Topic P05: Diagnostics of *Pasteurellaceae* and G– non-fermenters

To study: *Haemophilus, Pasteurella, Pseudomonas* and G– non-fermenters (from textbooks, www etc.)
From spring term: Microscopy, culture, biochemical identification, antigenic analysis

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
Gram stain – Task 1									
Task 2 Cul-ture	Growth on BA (Y/N)								
	Growth characte-ristics on BA (ChA*)								
	Endo agar (–/L–/L+#)								
	MH agar (colour)								
Task 3a Satelite phenomenon (+/–)									
Task 3b Factor test (X, V, X + V)									
Task 3c *H. influen.* agglutination result									
3d Susc. test	Penicill.								
	Vanc.								
Gluc. fermentation Task 4 (Hajna)									
Oxidase test Task 5a									
NEFERMtest 24 Task 5b (code)									
FINAL CONCLUSION									

*Use ChA (chocolate agar) for bacteria not growing on BA (blood agar)
#does not grow/does grow, Lactose- non-fermenter/does grow, Lactose fermenter

Task 1: Microscopy of suspicious strains

There are letter-labelled strains on the table. Gram-stain them and write your results in the table. The strain that is **not** a 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 which bacteria do grow on blood agar and which do not. Then, using the standard procedure, describe the colonies of all the strains on blood agar. In strains that do not grow on blood agar *(demonstrated by only one agar plate on the side table of the practical hall)*, describe their growth on chocolate agar instead.

Then describe the growth of bacteria on Endo agar. Use "–" no growth, "L–" for growing, but lactose non-fermenters, "L+" for lactose fermenters. Pay attention: some strains may mimic lactose positivity although they are lactose negative – they produce pigments, so the colonies are dark, but the surroundings are pale. In doubts compare with Hajna medium (Task 4): completely yellow colour = lactose and glucose fermenter, complete red colour = lactose and glucose non-fermenter, half-yellow-half-red = glucose fermenter, lactose non-fermenter.

As to MH agar: check only one strain, and only for eventual pigment presence. Use the plate from for Task 1 or from Task 6b, there is no special MH plate for Task 2

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

a) Satellite phenomenon of haemophili

Haemophili are typical for the so-called satellite phenomenon, which means that they are able to grow on blood agar only in the presence of a microbe able to release growth factors for the haemophili. Usually *Staphylococcus aureus* is used for this purpose. Draw the satellite phenomenon (performed for two strains) and connect the terms below with the features on your picture. Write your results to the basic table on the first page.
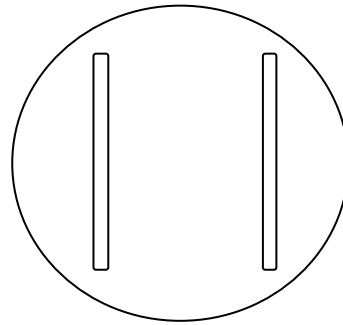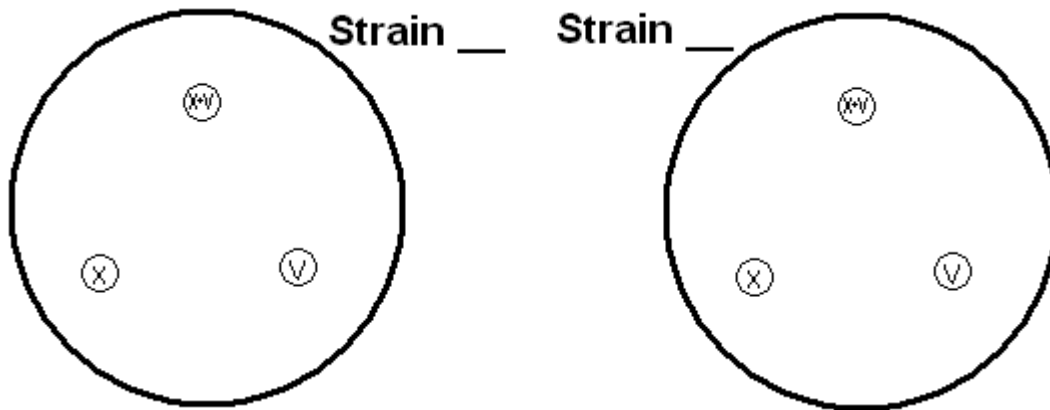

Staphylococcus aureus   Colonies of haemophili

b) Identification of the haemophili on the basis of the growth factors requirements

Determine the given strains according to their requirements of the 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 (from the slide-show) – write the result for all antigenic types to the table.

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

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

e) Confirmation of *Pasteurella multocida* determination using MALDI-TOF

Look at the MALDI-TOF results of our *Pasteurella* strain. Decide:
Determination of *Pasteurella multocida* by means of previous test **is – is not** (delete as appropriate) confirmed by MALDI-TOF test.

Task 4: Hajna medium (Triple Sugar Iron Agar in A. A. Hajna's modification)

Observe the results of culture of four strains on Hajna medium. Mark the strains able to ferment glucose (yellow colour) as "+", the strains unable to ferment it (red colour) as "–". Other results (lactose, sulphan) are not important for today's topic, but the lactose result (complete yellow vs. red-and-yellow) might be used for comparison between lactose-fermenters and lactose-non-fermenters in Task 2.

Task 5: Determination of G– glucose non-fermenters

a) Oxidase test

A demonstration of the oxidase test for the three strains determined as G– non-fermenters. Write down the results to the table (*Pseudomonas* should be always positive, *Burkholderia* is mostly positive but not necessarily; on the other hand, *Stenotrophomonas* tends to be negative).

The oxidase positive bacterium with typical odour and pigmentation (mostly green, less often blue or maroon) is almost certainly *Pseudomonas aeruginosa*. In this bacterium, it is not necessary to perform further biochemical testing, described in Task 5a. In the other two strains, this biochemical testing is necessary.

b) Detailed biochemical testing

Evaluate the given results of NEFERMtest 24, incubated two days prior (unlike the other biochemical tests, where it is one day) at 30 °C (again a difference, other tests require 37 °C). The way of code counting is different, too, as there are three rows in the test. The upper row is always "1" when positive, the medium row is "2" and the lowest one "4". The first number is for the oxidase test: write "1" when positive and "0" when negative. The results of "B" and "A" columns are NOT used for code counting. So, you obtain a 7-position code: The first number is "0" or "1" and the remaining six positions are for the results of the 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 6: Antibiotics susceptibility tests of pathogenic bacteria

Among your bacteria, there are five pathogens: two of the *Pasteurellaceae* family, three G– non-fermenters (but of them, you are supposed to measure zones for *Pseudomonas* only). Write the abbreviations of the antibiotics according to the card and measure the susceptibility zones for all the tested strains. Borderline zones are written on the cards; using them, interpret the strains as susceptible (S), resistant (R) and intermediate (I).

6a) Test for *Haemophilus* (*Haemophilus influenzae* was found to be strain ___)

*valid also for doxycyklin

Antibiotic	Zone ⌀ (mm)	Interpre-tation
Penicillin (P) S ≥ 12 / R < 12		
Co-amoxicillin (AMC) S ≥ 15 / R < 15		
Cefuroxime (CXM) S ≥ 26 / R < 25		
Nalidixic acid (NA) S ≥ 23 / R < 23		
Tetracyclin (TE)* S ≥ 25 / R < 22		
Co-trimoxazole (SXT) S ≥ 23 / R < 20		

6b) Test for *Pasteurella* (*Pasteurella multocida* was found to be strain ___)

Antibiotic	Zone ⌀ (mm)	Interpre-tation
Co-amoxicillin (AMC) S ≥ 15 / R < 15		
Cefotaxime (CTX) S ≥ 26 / R < 26		
Ciprofloxacin (CIP) S ≥ 27 / R < 27		
Tetracyclin (TE)* S ≥ 24 / R < 24		
Co-trimoxazole (SXT) S ≥ 23 / R < 23		
Penicillin (P) S ≥ 17 / R < 17		

6c) Test for *Pseudomonas* (*Pseudomonas aeruginosa* was found to be strain ___)

Antibiotic	Zone ⌀ (mm)	Interpre-tation	Antibiotic	Zone ⌀ (mm)	Interpre-tation
Piperacillin/tazobactam (TZP) $S \geq 18 / R < 18$			ciprofloxacin (CIP) $S \geq 26 / R < 26$		
gentamicin (CN) $S \geq 15 / R < 15$			ceftazidime (CAZ) $S \geq 17 / R < 17$		
ofloxacin (OFL) $S \geq 16 / R < 13$			colistin (CT) $S \geq 11 / R < 11$		
Note. Tazobactam acts as betalactamase inhibitor, but it also has its own antimicrobial effect.					

6d) Check-up for primary resistances for *Burhkohleria* and *Stenotrophomonas* strains

TABLE 2. Intrinsic resistance in non-fermentative Gram-negative bacteria; non-fermentative Gram-negative bacteria are also intrinsically resistant to benzylpenicillin, cefoxitin, cefamandole, cefuroxime, glycopeptides, fusidic acid, macrolides, lincosamides, streptogramins, rifampicin, daptomycin, and linezolid

In the diagram, prepared by EUCAST# you can see intrinsic (primary) resistances of the most common G– non-fermenters. On the side table you can see susceptibility tests for *Burkholderia* and *Stenotrophomonas*. You do not need to measure zones – they have been already measured.
EUCAST = The European Committee on Antimicrobial Susceptibility Testing

On the lid of your Petri dishes you can find:
1st column: intrinsic resistance for the given strain (re-written from the EUCAST table above): R = intrinsic resistance, – = no intrinsic resistance. **Only intrinsic resistances for antibiotics from our set (PS1) are written here.** *Note: The table above does not contain ofloxacin (OFX), but it is possible to consider strains primarily resistant to ciprofloxacin as resistant to ofloxacin, too. The table also does not contain namely gentamicin (CN), but its results can be derived from "Aminoglycosides".*
2nd column: results from measuring the zones and comparing them with reference zones: R = resistant, S = susceptible

Write on the next page, what is intrinsic resistance of *B. cepacia* and *S. maltophilia* according to EUCAST (copy from the first column).
Then check, if all intrinsic resistances are expressed in our test according to following table:

Intrinsic resistance (R)?	Measured as	Conclusion
–	susceptible (S)	the result is OK, the strain may be reported as "susceptible"
R	resistant (R)	the result is OK, the strain is to be reported as "resistant"
–	resistant (R)	the result is OK, the strain is to be reported as "resistant" (it is a secondary resistance)
R	susceptible (S)	**the result is not in accordance, the strain should be reported as "resistant", it should be understood as "false susceptibility"**

Finally, write if the strain is susceptible for any antibiotics (only such that may be reported as susceptible, not those measured susceptible, but they should be considered resistant as they have an intrinsic resistance!).

Write:

Strain ___ (*B. cepacia*) has, according to EUCAST, intrinsic resistance to antibiotics: _____

_____.

Susceptibility assessed by diffusion disc test

☐   is in accordance with this intrinsic resistance (= no "false susceptibility")

☐   is not in accordance with this intrinsic resistance for antibiotic(s): _____ *

Strain may be reported as "susceptible" to: _____

Strain ___ (*S. maltophilia*) has, according to EUCAST, intrinsic resistance to antibiotics: _____

_____.

Susceptibility assessed by diffusion disc test

☐   is in accordance with this intrinsic resistance (= no "false susceptibility")

☐   is not in accordance with this intrinsic resistance for antibiotic(s): _____ *

Strain may be reported as "susceptible" to: _____

In case of more discrepancies it is usually recommended to check the susceptibility by quantitative tests, eventually to check, whether the genus and species determination of the strain was performed correctly.

Task 7: Relations of bacteria to oxygen – comparison of *Enterobacteriaceae*, G– non-fermenters and anaerobes

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

Strain			
Growth in common broth			
Growth in VL-broth			
Conclusion			