

**TITLE:** EVALUATION OF CELL VIABILITY IN BIOFILMES: INNOVATIVES PRODUCTS WITH POSSIBLE APPLICABILITY IN DECONTAMINATION OF DENTAL UNIT WATERLINES

**AUTHORS:** MONTEIRO, R.M.<sup>1</sup>; AZEVEDO, M.P.F.<sup>1</sup>; OLIVEIRA, V.C.<sup>1,2</sup>; RAZABONI, A.M.<sup>2</sup>; WATANABE, E.<sup>1,2</sup>

**INSTITUTION:** <sup>1</sup>ESCOLA DE ENFERMAGEM DE RIBEIRÃO PRETO - USP, RIBEIRÃO PRETO, SP (AVENIDA DOS BANDEIRANTES, 3900, CEP: 14040-902, SÃO PAULO - SP, BRAZIL); <sup>2</sup>FACULDADE DE ODONTOLOGIA DE RIBEIRÃO PRETO – USP, RIBEIRÃO PRETO, SP (AVENIDA DO CAFÉ S/N, CEP: 14040-904, SÃO PAULO - SP. BRAZIL).

**ABSTRACT:**

In dentistry, dental unit waterlines represent a potential source of microbial contamination (water and the environment) due to the biofilm, which is considered an "amplifying system" of the low amount of microorganisms present in the public water supply. Standardized inoculum biofilms ( $10^8$ CFU/mL) of *Escherichia coli* (ATCC 25922), *Pseudomonas aeruginosa* (ATCC 27853) and *Staphylococcus aureus* (ATCC 29923) were exposed to negative control (saline), Wanitox A and Wanitox B for 24 hours. After 15 min of the addition of 100  $\mu$ L of the diluted solution of the *FilmTracer*<sup>™</sup> *LIVE/DEAD*<sup>®</sup> *Biofilm Viability kit* (Molecular Probes, Inc., Eugene, OR, USA), the reading of the live/dead cells in biofilms was performed by fluorescence microscope (Carl Zeiss Microscopy, Jena, Germany) with an increase of 630x. The images obtained by microscopy of the different fields were transferred to the Fiji program for the quantification of live and dead cells in biofilms. The data collected (each group with n=15) were transformed in percentage and submitted to the appropriate codification, validation by double typing, export to the BioEstat program (version 5.3), and statistical analysis (ANOVA and Tukey test) using level of significance  $\alpha = 5\%$ . According to the quantification of total cells and cell viability (live/green and dead/red cells): Regarding *E. coli*, for the number of total cells, there was a difference between Wanitox A and the negative control ( $p < 0.01$ ), as well as between Wanitox A and Wanitox B ( $p < 0.05$ ). For live cells, the difference between Wanitox A ( $p < 0.01$ ) and Wanitox B ( $p < 0.05$ ) with the negative control was evidenced, however none between Wanitox A and Wanitox B ( $p > 0.05$ ). According to dead cells, only Wanitox A and Wanitox B ( $p < 0.01$ ) were observed. Regarding *P. aeruginosa*, the three evaluated products showed no difference in the amount of total cells ( $p = 0.22$ ) and dead cells ( $p = 0.92$ ). However, Wanitox B had more live cells than the negative control and Wanitox A ( $p < 0.01$ ). In the *S. aureus* group, difference between Wanitox A and Wanitox B with negative control was showed for total and live cells ( $p < 0.01$ ), but there was no difference between Wanitox A and Wanitox B ( $p > 0.05$ ). However, the three evaluated products were different from each other ( $p < 0.01$ ) for dead cells. Our innovative products (Wanitox A and Wanitox B) to control biofilms in dental unit waterlines reduced the amount of live cells (*E. coli* and *S. aureus*) *in vitro*.

**Keywords:** biofilms, dental unit waterlines, dentistry.

**Development Agency:** FAPESP (Process n° 2013/02984-8).