P11 Bacterial biofilm

To study: Bacterial biofilm (from textbooks, www etc.)
From spring term: Microscopy, culture, biochemical identification, antibiotic susceptibility

Task 1: Microscopy of oral biofilm

Use a sterile slanted wooden skewer to obtain dental plaque. Smear it on **two** slides, fix them and Gram stain one of them. The second one is to be stained 5 minutes by alcian blue (a dye selectively binding the polysacharides). Draw the second picture according to the presentation. Describe and draw the objects. Note the clusters of bacteria and in the alcian blue stained slide also the extracellullar polysacharidic substance.

Gram staining	Alcian blue

Task 2: Effect of teeth cleaning on the oral biofilm

Rinse your mouth with a solution of the given stain according to the teacher's instructions and observe it. The stained spots are covered with biofilm. Describe the places, where the biofilm is the densest, or where the biofilm was not removed during cleaning teeth. After that, clean your teeth.
Result: Biofilm was mostly present at the following locations: _____

_____

Task 3: Diagnostics of microbes colonizing catheters

a) Qualitative method of multiplication in broth

A removed central venous catheter (CVC) was put into the cultivation medium and cultured for 24 hours. After that, the turbid cultivation medium was inoculated onto the blood agar. Assess the growth of microorganisms on blood agar.

b) Semi-quantitative method (Maki's method)

A removed CVC was rolled on the surface of the blood agar, which was subsequently cultured. Evaluate the growth of the microorganisms and count the grown colonies. More than 15 colonies is considered as significant, less than 15 colonies must be attributed to contamination. If there are apparently more than 100 colonies, do not count them and write down only ">100".

c) Quantification according to the catheter sonification

A removed CVC is immersed into 10 ml of saline and after that exposed to the ultrasound which destroys the biofilm structure and releases the individual bacterial cells. 100 microliters of the obtained suspension is inoculated directly onto blood agar and with a sterile loop spread out onto the whole agar surface. According to the teacher's instructions, perform the sonification of the catheter. Incubate the inoculated blood agars at 37 °C.
On the other prepared Petri dish count how many colonies grew on the blood agar and calculate the number of bacteria adhering onto the catheter surface. If there are obviously more than 100 colonies present, do not count them and write down simply "> 100".

Results:

	3a	3b	3c
Estimated number of organisms			

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

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

_____

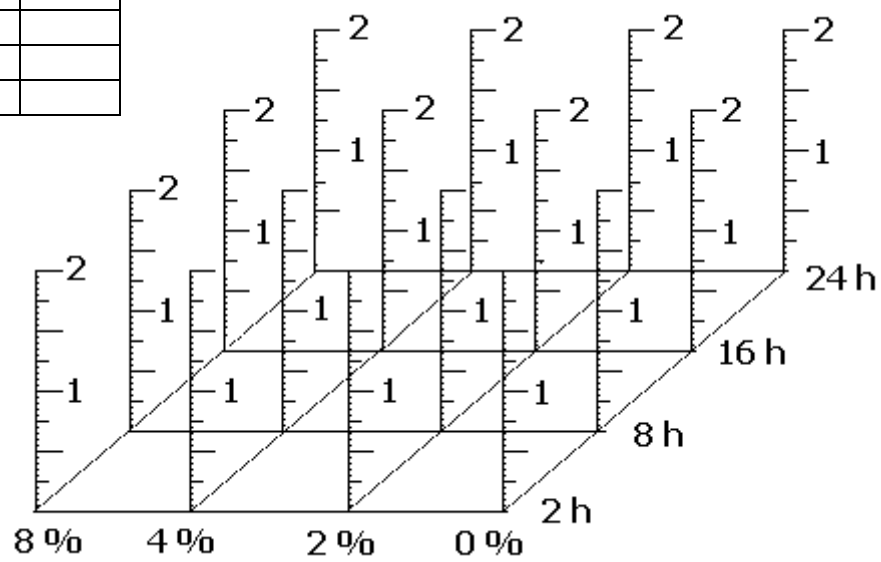
What is the purpose of quantification of a microbe isolated from a catheter? _____

Task 4: The influence of saccharides on the biofilm growth dynamics

The individual wells of a microtitration plate containing the BHI medium supplemented with 0 %, 2 %, 4 % and 8 % of glucose were inoculated with *Streptococcus mutans* strain. After 2, 8, 16 and 24 hrs of culturing at 37 °C the wells were washed three times. The biofilm layer, strongly adhering to the surface, was stained with gentiane violet for 20 minutes. The remaining dye was removed from the wells by careful washing. The intensity of the wells colouring is measured with a spectrophotometer and it corresponds to the thickness of the biofilm layer.
On a sheet of paper, you have the results of spectrophotometric measurement of the well colouring intensity. Based on the presented results, draw a 3D-graph of biofilm formation dynamics in correlation to the glucose concentration and the time. (For each time and concentration, six wells are measured; choose always an approximated average, it is not necessary to establish the average precisely.)

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 glucose concentration for the same time



How does supplementing the medium with glucose influence the biofilm?

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

The microtitration plate No. 1 contains the planktonic form of *S. epidermidis*. On the plate 2, the same strain was cultured in such a way that it formed a biofilm layer in the wells. After that, antibiotics were added to the wells in the same concentrations as in plate No. 1. After 18 hrs, the antibiotics were removed and into the wells a colorimetric medium was added. The presence of living bacterial cells leads to a colour change of the medium (red to yellow). According to the interpretation tables, determine the MIC for the planktonic form and the concentration of antibiotics able to affect the cells in the biofilm and so to eradicate them (minimal biofilm eradication concentration, MBEC). If in case of MBEC all wells are yellow, write that MBEC is e. g. ">1024", if 1024 mg/l is the highest concentration in the plate No 1.

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