

## Topic P08+09: Laboratory diagnostics of tuberculosis, actinomycetes, nocardiae and spiral bacteria

**To study:** *Mycobacterium*, *Actinomyces*, *Nocardia*, *Borrelia*, *Treponema*, *Leptospira* (textbooks, WWW etc.)

**From spring term:** Microscopy, culture, antibiotic susceptibility, PCR, antibody detection methods

### Task 08/1: Microscopy of acid-fast and partially acid fast microorganisms

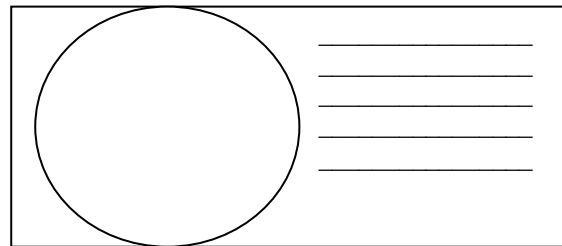
While entirely acid-fast microorganisms (*Mycobacterium*) cannot be stained using Gram staining, only partially acid-fast ones (*Actinomyces*, *Nocardia*) can be Gram stained, but they stain inconstantly; they also tend to have branched filamentous forms.

#### a) Staining of (negative) clinical material using Ziehl-Neelsen staining method

Ziehl-Neelsen staining is used for mycobacteria (*M. tuberculosis*, *M. leprae*), but also for some parasites (*Cryptosporidium parvum*, *Cyclospora cayetanensis*). The acid-fast organisms are stained only when heated during staining\*, but then they are not decolorized even by so called „acid alcohol“ (solution of alcohol with HCl or H<sub>2</sub>SO<sub>4</sub>). Decolorized background is then counterstained.

Stain the negative sputum according to the Ziehl-Neelsen method (methylene blue variant). It is not likely that acid-fast rods would be present. Observe in microscope (immersion). Draw the results; at least, you will see the background, e. g. leucocytes, epithelia and other objects. Do not forget do **describe** your picture (use lines)!

Describe also the staining procedure – fill in the following table with names of used reagents.



|    |   |           |
|----|---|-----------|
| 1. | During the staining the preparation is _____ until _____  |           |
| 2. | This reagent is made of _____                             | and _____ |
| 3. | Instead of this reagent, it is also possible to use _____ | _____     |

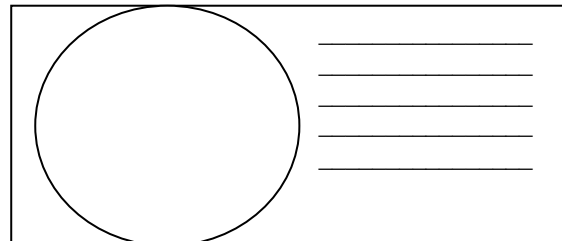
#### b) Microscopy of a mycobacterial culture

Examine microscopically (immersion oil, immersion 100× objective) the preparation of mycobacterial culture stained by Ziehl-Neelsen staining method.

Evaluate presence of red acid-fast rods.

Draw observed structures.

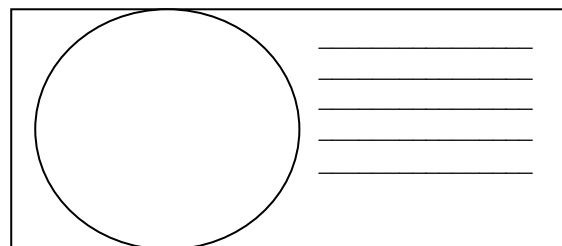
Do not forget do **describe** your picture (use lines)!



#### c) Microscopic examination of actinomycetes and nocardia strains

Examine microscopically the slide stained by Gram. Describe and draw observed formations. Observe high polymorphism of the microorganisms (from coccal shape, through rods to fibre/strings, often branched; Gram-positive, but often staining half Gram-negative).

Do not forget do **describe** your picture (use lines)!



### Task 08/2: Culture of mycobacteria, Actinomyces and Nocardia.

The culture requests of acid fast and partially bacteria are very different.

- ❖ For *Mycobacterium tuberculosis* we use special media: liquid media (Šula, Banič) and solid media (Ogawa, Löwenstein-Jenssen). The solid media are different from majority of other solid media used in medical microbiology; they do not contain agar, they are „solid“ because of coagulated egg proteins. Before culture, the medium should be specially treated.
- ❖ For *Nocardia* a current blood agar is sufficient.
- ❖ For *Actinomyces* we need VL-agar and culture in anaerobic box/anaerobic jar (see P07), as this organism is anaerobic.

\*Heating may be eventually substituted by use of highly concentrated carbolfuchsin and highly concentrated phenol; this modification of Ziehl Neelsen staining (Kinyoun modification) does not require heating.

**a) Describe media for mycobacterial cultivation**

| Medium name | liquid/solid | colour | notes |
|-------------|--------------|--------|-------|
|             |              |        |       |
|             |              |        |       |

**b) Describe and draw the growth of *Mycobacterium*, *Actinomyces* and *Nocardia* on (in) given media**

| Bacterium            | Medium name | Presence/absence of growth, eventually growth character (use your own words to characterize the growth) |
|----------------------|-------------|---|
| <i>Mycobacterium</i> |             |   |
|                      |             |   |
| <i>Actinomyces</i>   | blood agar  |   |
|                      | VL agar     |   |
| <i>Nocardia</i>      | blood agar  |   |
|                      | VL agar     |   |

**Task 08/3: Assessment of antimicrobial drugs susceptibility**

For treatment of mycobacterial infections, it is necessary to use special drugs, called antitubercotics. The way of testing is different from other bacteria, too: antitubercotics are added directly to the culture media. On the other hand, *Actinomyces* and *Nocardia* are treated by „normal“ antibiotics and also „normal“ diffusion disc test is used for testing.

**a) Assessment of mycobacterial susceptibility to antitubercotics**

By comparing with a control test-tube, read the results of antitubercotic susceptibility tests of *Mycobacterium tuberculosis* strain.

|                |  |  |  |                |
|----------------|--|--|--|----------------|
| Antitubercotic |  |  |  | Growth control |
| Growth Y/N     |  |  |  |                |
| Interpretation |  |  |  |                |

**b) Antibiotic susceptibility of *Nocardia* and *Actinomyces***

Perform in vitro susceptibility testing of *Nocardia* and *Actinomyces* to suitable antibiotics.

Into the table, write the abbreviation of the antibiotics according to a card and for all tested strains measure the susceptibility zones. On your card, you have limit zones – according to them, interpret the zones as susceptible (S) resistant (R) and dubious (D).

| Strain →               |             |                |             |                |
|------------------------|-------------|----------------|-------------|----------------|
| Antibiotic (full name) | Zone Ø (mm) | Interpretation | Zone Ø (mm) | Interpretation |
|                        |             |                |             |                |
|                        |             |                |             |                |
|                        |             |                |             |                |
|                        |             |                |             |                |
|                        |             |                |             |                |

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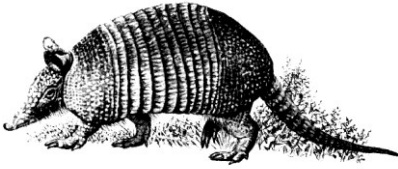
### Task 08/4: PCR in diagnostics of TB

As the culture of mycobacteria is complicated, PCR becomes a very important method in its diagnostics. Read a result of PCR TB diagnostics (from slideshow), write the results and interpret them.

| Patient No. | Sample band | Control band | Interpretation |
|-------------|-------------|--------------|----------------|
| 1           |             |              |                |
| 2           |             |              |                |
| 3           |             |              |                |
| 4           |             |              |                |

### Task 08/5: Diagnostics of leprosy

Leprosy is a disease that still affects millions of people in underdeveloped states. Its diagnostics is very difficult. Fill in the following table.

|   |                                |  |
|---|--------------------------------|--|
|  | The name of this animal is     |  |
|   | It is used to produce          |  |
|   | and this substance is used for |  |

Picture source: [http://www.1-costaricalink.com/costa\\_rica\\_fauna/nine\\_banded\\_armadillo.htm](http://www.1-costaricalink.com/costa_rica_fauna/nine_banded_armadillo.htm)

### Lyme borreliosis



Common table for Task 09/1, 2 and 3.

| Patient Letter | Short clinical description (1–3 words characterizing the situation) | ELISA (Task 09/1) |       |      |       | W. blotting (T09/2) |           | PCR (T09/3) (+/-) | Conclusion: final interpretation, recommendation for event. therapy |
|----------------|---|-------------------|-------|------|-------|---------------------|-----------|-------------------|---|
|                |   | IgM               |       | IgG  |       | IgM (+/-)           | IgG (+/-) |                   |   |
|                |   | Abs.              | (+/-) | Abs. | (+/-) |                     |           |                   |   |
| J              |   |                   |       |      |       |                     |           |                   |   |
| K              |   |                   |       |      |       |                     |           |                   |   |
| L              |   |                   |       |      |       |                     |           |                   |   |
| M              |   |                   |       |      |       |                     |           |                   |   |
| N              |   |                   |       |      |       |                     |           |                   |   |

### Task 09/1: Proof of antibodies to *Borrelia garinii* using ELISA

Read the results of patients with suspect Lyme borreliosis. Both IgG and IgM antibodies are assessed. In A1 field (corresponding to A1 well of the microtitration plate) you can see CAL level (borderline level; all absorbance levels above CAL are positive, all absorbance levels beneath CAL are negative). There are controls in B1 and C1. Patients J to N are in fields with coloured squares.

Write CAL level here, check, whether negative control is really negative and positive control really positive. Then read and interpret ELISA results for patients J, K, L, M, N (do not write them here, use main table above).

|                      |  |                                |  |  |   |
|----------------------|--|--------------------------------|--|--|---|
| CAL level (well A1): |  | K+ absorbance level (well B1): |  | <input type="checkbox"/> K+ is OK<br><input type="checkbox"/> K+ is not OK | <br>tick what is correct |
| <b>IgM</b>           |  | K- absorbance level (well C1): |  | <input type="checkbox"/> K- is OK<br><input type="checkbox"/> K- is not OK |   |
| CAL level (well A1): |  | K+ absorbance level (well B1): |  | <input type="checkbox"/> K+ is OK<br><input type="checkbox"/> K+ is not OK | <br>tick what is correct |
| <b>IgG</b>           |  | K- absorbance level (well C1): |  | <input type="checkbox"/> K- is OK<br><input type="checkbox"/> K- is not OK |   |

### Task 09/2: Proof of antibodies to *Borrelia garinii* using Western blotting

In patients diagnosed in the task No.1, the serum samples or CSF were performed by Western blotting. Read results according to instructions. Use the given pattern for evaluation of the reaction. A diagnostic scheme is always the same – ELISA is used for screening, whereas Western blotting is performed as a confirmation of ELISA results. Read the Western blot results of patients J to N and write the results to the main table.

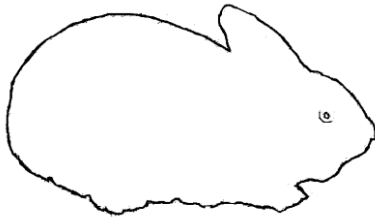
**Task 09/3: Diagnostics of Lyme borreliosis using polymerase chain reaction (PCR)**

According to the given photos of PCR product on the agarose gel, draw and record which of the tested samples is positive. Remark, that with regard to anamnesis, PCR reaction was performed only in two of our five patients. After that, interpret finally the total of all three examinations and write down a conclusion.

**Syphilis**

**Task 09/4: Direct proof of syphilis**

Direct proof of syphilis is only possible if suitable samples are sent to the laboratory. In some stages of the disease it is not possible to take anything for this purpose.



**a) Rabbit infectivity testing – RIT**

Write down the name of the rabbit used for the test.  
(It is derived from these islands: →→→→→→→→→.)

Exsudate from a suspect ulcer uses to be evaluated by darkfield microscopy and inoculated to rabbits testes. The tested animal starts to suffer from orchitis after 10 days after inoculation. Rabbit name:

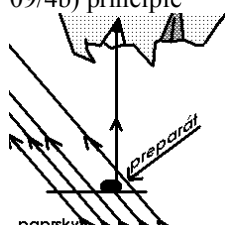

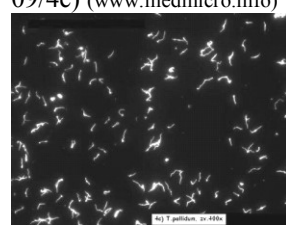


**b) Darkfield microscopy**

Look at a photograph of darkfield microscopy. For simplification you have it already filled.

**c) Direct immunofluorescence**

For simplification you have it already filled.

|  |   |  |
|--|---|--|
| <p>09/4b) principle</p>  | <p>09/4b) result (www.medmicro.info)</p>  | <p>09/4c) (www.medmicro.info)</p>  |
|--|---|--|

The causative agent of syphilis, *Treponema pallidum*, is NOT a culturable microorganism. The diagnostics is dependent on a stage of disease.

**Indirect diagnostics of syphilis**

Common table for Task 09/5 and 09/6.

| Patient Letter | Short clinical characterisation | Task 09/5 screening |        | Task 09/6 confirmation |     |     |           | Conclusion: final interpretation, eventually recommended therapy |           |
|----------------|---------------------------------|---------------------|--------|------------------------|-----|-----|-----------|--|-----------|
|                |                                 | RRR                 | MHA-TP | ELISA                  |     | WB  |           |  |           |
|                |                                 |                     |        | FTA-ABS                | IgM | IgG | IgM (+/-) |  | IgG (+/-) |
|                |                                 |                     |        |                        |     |     |           |  |           |
| A              |                                 |                     |        |                        |     |     |           |  |           |
| B              |                                 |                     |        |                        |     |     |           |  |           |
| C              |                                 |                     |        |                        |     |     |           |  |           |
| D              |                                 |                     |        |                        |     |     |           |  |           |
| E              |                                 |                     |        |                        |     |     |           |  |           |

**Task 09/5: Screening of syphilis – RRR and MHA-TP**

Pregnant women and blood donors undergo a screening using rapid reagin reaction (RRR) and *Treponema pallidum* microhemagglutination (MHA-TP). Read results of screening in a given group of persons and assess

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who of them need to be tested using confirmation tests. Record your results directly into the table.  
 RRR: flocculation in the well is positive; MHA-TP: agglutinate formation positive (see practical J07).

**Task 09/6: Confirmation of syphilis – FTA-ABS, ELISA and Western blotting**

Evaluate the results of FTA-ABS, ELISA a western blotting (WB) in patients who are suspect of syphilis (see the previous task). In ELISA reaction, count the cut-off and compare K-, + and patient values with it.  
 A1 field (A1 well) represents the blank.

|                                |  |                                    |  |                      |
|--------------------------------|--|------------------------------------|--|----------------------|
| Cut off level<br>(C1 + D1) / 2 |  | K- absorbance level<br>(B1 value): | <input type="checkbox"/> K- is OK<br><input type="checkbox"/> K- is not OK | ←                    |
| <b>IgM</b>                     |  | K+ absorbance level<br>(E1 value): | <input type="checkbox"/> K+ is OK<br><input type="checkbox"/> K+ is not OK | tick what is correct |
| Cut off level<br>(C1 + D1) / 2 |  | K- absorbance level<br>(B1 value): | <input type="checkbox"/> K- is OK<br><input type="checkbox"/> K- is not OK | ←                    |
| <b>IgG</b>                     |  | K+ absorbance level<br>(E1 value): | <input type="checkbox"/> K+ is OK<br><input type="checkbox"/> K+ is not OK | tick what is correct |

**Leptospirosis**

**Task 09/7: Direct proof of *Leptospira* sp.**

According to a given picture, describe and draw morphology of leptospires cultivated in the liquid Korthoff's medium for 2 weeks. Urine of a patient who is suspect of leptospirosis was used for the test.  
 For simplification, you have it filled already.

