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.* capsular type									
3d Susc. test	Penicill.								
	Vanc.								
Gluc. 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)
[#]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)

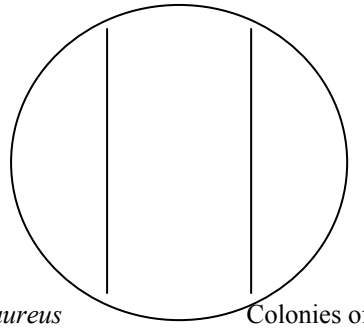
Task 2: Cultivation on agar media

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*, describe their growth on chocolate agar instead. Then describe the growth of bacteria on Endo agar (only "–" for not growing bacteria, "+" for growing ones; lactose fermentation cannot be seen, as the strains do not have isolated colonies). On MH agar check only one strain and only for eventual pigment presence (the plate serves also for Task 6b).
**demonstrated by only one agar plate on the side table of the practical hall*

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

a) Satellite phenomenon of haemophili

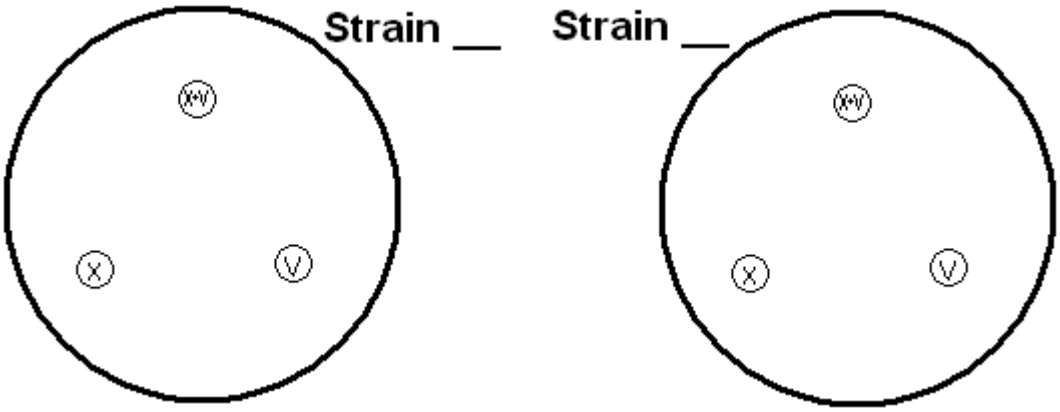
Haemophili are typical for the so-called satellite phenomenon, which means that they are able to grow on blood agar only in the presence of a microbe able to release growth factors for the haemophili. Usually *Staphylococcus aureus* is used for this purpose. Draw the satellite phenomenon and connect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



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).

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.

Task 4: Hajna medium

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 "–".

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 *Pasteurellaceae*

Strain →				
Antibiotic	Zone ∅ (mm)	Interpre-tation	Zone ∅ (mm)	Interpre-tation
Ampicillin (AMP) S ≥ 16 / R < 16				
Co-amoxicillin (AMC) S ≥ 16 / R < 16				
Cefuroxime (CXM) S ≥ 25 / R < 25				
Chloramfenicole (C) S ≥ 28 / R < 28				
Tetracyclin (TE)* S ≥ 25 / R < 22				
Co-trimoxazole (SXT) S ≥ 23 / R < 20				

Large, confluent zones should not be measured, but considered just "susceptible".

6b) 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.					

6c) 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

Rule no.	Organisms	Ampicillin	Amoxycillin-clavulanate	Ticarcillin	Ticarcillin-clavulanate	Piperacillin	Piperacillin-tazobactam	Cefazolin	Cefotaxime	Ceftriaxone	Ceftazidime	Ertapenem	Imipenem	Meropenem	Ciprofloxacin	Chloramphenicol	Aminoglycosides	Trimethoprim	Trimethoprim-sulphamethoxazole	Fosfomycin	Tetracyclines/tigecycline	Polymyxin B/colistin
2.1	*Acinetobacter baumannii*, *Acinetobacter calcoaceticus*	R[a]	R[a]	–	–	–	–	R	R	R	–	R	–	–	–	–	–	R	–	R	–	–
2.2	*Achromobacter xylosoxidans*	R	–	–	–	–	–	R	R	R	–	R	–	–	–	–	–	–	–	–	–	–
2.3	*Burkholderia cepacia* complex[b]	R	R	R	R	–	–	R	–	–	–	R	R	–	R	R	R[c]	R	–	R	–	R
2.4	*Elizabethkingia meningoseptica*	R	–	R	R	–	–	R	R	R	R	R	R	R	–	–	–	–	–	–	–	R
2.5	*Ochrobactrum anthropi*	R	R	R	R	R	R	R	R	R	R	R	–	–	–	–	–	–	–	–	–	–
2.6	*Pseudomonas aeruginosa*	R	R	–	–	–	–	R	R	R	–	R	–	–	–	R	Note[f]	R[e]	R[e]	–	R	–
2.7	*Stenotrophomonas maltophilia*	R	R	R	–	R	R	R	R	R	R[g]	R	R	R	–	–	R[c]	R[d]	–	R	–	–

R, resistant.
[a] *Acinetobacter baumannii* may appear to be susceptible to ampicillin–sulbactam, owing to the activity of sulbactam against this species.
[b] *Burkholderia cepacia* complex includes different species. Some strains may appear to be susceptible to some β-lactams *in vitro*, but they are clinically resistant and are shown as R in the table.
[c] *Burkholderia cepacia* and *Stenotrophomonas maltophilia* are intrinsically resistant to all aminoglycosides. Intrinsic resistance is attributed to poor permeability and putative efflux. In addition, most *Stenotrophomonas maltophilia* isolates produce the AAC(6′)-Iz enzyme.
[d] *Pseudomonas aeruginosa* is intrinsically resistant to kanamycin and neomycin, owing to low-level APH(3′)-IIb activity.
[e] *Pseudomonas aeruginosa* is typically resistant to trimethoprim and moderately susceptible to sulfonamides. Although it may appear to be susceptible *in vitro* to trimethoprim–sulphamethoxazole, it should be considered to be resistant.
[f] *Stenotrophomonas maltophilia* may show low ceftazidime MIC values but should be considered to be resistant.
[g] *Stenotrophomonas maltophilia* is typically susceptible to trimethoprim–sulphamethoxazole but resistant to trimethoprim alone.

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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 – the reference zones are already drawn on the Petri dishes, so only compare real zones with those drawn on the Petri dish. Write on the next page, what is intrinsic resistance of *B. cepacia* and *S. maltophilia* according to EUCAST, but **write only resistance for bacteria tested in our atb susceptibility test**. Then check, if all intrinsic resistances are expressed in our test (= "is in accordance", not necessary to add anything more) or if there is any problem (a strain looks susceptible, although it is supposed to have an intrinsic resistance) – if so, report what is/are the discrepant antibiotic(s).

Write:

Strain ___ (*S. maltophilia*) should have intrinsic resistance to antibiotics: _____

_____.

Susceptibility assessed by diffusion disc test

☐ is in accordance with this intrinsic resistance

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

Strain is susceptible to: _____

Strain ___ (*B. cepacia*) should have intrinsic resistance to antibiotics: _____

_____.

Susceptibility assessed by diffusion disc test

☐ is in accordance with this intrinsic resistance

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

Strain is susceptible to: _____

Note: In practice, if the susceptibility is not in accordance (a strain looks like susceptible, although it should be intrinsically resistant) is so or so considered to be resistant. 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.

EUCAST = The European Committee on Antimicrobial Susceptibility Testing

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			