Name: Date:

Diagnostics of *Haemophilus* and G– non-fermenting rods

Table for major results of Task 1 to Task 5:

Strain		K	L	M	N	P	Q	R	S
Gram stain – Task 1									
Cul-ture Task 2	Growth on BA (Y/N)								
	Growth characte-ristics on BA (ChA*)								
	Endo agar (–/L-/L+)								
	MH agar (colour)								
Task 3a Satellite phenomenon (+/–)									
Task 3b Factor test (X, V, X + V)									
Glc fermentation Task 4 (Hajna)									
Oxidase test Task 5a									
NefermTest 24 (Task 5b)									
FINAL CONCLUSION									

*Use ChA (Chocolate agar) for bacteria not growing on BA (blood agar)

Task 1: Microscopy of strains

There are letter-labelled strains on the table. Gram-stain them and write your results to the table. Strain that is NOT G– rod should not be used in tasks 3 to 5 (but in Task 2 it should be described, for comparison)

Task 2: Cultivation on agar media

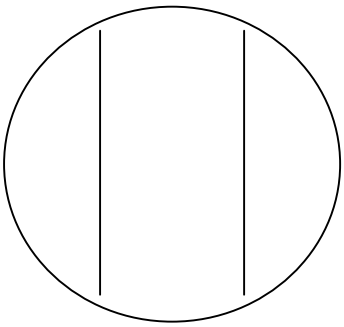
First write down, what bacteria do grow on blood agar and what bacteria do not. Then, using standard procedure, describe colonies of all strains on blood agar. In strains that did not grow on blood agar, describe their growth on Chocolate agar instead. Then describe growth of bacteria on Endo agar (only „–“ for not growing bacteria, „+“ for growing ones; lactose positivity/negativity cannot be seen, as the strains do not have isolated colonies) and on MH agar (only „–“ or „+“, and eventually presence of specific colour).

Name: Date:

Task 3: Identification of *Haemophilus* and their more precise determination

a) Satellite phenomenon

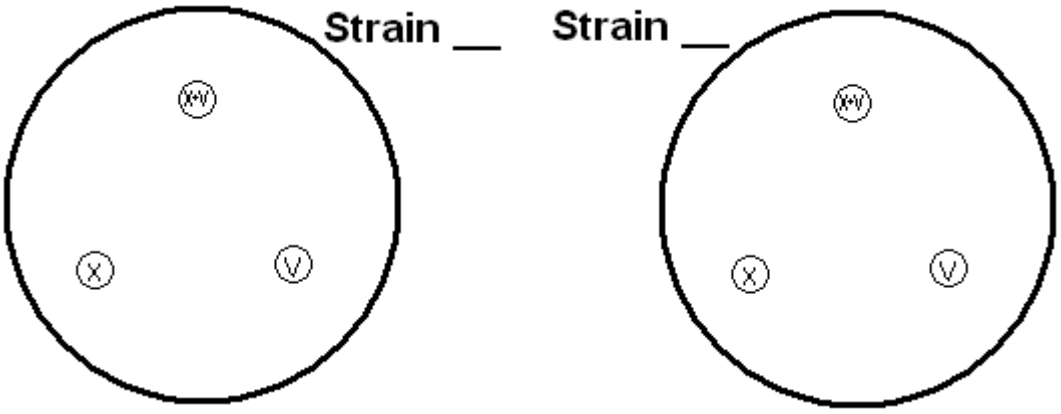
Haemophili are typical by so named satellite phenomenon. That means that they are not able to grow on blood agar, unless a strain able to release growth factor necessary for haemophili is present. Usually *Staphylococcus aureus* is used for this purpose. Draw the satellite phenomenon and connect the terms below with the features on your picture



Staphylococcus aureus Colonies of haemophili

b) Identification of the hemophili on the basis of growth factors necessity

Determine the given strains according to their requirements of growth factors. Draw the growth factor tests for both strains.



Task 4: Hajna medium

Observe the results of culture of four strains on Hajna medium. Mark strain able to ferment glucose (yellow colour) as „+“, strains unable to ferment (red colour) as „–“

Task No. 5 Determination of G– glucose non-fermenters

a) Oxidase test

Demonstration of oxidase test for three strains shown to be G– non-fermenters. Write down your results to the table. (*Pseudomonas*, should be always positive, *Burkholderia* is usually positive, too, but not necessarily; on the other hand, *Stenotrophomonas* uses to be negative).

Name: Date:

Oxidase positive bacteria with typical odour and pigmentation (mostly green, less often blue of maroon) is quite sure *Pseudomonas aeruginosa*. In this bacterium it is not necessary to perform further biochemical testing, described in Task 5a. In other two strains this biochemical testing is necessary.

b) Detailed biochemical testing

Evaluate given results of NEFERMtest 24, been prepared two days before (difference from other biochemical tests) at 30 °C (another difference, other test require 37 °C). The way of code reading is different, too, as there are three rows in the test. Always upper row is „1“ when positive, medium row is „2“ and lowest one „4“. First number is for oxidase test: write „0“, when negative, and „1“, when positive. Results of „B“ and „A“ columns are NOT used for code reading. So, you obtain 7 position code: first number is „0“ or "1“, and six more positions are for results of tests in columns H to C.

Strain:		OX	H	G	F	E	D	C	B	A	Code:	
	1										Identification:	
	2										% of probability:	
	4										Typicity index:	
	Code											
Strain:		OX	H	G	F	E	D	C	B	A	Code:	
	1										Identification:	
	2										% of probability:	
	4										Typicity index:	
	Code											

Notes:

Task No. 6: Susceptibility tests of pathogenic bacteria to antibiotics

Among your bacteria, there are five pathogens: two of *Pasteurellaceae* family, three G– non-fermenters. Write full names of antibiotics and measure susceptibility zones. Interpret the strains as susceptible (S) resistant (R) and dubious (D). (Dubious are such stains that have the zone just the same as is the limit.)

Test for *Haemophilus*

Strain →			Strain →		
Antibiotic (full name)	Zone ∅ (mm)	. Interpr.	Antibiotic (full name)	Zone ∅ (mm)	Interpr.

Test for Gram non-fermenters:

Strain →						
Antibiotic (full name)	Zone ∅ (mm)	Interpr.	Zone ∅ (mm)	Interpr.	Zone ∅ (mm)	Interpr.

Name: Date:

Task No. 7 Relations of bacteria to oxygen – comparison of enterobacteria, G– non-fermenters and anaerobes

Look at the broth cultivated under aerobic and anaerobic conditions (layer of paraffin oil on the surface), evaluate bacterial growth and its character.

Strain			
Growth in broth			
Growth in VL-broth			
Conclusion			

Check-up questions:

1. Under what conditions are the hemophili able to growth on Blood agar? Why?

2. What is the most typical material for *Pasteurella multocida* findings?

3. Which hemophilus species is the most pathogenic? Which diseases it causes?

4. Why we have less newborn infections caused by *Haemophilus* in recent years?

5. Why it is usually not necessary to perform biochemical tests in *Pseudomonas aeruginosa*?

6. What are the most typical infections caused by G– non-fermenters?

7. Make proposal of antibiotics suitable for treatment of infections caused by G– non-fermenters.