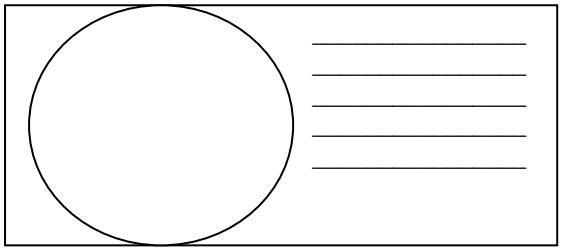
# Topic P08: Laboratory diagnostics of tuberculosis, actinomycetes and nocardiae

## Task 1: Microscopy of acid-fast and filamentous microoorganisms

While entirely acid-fast microorganisms (*Mycobacterium*) cannot be stained at all according to Gram, only partially acid-fast ones (*Nocardia*) or not at all (*Actinomyces*) can be Gram-stained. Typical morphology of *Nocardia* and *Actinomyces* is that of branched filaments, but sometimes they might be shorter, or even coccoid.

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



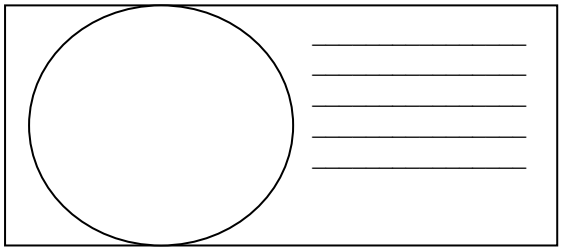
Ziehl-Neelsen staining is used for mycobacteria (*M. tuberculosis, M. leprae*), but also for some parasites (*Cryptosporidium parvum*, *Cyclospora cayetanensis*). The acid-fast organisms are stained only when heated during staining or in the „cold" variant (according to Kiyoun) at use of concentrated carbolfuchsin and concentrated phenol. On the other hand, after that, they are not decolorized even by so called „acid alcohol" (solution of alcohol with HCl or $H_2SO_4$). Decolorized background is then counterstained by a blue or green contrast dye.

Stain the negative sputum according to the Ziehl-Neelsen method (classic „hot" 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.

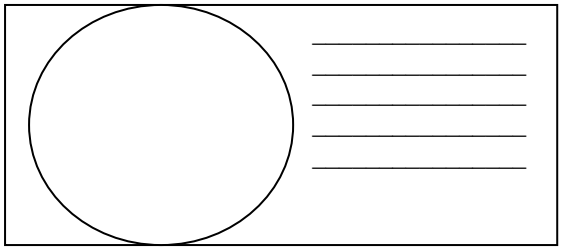
| | | |
|---|---|---|
| 1. | During the staining the preparation is | until |
| 2. | This reagent is made of | and |
| 3. | Instead of this reagent, it is also possible to use | |

### 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 2: Culture of mycobacteria, *Actinomyces* and *Nocardia*.

The culture requests of acid fast and partialy 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 culturing, the examined specimens should be rid of other microbes, usually by NaOH ("pickling")
- For ***Nocardia*** a current blood agar is sufficient.
- For ***Actinomyces*** we need VL-agar and culture in anaerostat/anaerobic jar (see P07), as this organism is microaerophilic with so low need for oxygen that anaerobic conditions are optimal for it.

**a) Describe the media for mycobacterial cultivation**

| Medium name | Liquid/solid | Colour | Notes |
|---|---|---|---|
| | | | |
| | | | |

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

| Bacterium | Medium name | Presence/absence of growth, possibly also growth character (use your own words to characterize the growth) |
|---|---|---|
| *Mycobacterium* | | |
| | | |
| *Actinomyces* | blood agar (labelled "KA") | |
| | anaerobic WCHA agar (labelled "WCHA") | |
| *Nocardia* | blood agar (labelled "KA") | |
| | anaerobic WCHA agar (labelled "WCHA") | |

## Task 3: Determination of antimicrobial drugs susceptibility

For treatment of mycobacterial infections, it is necessary to use special drugs, called antituberculotics (and not standard antibiotics). The way of testing is different from other bacteria, too: antituberculotics 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) Determination of susceptibility to antituberculotics**

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

| Antituberculotics | | | | 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.

Complete the table with the abbreviations of the antibiotics according to the card and for all the tested strains, measure the diameter of the susceptibility zones. On your card, you have limit zones – according these, interpret the zones as susceptible (S) or resistant (R). There are no "intermediate" interpretations this time.

| Strain → | | | | |
|---|---|---|---|---|
| Antibiotics (full name) | Zone ∅ (mm) | Interpretation | Zone ∅ (mm) | Interpretation |
| | | | | |
| | | | | |
| | | | | |
| | | | | |
| | | | | |
| | | | | |

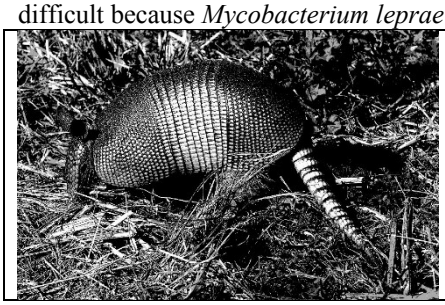
## Task 4: PCR in the TB diagnostics

As the culture of mycobacteria is rather prolonged (on average 6 weeks), PCR becomes a very important method in the diagnostics of TB.
Read a result of PCR TB diagnostics (from the slideshow), write down the results and interpret them.

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

## Task 5: Diagnostics of leprosy

Leprosy is a disease that still affects millions of people in underdeveloped countries. Its laboratory diagnostics is difficult because *Mycobacterium leprae* does not grow on artificial media. Fill in the following table.



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

**By Hans Stieglitz - Own work, CC BY-SA 3.0, https://commons.wikimedia.org/w/index.php?curid=11037077. Adapted.**

## Task 6: Indirect TB detection by means of QUANTIFERON©-TB Gold test

It is a test of induced interferon gamma release checking and by means of this, checking of the cell-mediated immunity. **Test principle:** It was proven that in TB, including latent TB, tuberculosis antigens activate T-lymphocytes and they produce big amounts of interferon gamma. Similarly those T-lymphocytes may be activated non-specifically by so called mitogene; that is why mitogene is used as a positive control (MIT). As a negative control we use a test tube containing nothing (NIL). The test tube with proper TB antigen is labelled "TB". Interferon itself is detected by ELISA reaction.
Interpret the Quantiferon-TB Gold examination in four patients with use of interpretation table.

Anna: MIT = 4.8 TB = 1.2 NIL = 1.1 Your interpretation: _____

Berta: MIT = 5.3 TB = 4.8 NIL = 2.1 Your interpretation: _____

Cecil: MIT = 0.9 TB = 0.9 NIL = 0.8 Your interpretation: _____

Dimos: MIT = 8.4 TB = 8.3 NIL = 8.2 Your interpretation: _____

(all values are in IU/ml)

**Interpretation table (according to test recommendations; simplified!)**

| NIL | TB minus NIL | MIT minus NIL | Final test interpretation | Presence of *M. tuberculosis* infection |
|---|---|---|---|---|
| ≤ 8,0 | < 0.35 | ≥ 0.5 | negative | Not likely |
| | ≥ 0.35 | any value | positive | Likely |
| | < 0.35 | < 0.5 | unsure | Cannot be determined |
| > 8,0 | any value | any value | | |

***Note:*** *Updated variant of QUANTIFERON test contains four (and not three) test tubes, as "TB" is replaced by two types of antigens. Nevertheless, for simplification, we count here with the classic variant of the test.*