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S. aureus biofilm removal multi-assay V.1

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We are still developing and optimizing this protocol

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Disclaimer

Abstract

The following protocol describes culture and treatment of the S.aureus-based biofilm and techniques that can be used to asses its eradication/removal. The protocol covers basic preparation and three techniques, namely: growth control, colony forming assay, and crystal violet staining.

Troubleshooting

Biofilm culture

1d 0h 5m

1 Culture bacteria strains in **TSB** at 35 °C for approximately 04:00:00 in order to reach mid-log phase (OD_{600} 0.5-0.6). 4h

2 Pellet the cells by centrifugation (4500 rpm, 20°C, 00:05:00) then wash twice with potassium phosphate buffer (**PPB**; [M] 100 millimolar (mM) , pH 7). 5m

For washing, redisperse and repeat the centrifugation. First two washes with 3 mL of **PPB**, then redisperse in 3 mL of**PPB**

3 Adjust bacterial suspensions to 10^7 CFU/mL in **PPB** (OD_{600}= 0.01, ref: **PPB**).

4 Initiate biofilm formation in 24-well plate by covering surface with 1 mL of the adjusted cell suspension, and incubate the plate at Room temperature for 02:00:00 to allow the bacterial adhesion. Remove the content of the wells after this time. 2h

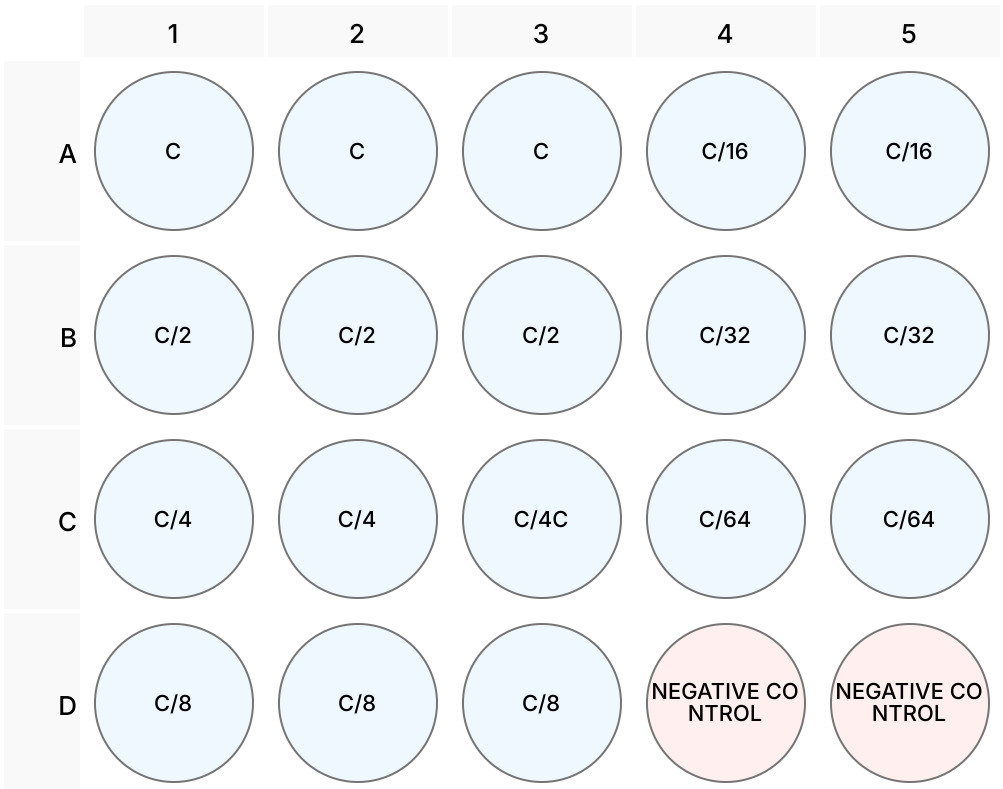
5 Cover the wells with 1 mL of **TSB**, and put a protective film before putting the lid to minimize the evaporation. Incubate the bacteria for at least 18:00:00 18h

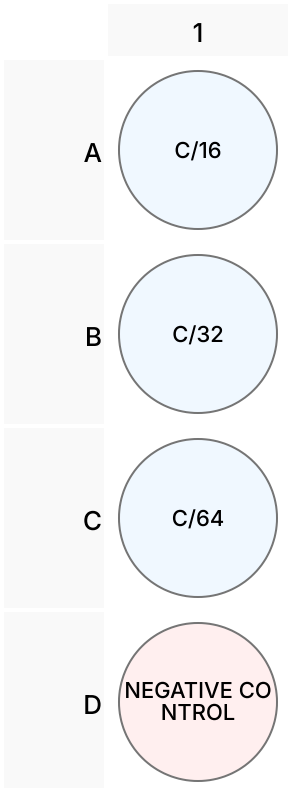
Treatment of the biofilm

6 Prepare your substance in either **PBS** or **NaCl (** [M] 9 Mass Percent **)** by serial dilution ranging from C to $C/(X^2)$ where X is the number of concentrations tested. A 24-well plate can hold 7 concentrations + negative control.

7 After the biofilm matured, remove the 24-well plate from the incubator.

8 Gently remove the **TSB** using either pipette or vacuum aspirator. After removing medium from a cell, immediately wash it with **PPB**, **PBS** or **NaCl** two times. After last washing add 1 mL of your sample. Repeat for concentration in triplicate. Same as before, apply protective film before incubating the plate.





9 Treat the biofilms at suitable temperature and time. This two factors have to be estimated prior to the treatment, for example by means of crystal violet staining (see below).

10 After the time has passed, remove the plate, and start removing the content of the well. After each removal, wash the remaining biofilm three times with either **PBS**, **NaCl** or **PPB**. Remove remaining liquid after and perform on of the techniques described below.

Growth control

11 Add 0.5 mL of **TSB** to each well and indubate the plate at 37 °C for 06:00:00 08:00:00 14h

12 Transfer the supernatant to 96-well plate and measure OD_{600}, dilute with **NaCl** if necessary.

Colony forming assay 4h

13 Add 1 mL of either **PBS**, **NaCl** or **PPB** to the well with a pipette, directly onto the bioflm, so the thrust of the liquid damage the biofilm.

14 Scratch the bottom of the well with a pipette tip and transfer the content to eppendorf tubes. Vortex-shake eppendorfs for couple of seconds or until reaching homogeneity.

15 Serially dilute the content of the eppendorf tubes in 96-well plate.

16 Plate 20µL of the content of the wells on the **BHI** medium prepared in the square Petri dish.

17 Incubate Petri dish Overnight 4h

18 Next day, count the colonies and asses the CFU/mL or CFU/cm^2.

Crystal violet staining + brightfield microscopy 1h 30m

19 Dry the biofilms for 00:30:00 in Room temperature 30m

20 After that time, fix the biofilms by adding 200 µL of methanol to each well. Wait 00:15:00 and remove any remaining methanol. 15m

21 Add 250 µL of [M] 0.06 Mass Percent solution of crystal violet to each well and wait 00:15:00 . 15m

22 Remove any non-absorbed dye by washing gently stained biofilms with either **PBS**, **NaCl** or **PPB**.

22.1 At this moment you can perform brightfield microscopy of the biofilm to image better its structure.

23 Add 1 mL of acetic acid to the wells and gently shake 120 rpm, Room temperature them horizontally for 00:30:00 . 30m

24 Remove 200 µL od the solubilised pigment with a pipette and transfer it to 96-well plate and dilute serially with water.

25 Record the absorption of the wells at **570 nm**. The percent of the remaining biomass is calculated by dividing the A_{570} of the sample to A_{570} of the control.