

Name: _____

Date: _____

Oral microbiology III - biofilm

Task 1: Microscopy of dental plaque

Using a sterile stick, get dental plaque. Make a smear and Gram stain. Describe and draw the objects. Mention clusters of bacteria, especially their shape and colour.

Task 2: Effect of teeth cleaning to oral biofilm



Wash your mouth by a solution of given stain according to teacher's instructions and observe. Stained places are covered

by biofilm. Describe places, where biofilm is the most dense.

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 hours. After that, the turbid cultivation medium 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 of microorganisms and count grown colonies. As significant take amount of colonies >15, less than 15 colonies should be considered to be contamination. If there are clearly more than 100 colonies, do not count them and write down simply „> 100“.

c) Quantification according 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 microliters 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 thermostat to 37 °C.

Onto prepared Petri dish, count how many colonies grew onto blood agar and count the number of bacteria adhering onto the catheter surface. If there are clearly more than 100 colonies, do not count them and write down simply „> 100“

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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 isolated from a catheter? _____

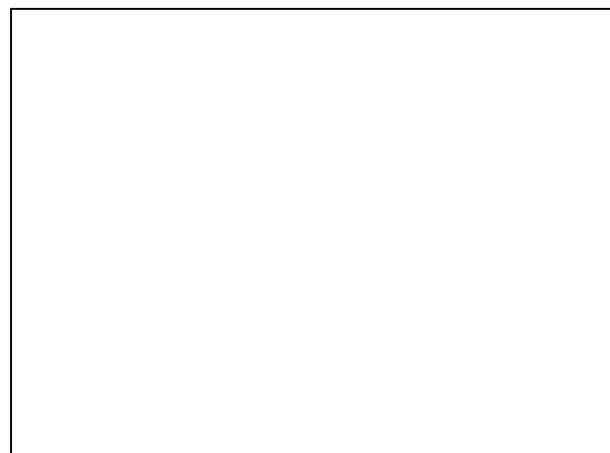
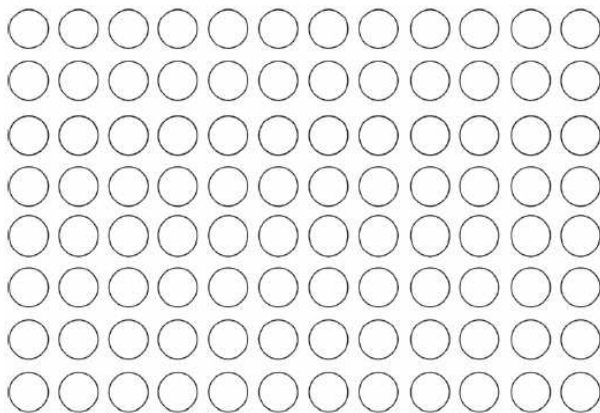
Task 4: Influence of saccharides concentration on biofilm growth dynamics

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 measurement of intensity of well colours. From given result, draw a graph 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 glucose concentration an the same time



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Check up questions:

1. What is biofilm?
2. What are the main components of the biofilm?
3. What is the importance of oral biofilm with relation to dental caries?
4. What more diseases are influenced by a biofilm?
5. What complicates treatment of biofilm infections?