

**TITLE:** STANDARDIZATION OF *STAPHYLOCOCCUS AUREUS* BIOFILMS

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Biofilm formation plays an important role in the pathogenesis of nosocomial infections caused by *Staphylococcus aureus* because of its ability to colonize resident medical devices. This event is an important virulence factor being the major cause of chronic infections and increased antimicrobial tolerance. The objective of this study is to standardize a technique for the formation of *S. aureus* biofilms in polystyrene flat bottom 96 well-microplate. A standard suspension of  $1 \times 10^8$  cfu/ml *S. aureus* (ATCC 29213 or ATCC 6538) in Tryptic Soy Broth (TSB) supplemented or not with 2% glucose and 2% NaCl was dispensed (100  $\mu$ L) into the wells of the polystyrene flat bottom 96 well-microplate, incubated at 35 ° C, under agitation (150 RPM) for up to 96 h . At each 24 h the supernatant was removed and the biofilm was washed with PBS and quantified by the violet crystal staining technique and the optical density (O.D.) measured at 590 nm. The data analyzed indicate that in the medium supplemented with 2% glucose and 2% NaCl lead to the better biofilm formation (O.D ~ 3.0) when compared to TSB without supplementation (O.D. ~ 1.5). Both *S. aureus* strains of in TSB supplemented presented robust biofilms at 24h and remained up to 96h, but the ATCC 6538 strain presented greater adhesion at the microplate surface in detriment of the ATCC 29213 strain. Studies have shown that supplementation of the TSB medium plays a key role in the formation of *S. aureus* biofilm because it is related to the *ica* locus expression encoding the enzymes involved in the N-acetylglucosamine oligomer biosynthesis, important to extracellular matrix production and biofilm adhesion. In this way, the standardization of biofilm formation is an important step to study new therapeutic strategies to combat *S. aureus* infection. Fighting biofilms is a considerable challenge for both researchers and clinicians. Processes involved in the formation, maintenance and dispersion of microbial biofilms could be targets for the discovery of new inhibitors that could be used alone or in combination with conventional antimicrobial agents.

**Key words:** Biofilm, *Staphylococcus aureus*, nosocomial infections

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