

## P 12 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

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 polysaccharides). Describe and draw the objects. Mention clusters of bacteria and in alciane blue stained preparation also extracellullar polysaccharidic substances.

Gram staining	Alciane blue
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### 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 considered 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? \_\_\_\_\_



## Topic P12

### **Check-up questions:**

1. What the biofilm is?
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?
6. What are the probable causes of higher resistance of biofilm to antibiotics?
7. What are focal infections?
8. How can we measure absorbance (in ELISA or in detection of biofilm in wells)?