Topic J03: Cultivation of bacteria and yeasts

Materials for study (from textbooks, internet etc.): Bacterial culture. Use also your notes from Chemistry and Biochemistry (e. g. Schiff reagent etc.)

Task 1: Characterisation of media and their production

Task 1a: Most important media in medical microbiology

Look at given media and write here the type of medium according to explanation given by your teacher. Do not forgot to write, whether the medium is solid or liquid and whether it is in a Petri dish or in a test tube.

Name of medium	Liquid/solid Petri dish/test t.	Colour	Type of medium (selective, …)	Used for bacteria*:
1. Broth				
2. VL-broth				
3. Selenite broth				
4. Sabouraud agar				
5. Löwenstein-Jenssen medium				
6. Blood agar (BA)				
7. Endo agar (EA)				
8. Mueller Hinton (MH medium)				
9. BA with 10 % NaCl				
10. VL agar (VLA)				
11. XLD				
12. Chocolate agar				
13. Levinthal agar				
14. Slanetz-Bartley medium				

*not necessary to fill everywhere, only in media used for diagnostics of certain bacteria

Task 1b: Manufacturing of blood agar.

Look at the video. Fill in missing parts of following text:

If we want to manufacture blood agar, we have to mix together following components:

_____

Now, the components are heated using Arnold apparatus, and sterilised. Now, we let the temperature to decrease.

At temperature beneath 55 °C we add _____. Then we pour the agar into

_____ or we use _____.

Eventual more notes to blood agar manufacturing:

Task 2: Influence of physical and chemical conditions on the bacterial growth

Task 2a: Influence to oxygen

Five strains (J, K, L, M, N) were cultured on agar plates in four types of conditions:
a) normal athmosphere
b) elevated CO_2 concentration
c) decreased oxygen concentration
d) no oxygen at all (oxygen replaced by a mixture of other gases)
Write „G" (growing) or "N"(not growing); assess, which strain is strictly aerobic, facultatively anaerobic, strictly anaerobic, microaerophilic, capnophilic
Result:

Strain	normal air	elevated CO_2	traces of oxygen only	no oxygen	conclusion
J					
K					
L					
M					
N					

Task 2b: Influence of salts and antibiotics

You can see three strains on various types of media. Describe the presence/absence of growth
Mark: GROWS – DOES NOT GROW

Strain	Blood agar (BA)	BA + NaCl (6,5 %)	BA + NaCl (10 %)	Slanetz Bartley agar (Na-azide)	BA with amicacine
E – *Enterococcus*					
SR – *Streptococcus*					
ST – *Staphylococcus*					

Task 2c: Influence of temperature

Like the previous task, only with the same medium, but different temperature.
Mark: GROWS – DOES NOT GROW

Strain	4 °C	37 °C	42 °C
PSAE			
PSFL			

Task 3: Properties of the two most common diagnostic and selective-diagnostic media

Task 3a: Blood agar – viridation and hemolysis

Blood agar may be considered to be an enriched medium (with RBCs) but it is also a diagnostic medium. Following changes may be observed on it:
Total haemolysis – bacterie with their activity destroy the erythrocytes around them tolally, blood agar becomes serum-colloured, it is transparent
Partial haemolysis – bacterie using their activity destroy erythrocytes only partially, blood agar around colonies is only half-translucent and its colour is yellowish (no greenish tone)
Viridation – change of red blood colour to a green one; agar around colony becomes greenish
No change – majority of bacteria do not change the agar
Describe haemolytical properties of four strains on blood agar. Read against light. Observe the colour of the haemolysis, not the colour of the bacterial colony itself.

Streptococcus pyogenes (SRPY)	
Streptococcus agalactiae (SRAG)	
Streptococcus pneumoniae (SRPN)	
Enterococcus faecalis (ECFS)	

Task 3b: Endo agar – presence/absence of growth, lactose fermentation

Describe growth/no growth, and changes of the medium surrounding the colonies.

Staphylococcus epidermidis (STEP)	
Escherichia coli (ESCO)	
Salmonella Enteritidis (SAEN)	

Task 4: Description of morphologic characteristics of colonies

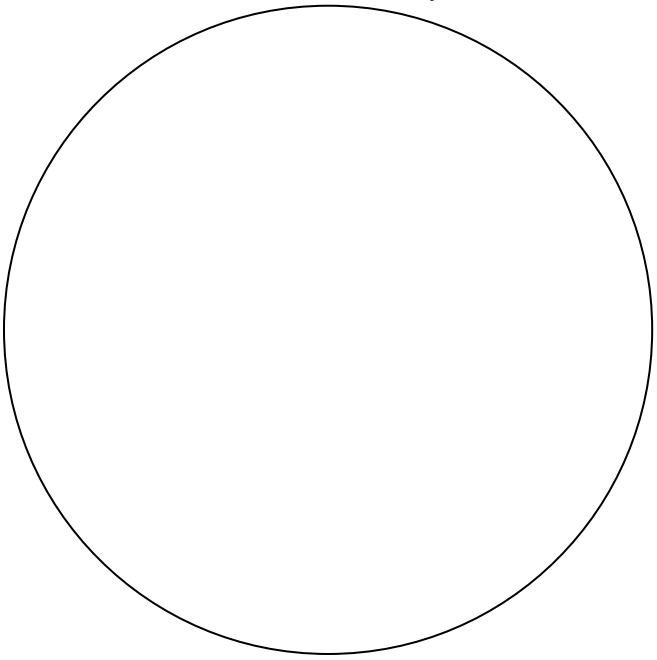
Describe three strains of bacteria. If it is impossilbe to fill a cell, enter a reason (e. g. „too small“)

	Strain	Strain	Strain
Size			
Colour			
Shape			
Profile			
Surface			
Edges			
Translucency			
Consistency			
Odour			
Surroundings			

Task 5: Inoculation of samples and strains on solid media

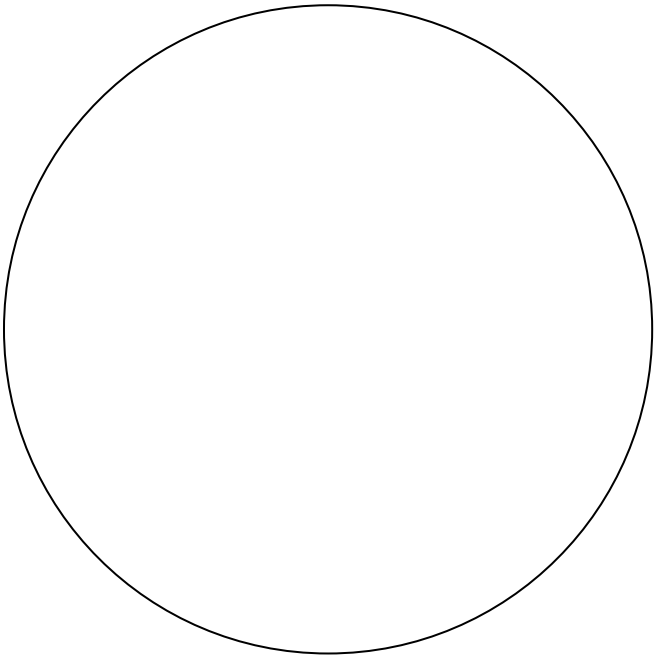
Task 5a: Inoculation of a swab

Inoculate a swab on the medium. Draw your result.



Task 5b: Inoculation of a strain

Inoculate a strain on the medium. Draw your result.



Check-up questions:

1. Why VL broth is covered by parafin oil?

2. Why red blood cells are used only after the agar gets cold?

3. Why gelatine is usually not used at making solid media?

4. Microaerophilic and capnophilic conditions: is it the same?

5. Is the ability of staphylococci to grow at high NaCl concentrations related with its adaptation related to the macroorganism?

6. What characteristics cannot be seen by one's eye? And what characteristic requires touching the colony?

7. Why it is so important to obtain isolated colonies at cultivation?

8. Blood agar is made of "basis for blood agar" (in fact it is nutrient agar) and defibrinated sheep blood. Is it possible to add blood to other bases?