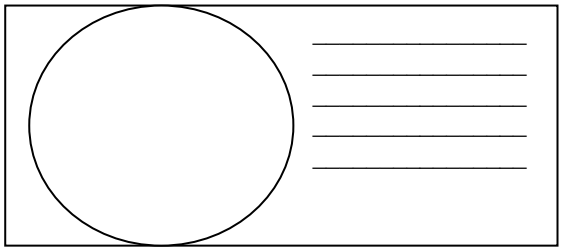
Topic P08: Laboratory diagnostics of tuberculosis, actinomycosis and nocardiosis

To study: *Mycobacterium, Actinomyces, Nocardia* (from textbooks, www etc.)
From spring term: Microscopy, culture, antibiotic susceptibility, PCR

Task 1: Microscopy of acid-fast and partially acid-fast 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, but they stain irregularly; they also tend to form branched filaments.

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



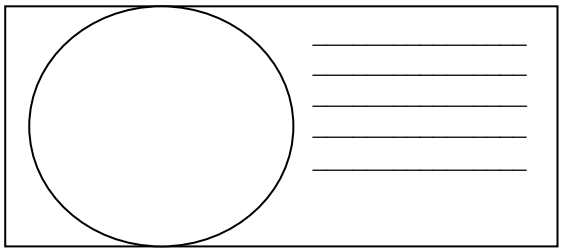
Ziehl-Neelsen staining is used for mycobacteria (*M. tuberculosis, M. leprae*) and also for some parasites (*Cryptosporidium parvum*, *Cyclospora cayetanensis*). The acid-fast organisms are stained only when heated during staining*, but then they are not decolorized even by so-called "acid alcohol" (mixture of alcohol with HCl or H_2SO_4). Decolorized background is then counterstained by a different dye.

Stain the negative sputum according to the Ziehl-Neelsen method (methylene blue variant). The presence of acid-fast rods is unlikely. Observe in the microscope (immersion). Draw the results; you will see mainly the background, e.g. leucocytes, epithelia and other objects. Do not forget to **describe** your picture (use the lines)!

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

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

b) Microscopy of a mycobacterial culture

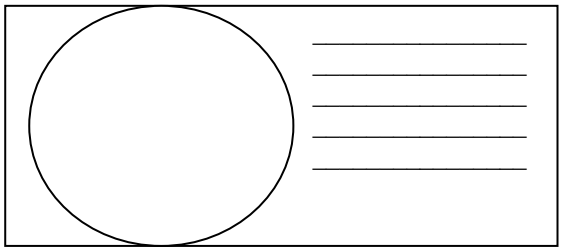


Examine microscopically (immersion 100× objective) the preparation from a mycobacterial culture stained by Ziehl-Neelsen staining method.

Evaluate the presence of red acid-fast rods. Draw the observed structures.

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

c) Microscopic examination of actinomycetes and nocardia strains



Examine microscopically the Gram-stained slide. Describe and draw the observed objects. Notice high polymorphism of the microorganisms (from cocci through rods to filaments, often branched; Gram-positive, but in many cases staining partially Gram-negative).

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

Task 2: Mycobacteria, *Actinomyces* and *Nocardia* cultivation

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

- For ***Mycobacterium tuberculosis***, we use special media: in the CR liquid Šula medium and solid media Ogawa and Löwenstein-Jensen. The solid media are different from the 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.
- For ***Nocardia***, common 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.

**Heating may be substituted by using highly concentrated both carbolfuchsine and phenol; this Kinyoun modification of Ziehl-Neelsen staining does not require heating.*

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 the treatment of mycobacterial infections, it is necessary to use special drugs, called antituberculotics. 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 common antibiotics and so the common diffusion disc test is used for the 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	

Picture source: http://www.1-costaricalink.com/costa_rica_fauna/nine_banded_armadillo.htm

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 mitogenem; 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 labeled "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 infection *M. tuberculosis***
≤ 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		