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AmB quantitation in 24 hrs. biofilm.

1- Add 2 ml of RPMI to each well of a 6 well plate.
2- Prepare a 10^6/ml cell suspension and add 100ul of the suspension to each well.
3- Incubate at 37 C for 30-60 min without shaking.
4- Cover the plate with foil and place in the shaker incubator (50 rpm) at 37C overnight.
5- Next day careful, remove the RPMI and add 1 ml of fresh RPMI containing X ug/ml of amphotericin. Incubate at 37C for the desire time (1, 2, 4 or 6 hrs.)
6- At the end of the time-point remove RPMI and save. (label tube Sup)
7- Rinse the biofilm with 1 ml pf 1X PBS, save the rinse. (Label tube rinse)
8- Add 1-2 ml of PBS, with a spatula scrape the biofilm, and pipette it into a 15 ml conical tube. (this tube contains the biofilm)
9- Sonicate the above tubes for 20 min to remove the matrix.
10- Spin tubes at 4K rpm for 10 min.
11- Remove matrix and place in a fresh tube. (labeled matrix)
12- Resuspend the cell pellet in 1.2 ml of PBS.
13- Add the 1.2 ml from step 12 to a tube containing glass beats.
14- Using the beater beat 3 times. (see how is done first)
15- Remove the fluid from the glass beating tube and place in fresh tube.
16- Centrifuge for 10 min @ 10K.
17- Remove supernatant, (this is the intracellular material). Pellet in the bottom of the tube is the cell wall material.
18- On a 96 well plate, prepare a standard curve with known amounts/concentrations of Amphotericin 100ul/well. Test the samples collected from steps 6,7,11 & 17 in triplicates.
19- Read the absorbance at 328 & 415 nm.
20- Plot the standard curve and calculate the values/concentrations of your samples.