

Supplementary Files

# In Vitro Evaluation of Polihexanide, Octenidine and NaClO/HClO - Based Antiseptics Against Biofilm Formed by Wound Pathogens

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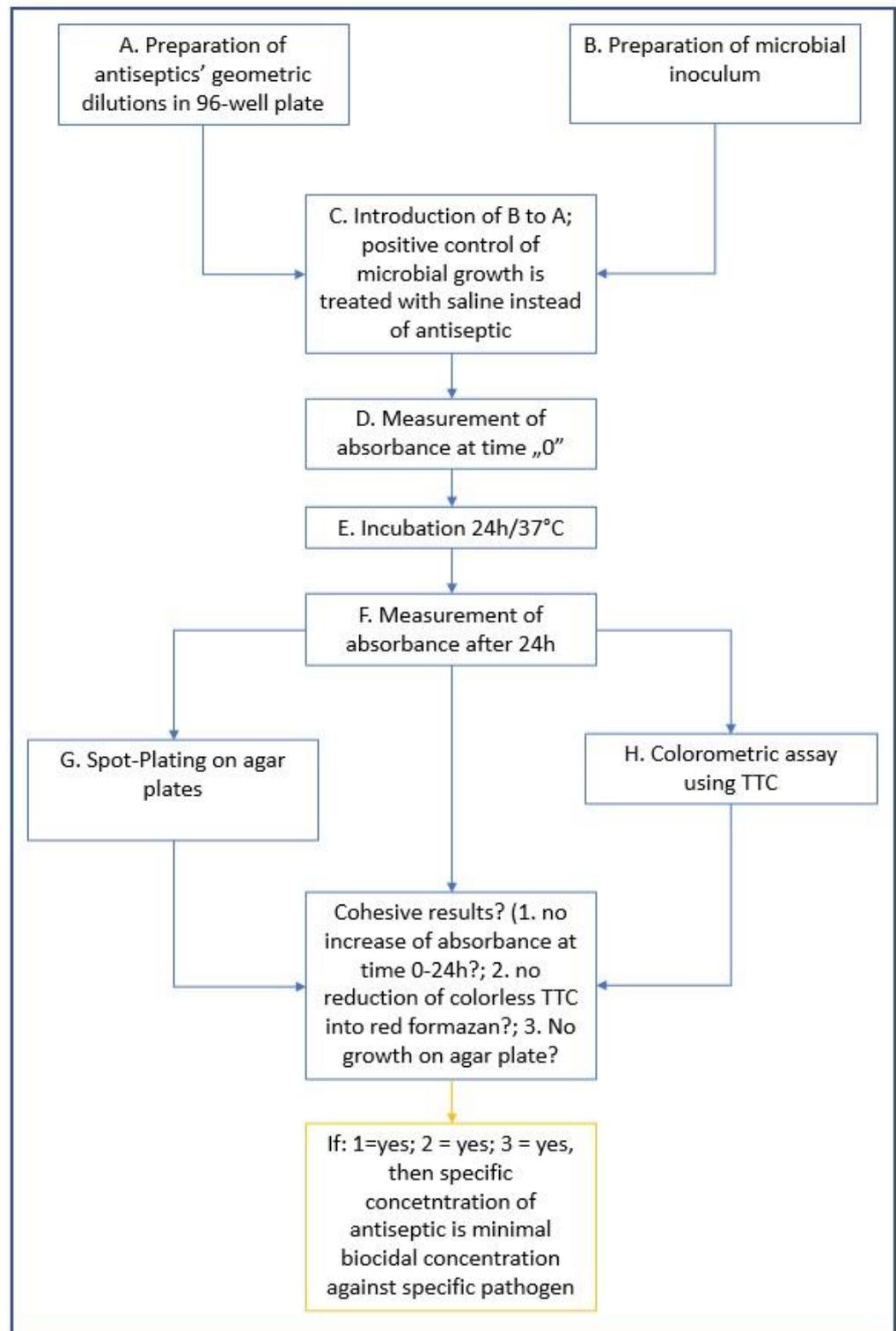
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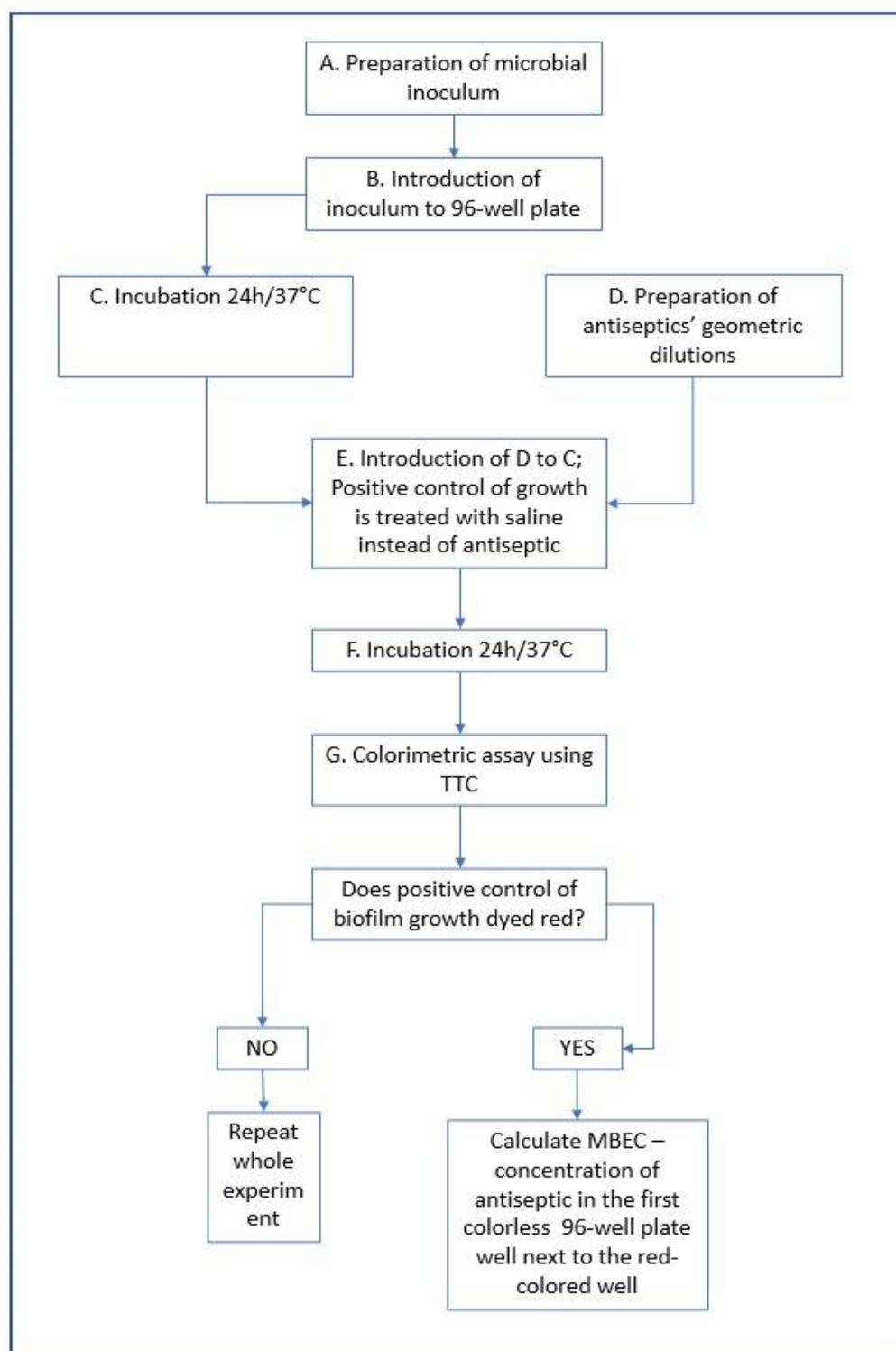


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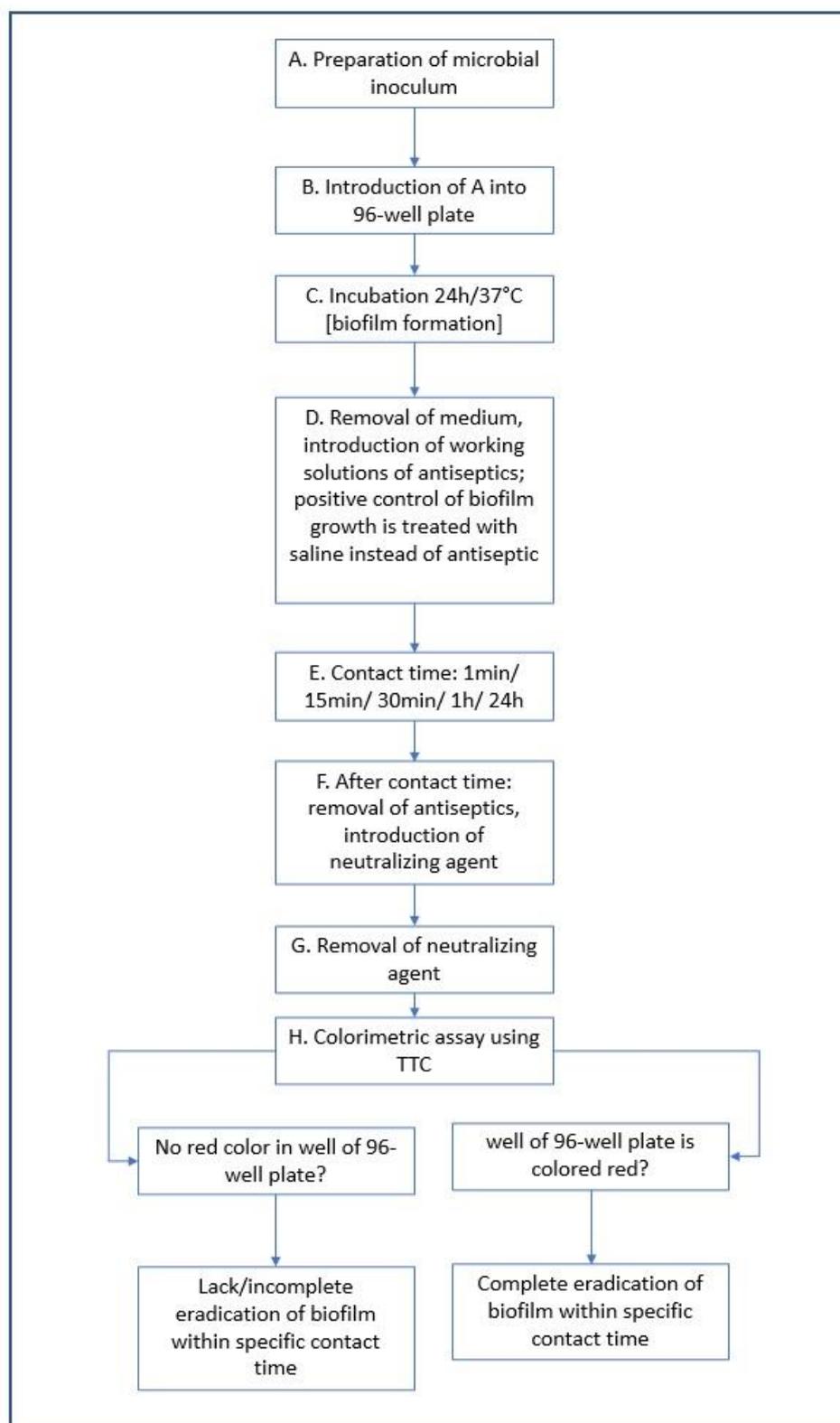
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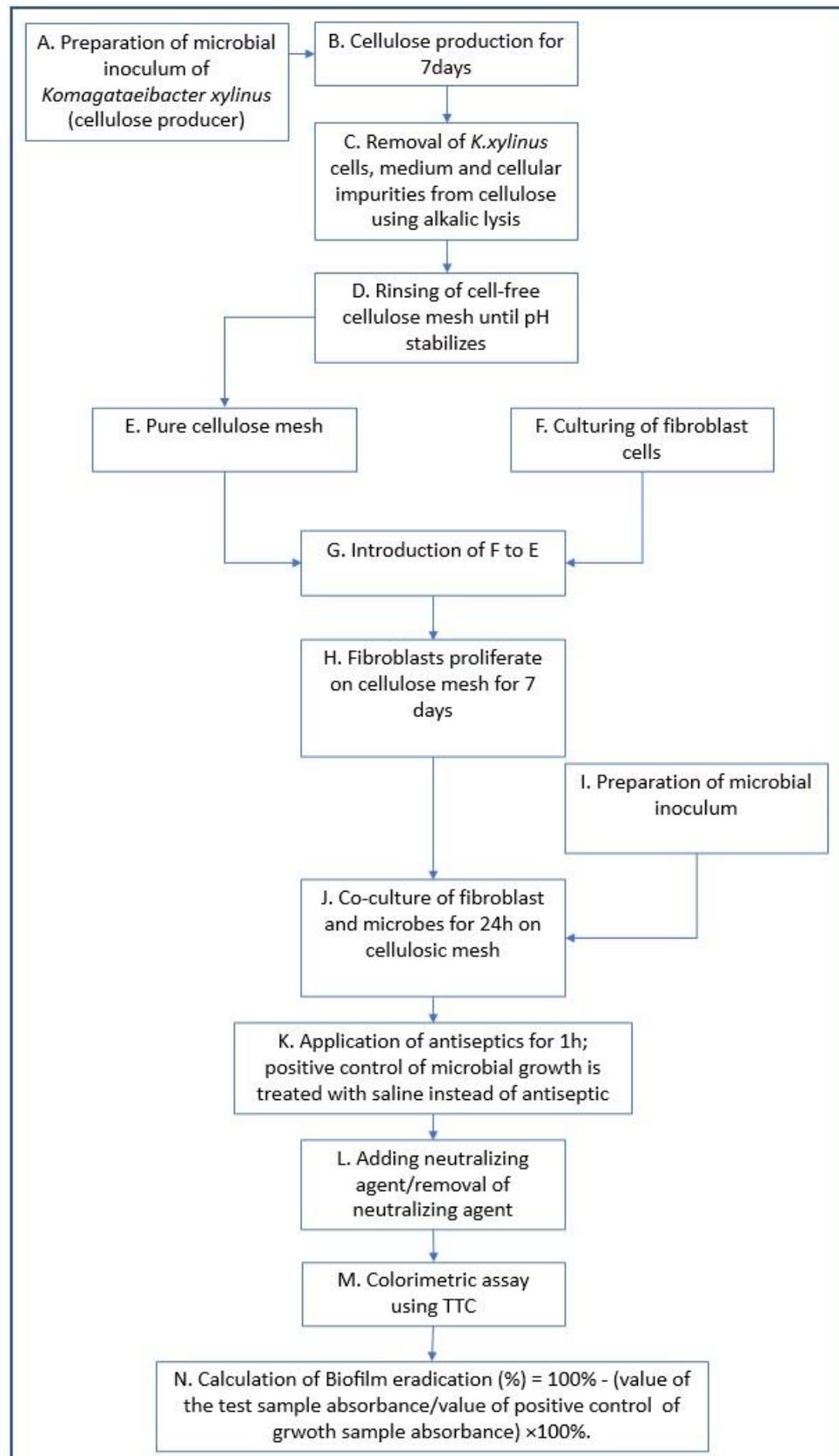
**Figure S1.** Flow chart of Minimal Biocidal Concentration performance; TTC – tetrazolium chloride.



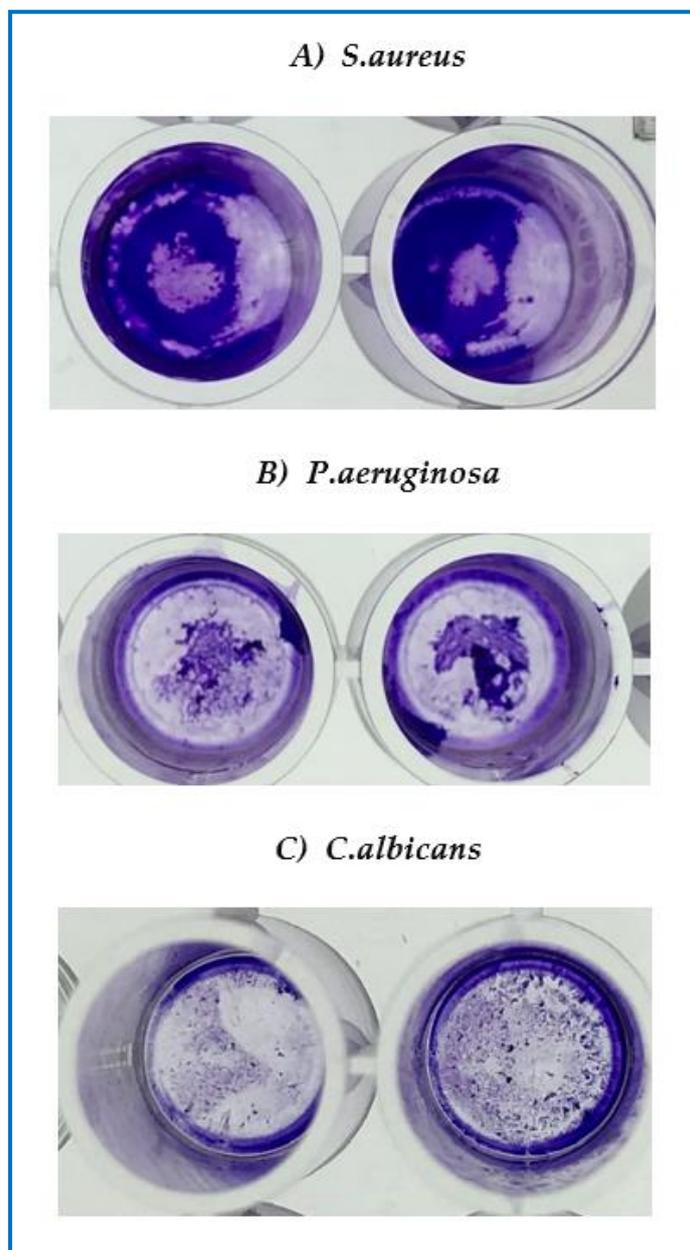
**Figure S2.** Flow chart of Minimal Biofilm Eradication Concentration performance; TTC – tetrazolium chloride.



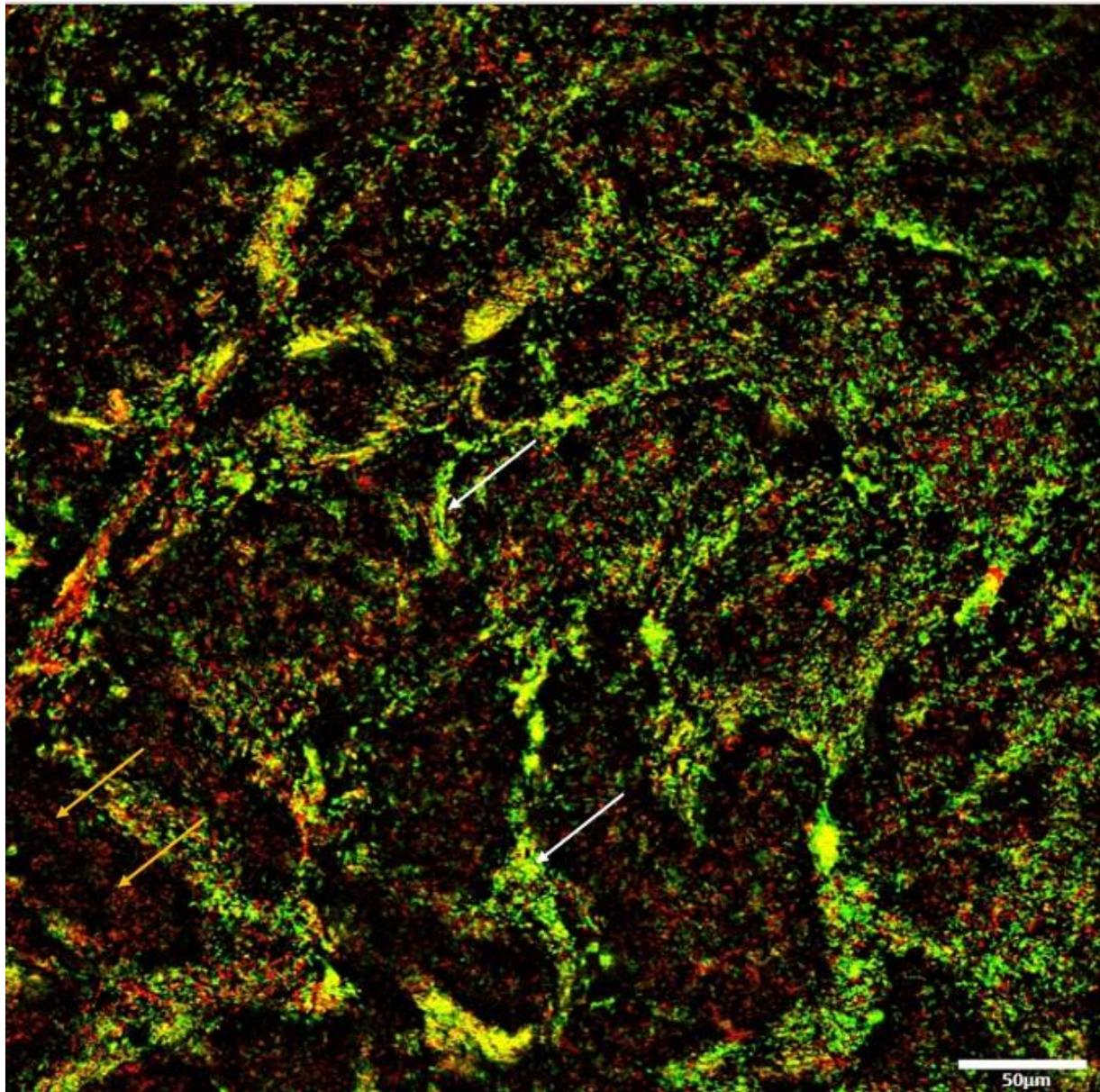
**Figure S3.** Flow chart of Biofilm-Oriented Antiseptic Test performance; TTC – tetrazolium chloride.



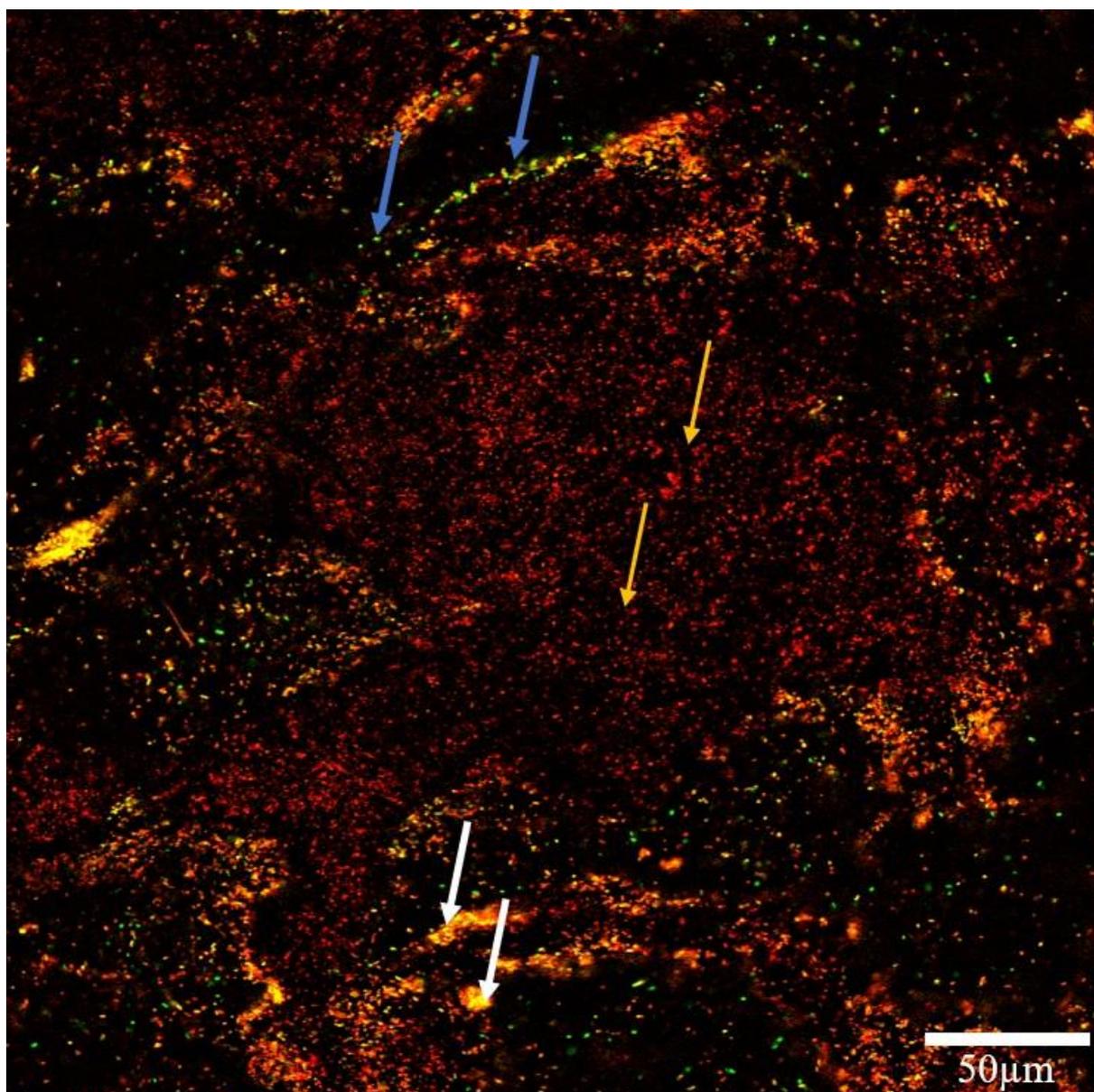
**Figure S4.** Flow chart of assessment using Cellulose-Based Biofilm Model; TTC – tetrazolium chloride.



**Figure S5.** The Cristal Violet-dyed biofilm formed by *S.aureus* PRT-9, *P.aeruginosa* PRT-9, *C.albicans* PRT-9 on the bottom of polystyrene well. Please note that majority of biofilm biomass is located on the flat surface of well. The standard Crystal Violet technique for biofilm dyeing was performed according to the protocol presented by Merritt JH, Kadouri DE, O'Toole GA. *Growing and analyzing static biofilms*. Curr Protoc Microbiol. 2005;Chapter 1:Unit-1B.1. doi:10.1002/9780471729259.mc01b01s00.



**Figure S6.** The untreated with antiseptics (positive control of growth) staphylococcal biofilm dyed with Live/Dead staining kit. White arrows indicate aggregates of live staphylococcal cells (dyed green), while orange arrows indicate aggregates of dead staphylococcal stains (dyed red). Please note that staphylococcal biofilm untreated with antiseptics consists of live and dead cells, however the number of live cells prevails over dead cells.



**Figure S7.** Staphylococcal biofilm treated with O-antiseptic for 1h and dyed with Live/Dead staining kit. Blue arrows indicate aggregates of live staphylococcal cells (dyed green); orange arrows indicate aggregates of dead staphylococcal stains (dyed red), while white arrows indicate aggregates of staphylococcal cells with partially damaged cell wall (incorporation of two dyes). Please note the higher share of black (undyed) surfaces in this picture comparing to picture presented in Fig.S6. These are surfaces, where complete biofilm removal occurred.