

**TITLE:** ANALYSIS OF ANTIBIOFILM ACTIVITY BY CONSOCAL LASER MICROSCOPY: INNOVATIVE PRODUCTS WITH APPLICABILITY IN WATERLINES OF DENTAL EQUIPMENT

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**ABSTRACT:** Health care is constantly challenged in the dental environment, with microbial contamination and the formation of biofilm waterline of the dental equipment being challenges that must be faced with disinfection protocols with chemical agents. The objective of this research was to analyze in vitro the antibiofilm activity of innovative products with promising applicability in the waterline of dental equipment. For the analysis of the antibiofilm activity, 200µL of Mueller Hinton Broth (MHb) containing standardized bacterial inocula (106UFC/mL) of *Escherichia coli* (ATCC 25922), *Pseudomonas aeruginosa* (ATCC 27853) and *Staphylococcus aureus* (ATCC 29923) in black plates were used. 96 glass-bottom wells. The plates were incubated at 37°C for 48h in an oven with orbital shaking at 90rpm. After 24h, the MHb was replaced by another freshly prepared culture medium. After the incubation period, the wells were washed twice with PBS to remove planktonic cells. The three products were kept in contact with the biofilm for 24 hours. After removing the products, the wells were washed twice with PBS and the remaining biofilm was stained with 100µL of the FilmTracer™ LIVE/DEAD™ Biofilm Viability Kit solution for 15 minutes. Subsequently, 15 randomly distributed images were acquired with an Operetta CLS High-Content microscope for each well. There was a significant reduction in the total biofilm after the use of the products ( $p < 0.001$ ), compared to the control. Despite the significant reduction, residual biofilm aggregates were still observed, covering a large portion of the surfaces, even after using the products. Considering the amount of live cells of *P. aeruginosa* and *E. coli*, Product A and Product B, alone or together, showed similar results. In addition, Product AB and the sequential use of Products A+B+AB did not promote difference in the amount of living *S. aureus* cells, compared to the control, indicating that the combination of products did not enhance the antibiofilm activity. It is concluded that the three products individually or in sequential use are innovative and demonstrate promising antibiofilm activity for applicability in water lines of dental equipment.

**Keywords:** antibiofilm activity, dental unit, chemicals.

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