. Biofilm Formation Verification

After swabbing the stainless steel plate, a contrast was observed between two regions of the surface: one opaque and the other shinier **(Figure 5)**. This distinction was visible to the naked eye but was enhanced by the use of the equipment, confirming effective biofilm formation by all microorganisms tested.



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

CONCLUSION

Using the LOC equipment, it was possible to observe the biofilm formation process of Staphylococcus aureus, Candida albicans, Escherichia coli, and Pseudomonas aeruginosa on stainless steel surfaces from the early incubation stages.

The biofilms were characterized by an opaque layer on the stainless steel surface, which exhibited fluorescence and a white-green coloration when illuminated. Thus, the **LOC – Light On Cells** device can effectively be used to identify bacterial and fungal biofilms on contaminated surfaces.

REFERENCES

- LIN, H. L. et al. Revisiting with a relative-density calibration approach the determination of growth rates of microorganisms by use of optical density data from liquid cultures. Applied and Environmental Microbiology, v. 76, 2010. doi: 10.1128/AEM.00824-09

- PIMENTEL-FILHO, N. J. et al. Bovicin HC5 and nisin reduce Staphylococcus aureus adhesion to polystyrene and change the hydrophobicity profile and Gibbs free energy of adhesion. International Journal of Food Microbiology, v. 190, p. 1–8, 2014. doi:10.1016/j.ijfoodmicro.2014.08.004

- RODRÍGUEZ-TUDELA J. L. et al. Standardization of antifungal susceptibility variables for a semiautomated methodology. Journal of Clinical Microbiology, v. 39, p. 2513-2517, 2001. doi: 10.1128/JCM.39.7.2513-2517

- ŽIVKOVIĆ, V. et al. To biofilm or no to biofilm? SEEMEDJ, v. 2, 2018. doi: 10.26332/seemedj.v2i1.69