ABSTRACT

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Subject of study: Bioanalytical laboratory diagnostics in healtcare

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Title: Evalution of the effectiveness of selected methodical approaches for disagregation of staphylococcal biofilm biomass

Background: Evaluation of the impact of selected biochemical and physical approaches on the disaggregation of the biofilm community formed by the bacterium *Staphylococcus aureus*. For the biochemical approach was chosen enzyme proteinase K, the impact of sonication, shaking with/without glass beads was studied within the physical approaches.

Methods: Staphylococcal biofilm was formed *in vitro* in two methodical configurations – in the wells of a microtiter plate and on pegs (analogy to the Calgary Biofilm Device). To evaluate the degree of consistency of the obtained data, which reflects the homogenity of the biofilm biomass disaggeragation, the „recovery" method, and the method of metabolic activity evaluation, using the Alamar Blue indicator. To express the degree of effectiveness of the chosen approaches on the overall disaggregation of the biofilm community, the method of quantification of biofilm biomass by crystal violet staining was chosen.

Results: In the case of the biochemical approach (proteinase K enzyme), a statistically significant effect on the disaggregation of the staphylococcal biofilm, formed both within the wells of the plate and on the pegs, was demonstrated. In the case of physical approaches, a statistically significant effect was demonstrated only in the case of sonication - shaking without beads - sonication, on the biofilm formed within the wells of the plate. Furthermore, it has been shown that both biochemical and physical approaches do not contribute to the homogeneous disaggregation of staphylococcal biofilm biomass. The disaggregation approaches chosen are not suitable for any subsequent steps to the evalutation of number of viable biofilm-forming cells, such as seeding methods. It was also revealed that most of the chosen physical approaches (except the shaking step with beads alone, for the pegs biofilm model), do not have statistically significant impact on the viability of bacteria in the biofilm community.

Conclusions: The chosen studied approaches in themselves seem to be insufficient for significant disaggregation. Both the biochemical and physical approaches do not guarantee the acquisition of homogeneous quantities of biofilm-forming bacteria. In the future, other biochemical approaches will be evaluated, together with approaches combining both biochemical and physical effects.

Key words: bacterial biofilm, biofilm dispersion, extracellular polymeric substances, proteinase K, sonication