# 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, antigen 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 | | | | | | | | | |
| Cul-ture Task 2 | 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 Capsullar type *Haemophilus* | | | | | | | | | |
| 3d Susc. test | Penic. | | | | | | | | |
| | Vanco. | | | | | | | | |
| Glc fermentation Task 4 (Hajna) | | | | | | | | | |
| Oxidase test Task 5a | | | | | | | | | |
| NefermTest 24 (Task 5b) | | | | | | | | | |
| **FINAL CONCLUSION** | | | | | | | | | |

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

## 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– rod should not be used in tasks 3 to 5 (but in Task 2 it should be described, for comparison)

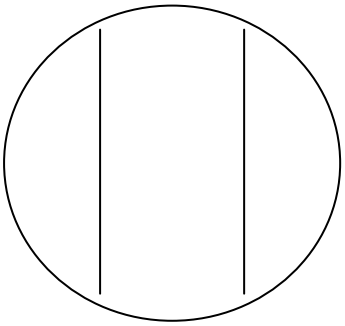
## Task 2: Cultivation on agar media

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 strans 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).

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

### a) Satelite phenomenon in hemophili

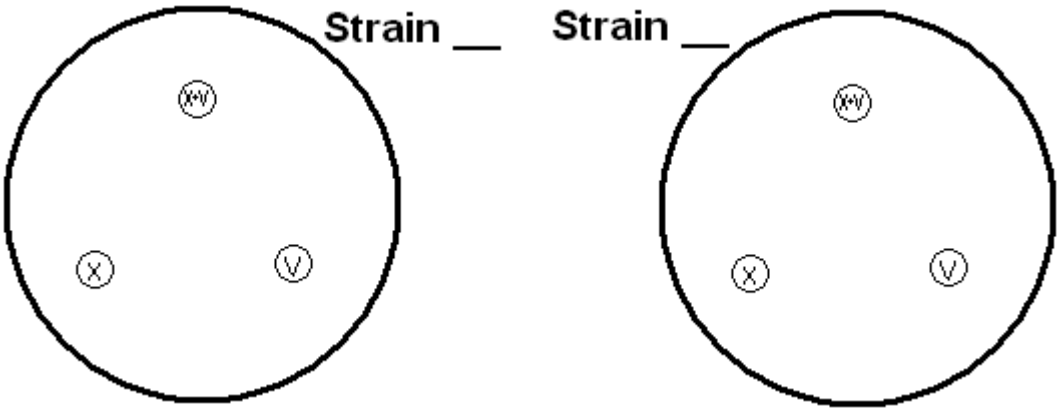
Haemophili are typical by so named satelite phenomenon. That means that they are able to grow on blood agar, but in presence of a strain able to release growth factor from haemophili. Usually *Staphylococcus aureus* is used fot this purpose. Draw the satelite phenomenon and connect the terms below with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




## 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 penicilin, 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 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 allways positive, *Burkholderia* is usually positive, too, but not necesarilly; 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.

## b) Detailed biochemical testing

Evaluate given results of NEFERMtest 24, beeng incubated two days berfore (difference from other bicochemical 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. Allways 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“ collumns 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 collumns 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 abbreviations of antibiotics according to the card and measure susceptibility zones for all tested strains. Borderline zones are written on the cards; using them, interprete the strains as susceptible (S) resistant (R) and dubious (D). (Dubious are succh stains that have the zone just the same as is the limit.)

### Test for *Pasteurellaceae*

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

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

### Test for Gram non-fermenters:

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

## 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. When 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 in recent years we have less infections caused by *Haemophilus* in Czechia?

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

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

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