P12 Bacterial biofilm

Task 1: Microscopy of oral biofilm

Using and sterile stick, get dental plaque. Make smear to a slide, fixate it and Gram stain it. Instead of the second one you have a picture in the presentation. The slide was stained 5 minutes by alcian blue (and dye selectively binding the polysacharides). Describe and draw the objects. Mention clusters of bacteria and in alciane blue stained preparation also extracellullar polysacharidic substances.

Gram staining	Alciane blue

Task 2: Effect of teeth cleaning to oral biofilm

Wash your mouth by a solution of given stain according to teachers instructions and observe. Stained places are covered biofilmem. Describe places, where biofilm is the most denese, eventually where the biofilm was not destroyed at cleaning teeth. After that clean your teeth.

Result: Biofilm was mostly present at following places: _____

_____

Task 3: Diagnostics of microbes colonizing catheters

a) Qualitative method multiplication in broth

Extracted central venous catheter (CVK) was put into cultivation medium and cultured 24 hodin. After that, the turbid cultivation médium was inoculated onto blood agar. Assess growth of microorganisms onto blood agar.

b) Semi-quantitative method (Maki method)

Extracted CVK was rolled on the surface of the blood agar, which was cultured after that. Evaluate growth microorganisms and count grown colonies. As significant take amout of colonies >15, less than 15 colonies should be consedered to be contamination. If there are clearly more than 100 colonies, do not count them and write down simply „> 100“

c) Quantification acording to catheter sonification

Extracted CVK is put into 10 ml of saline and after that ultrasound effects on it, destroying the biofilm structure and releasing individual bacterial cells. 100 microlitrs of such suspension should be inoculated directly onto blood agar and diluted by a sterile loop onto the whole agar surface. According to teacher's instructions, perform sonification of catheter. Inoculated blood agars place into the termostat to 37 °C.
Onto prepared Petri dish, count how many colonies grew onto blood agar and count the number of bacteria adhering onto the cathether surface. If there are clearly more than 100 colonies, do not count them and write down simply „> 100“

Results:

	3a	3b	3c
Estimated number of organisms			

Which of the methods enables to detect and to quantify not only bacteria present onto the surface of the catheter, but also in its lumen? _____

_____

What methods enable us to quantify the amount of bacteria adhering to the catheter surface?

_____

What is the sense of quantification of a microbe izolated from a catheter? _____

Task 4: Influence of presence of saccharides onto biofilm growth dynamics

Into individual wells of a microtitration plate with BHI medium supplemented by 0 %, 2 %, 4 %, 8 % of glucose, Streptococcus mutans strain was inoculate. After 2, 8, 16, 24 hrs of culture at 37 °C the well were three times washed. The biofilm layer, strongly adhered onto the surface was stained by 20 minute action of gentiane violet. The remaining dye was removed from wells by a careful washing. Intensity of colour of wells is measured by a spectrophotometer and correspond to the thickness of the biofilm layer.
On a sheet of paper you have results of spectrophotometric measurment of intensity of well colours. From given result, draw a 3D-graphics of dynamics of biofilm formation in correlation to glucose concentration and time. (For each time and concentration, six wells are measured; choose always an approximated average, it is not necessary to count very preciselly.)

Average values*	2 h	8 h	16 h	24 h
0 %				
2 %				
4 %				
8 %				

*absorbance values, approx. average of all six wells that were kept at the same glukose concentration an the same time

How supplementation of medium by glucose influences the biofilm?

Task 5: Susceptibility of biofilm-positive microbes to antimicrobial agents (comparison of planctonic and biofilm life form)

On the plate No. 1 you have for the planctonic form of *S. epidermidis*. The same strain was cultured so that it formed a biofilm onto the wells of microtitration plate. Onto that biofilm, antibiotics in the same concentrations as in plate No. 1 effected. After 18 hrs. of effect, antibiotics were destroyed and into the wells, colorimetric medium was added (plate No. 2). Presence of living bacterial cells leads to colour change of the medium (red to yellow). According to interpretation tables, assess MIC for planktonic form and concentration of antibiotics able to attack cells in biofilm and so to eradicate them (minimal biofilm eradication koncentration, MBEC). When, in case of MBEC, all wells are yellow, write in MBEC e. g. „> 1024“, if 1024 mg/l is the highest concentration in the second well.

Antibiotic	*S. epidermidis* – planctonic form	*S. epidermidis* – growth in biofilm form
	MIC	MBEC