Topic P05–06: Diagnostics of G– bacteria other than those from P04

To study: *Haemophilus, Pasteurella, Pseudomonas*, G– non fermenters, *Neisseria, Moraxella, Legionella, Bordetella, Brucella, Francisella* (from textbooks, WWW etc.)
From spring term: Microscopy, culture, biochemical identification, antigen analysis, antibody detection

Table for major results of Task 1 to Task 5 (to be filled step by step):

Strain		K	L	M	N	P	Q	R	S	T	U	V	W
Gram stain – Task 1													
Cul-ture Task 2	Growth on BA (Y/N#)												
	Growth char. on BA (ChA*)												
	Endo agar (–/L-/L+)												
	MH agar (colour)												
Task 3a Satelite phenomenon (+/–)													
Task 3b Factor test (X, V, X + V)													
Task 3c Capsular type *Haemophilus*													
3d Susc. test	Penic.												
	Vanco.												
Glc fermentation Task 4 (Hajna)													
Oxidase test Task 5a													
NEFERMtest 24, NEISSERIAtest and INAC (Task 5b+c)													
FINAL CONCLUSION													

*Use ChA (Chocolate agar) for bacteria not growing on BA (blood agar)
#For a strain not growing on BA, but growing on BA+, write **„+ only“**

Task 1: Microscopy of suspicious strains

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

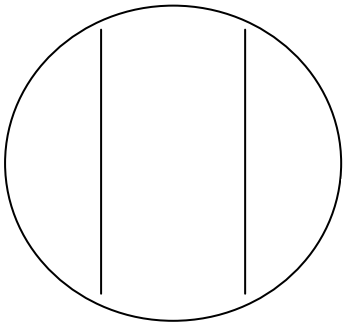
Task 2: Cultivation on agar media

First write down, what bacteria do grow on blood agar and what bacteria do not. One strain would not grow on poor variant of blood agar, but it is able to grow on rather rich blood agar (BA+). 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 or BA+ 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).

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

a) Satelite phenomenon in hemophili

Haemophili are typical by so named satellite phenomenon. That means that they are able to grow on blood agar, but in presence of a strain able to release growth factor from haemophili. Usually *Staphylococcus aureus* is used for this purpose. Draw the satellite phenomenon and connect the terms below with the features on your picture

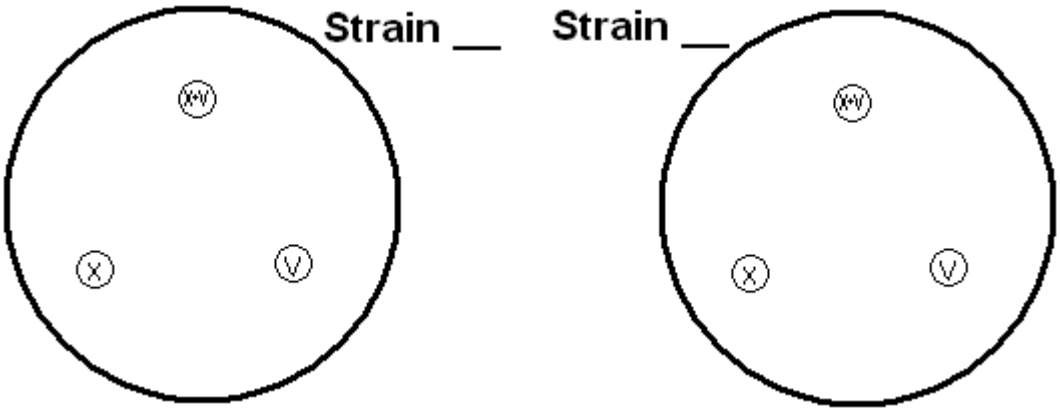


Colonies of haemophili

Staphylococcus aureus

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.



c) The detection of *H. influenzae* capsule antigens

Describe the result of agglutination of *H. influenzae* capsule antigens by means of latex agglutination.

d) The detection of *P. multocida* using typical antibiotic susceptibility pattern

Very typical for *P. multocida* is its susceptibility to penicillin, very rare among G– rods. On the other hand, it is resistant to much stronger (but for G+ bacteria only) antibiotic – vancomycin. Fill the table

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 it (red colour) as „–“

Task 5: Determination of G– glucose non-fermenters and G- cocci

a) Oxidase test

Demonstration of oxidase test for three strains shown to be G– non-fermenters, and for all strains shown to be G– cocci (neisseriae or moraxellae). Write down your results to the table.

For G– non fermenters: *Pseudomonas*, should be always positive, *Burkholderia* is usually positive, too, but not necessarily; on the other hand, *Stenotrophomonas* uses to be negative. Oxidase postitive 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 furhter biochemical testing, described in Task 5a. In other two strains this biochemical testing is necessary.

For G– cocci: Both *Neisseria* and *Moraxella* use to be oxidase positive, although in *Moraxella* it is possible to see slightly delayed reaction.

b) Detailed biochemical testing

Evaluate given results of NEFERMtest 24, being incubated two days before (difference from other biochemical tests) at 30 °C (again a difference, other test require 37 °C). The way of code counting 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 „1“, when positive, and „0“, when negative. Results of „B“ and „A“ columns are NOT used for code counting. 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											

Evaluate also results of NEISSERIAtest (Lachema). Here it is much simpler, the test has one row only. The test was inoculated one day before. Read it according to the scheme. The first well contains a negative control, so the proper test starts in the SECOND well! Dropping of Lugol solution was already done, you should not do it yourselves. Remark low biochemical activity of some *Neisseria*. Compare the result with cultivation conditions (the strain, found to be *N. gonorrhoeae*, should grow on chocolate agar only; the strain, found to be *N. meningitidis*, on chocolate and modified blood agar only).

Strain:	H	G	F	E	D	C	B	A			
	NEC									Code:	Identification:
	×	1	2	4	1	2	4	1			
	×										
Strain:	H	G	F	E	D	C	B	A			
	NEC									Code:	Identification:
	×	1	2	4	1	2	4	1			
	×										
Strain:	H	G	F	E	D	C	B	A			
	NEC									Code:	Identification:
	×	1	2	4	1	2	4	1			
	×										
Strain:	H	G	F	E	D	C	B	A			
	NEC									Code:	Identification:
	×	1	2	4	1	2	4	1			
	×										

c) Indoxylacetate test

The strain shown to be *Moraxella catarrhalis* could be identified also by a simpler way. Look at a demonstration of indoxylacetate test (positive for *M. catarrhalis*, negative for *Neisseria*)

Task 6: Susceptibility tests of pathogenic bacteria to antibiotics

For time reasons, read only susceptibility tests of *Pseudomonas*, although normally, of course, tests for pathogens from *Pasteurellaceae* family and G– cocci would be performed, too.

Test for pseudomonas (*Pseudomonas aeruginosa* was found to be strain ___)

Antibiotic	Zone ∅ (mm)	Interpretation	Antibiotic	Zone ∅ (mm)	Interpre-tation
Piperacillin/tazobactam (TZP) S ≥ 18 / R < 18			ciprofloxacin (CIP) S ≥ 25 / R < 22		
gentamicin (CN) S ≥ 15 / R < 15			ceftazidime (CAZ) S ≥ 16 / R < 16		
ofloxacin (OFL) S ≥ 16 / R < 13			colistin (CT) S ≥ 11 / R < 11		
Note. Tazobactam acts as betalactamase inhibitor, but it also has its own antimicrobial effect.					

Task 7: Direct detection of meningitis agents antigens in the cerebrospinal fluid (demonstration of a diagnostic kit and observation of a videoclip)

Meningococcal meningitis is a dangerous disease. It is not possible to wait for culture results, so we need a quick diagnostic method. Besides microscopy, latex agglutination is a very important method for this purpose.

a) Demonstration of a latex agglutination kit

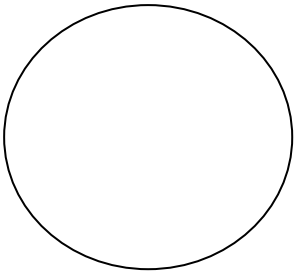
Observe the kit and write down the names of bacteria that can be found using this method.

b) Videoclip

Look at the videoclip. In our example, the pathogen was found to be __________________________________

Task 8: Diagnostics of *Bordetella*, *Brucella*, *Legionella* and *Francisella*

a) Culture diagnostics of *Bordetella*



There is a special medium for *Bordetella pertussis*, and a special way of inoculation is used here. Unlike many other bacteria, *Bordetella* is resistant to penicillin; so we start by making a drop of penicillin solution in the middle of the agar plate. The swab is mixed with the drop, and inoculated in a spiral form. Then the loop is used to make radial rays. Write down the name of the medium, and re-draw the way of its inoculation from your slideshow.

Name of the medium: _____

b) Demonstration of a culture medium for *Legionella*

Observe the culture medium for *Legionella*. Write down some data about it:

Abbreviation	What the individual letters of the abbreviation mean	Colour

c) Antibody detection in tularemia

For *Francisella*, we perform indirect diagnostics of using agglutination. The task is not performed in this double practiacal session.

d) Diagnostics of antibodies against brucellosis

Diagnostics of brucellosis (Bang disease – caused by *B. abortus*) was performed using indirect diagnostics – ELISA in both IgG and IgM antibodies. The absorbance was measured by a spectrophotometer and the results were converted into "positive", "borderline" or "negative" values using an expert system. Results can be found on your table. Try to interpret them together.

Patient	IgM result	IgG result	Final conclusion
Alice			
Bob			
Claudia			
David			

Note: Brucellosis is quite rare disease and many laboratories, including our laboratory, does not perform the diagnostics. Therefore the worksheets used for this task are not real Brucella diagnostics worksheets, but adapted worksheets of another serology reaction. On the other hand, the true worksheets for Brucella diagnostics would look the same of very similar.