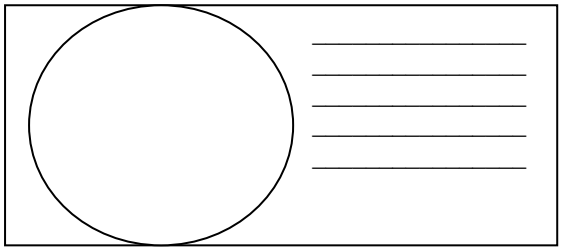
Topic P08: Laboratory diagnostics of tuberculosis, actinomycetes and nocardiae

Task 1: Microscopy of acid-fast and partially acid fast microoorganisms

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

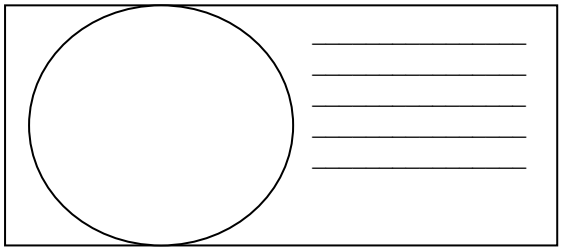
a) Staining of (negative) clinical material using Ziehl-Neelsen staining method



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 decororized even by so clled „acid alcohol“ (solution of alcohol with HCl or H_2SO_4). Decolorized bacground is then counerstained.
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 bacground, 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.

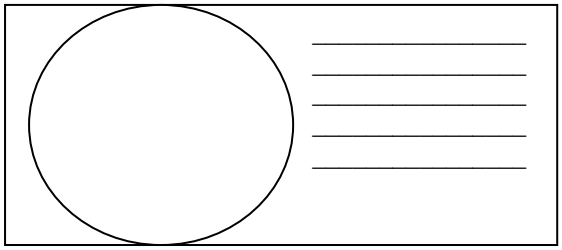
1.	During the staining the preparation is	until
2.	This reagent is made of	and
3.	Instead of this reagent, it is also possible to use	

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 culture, the medium should be specially treated.
- 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 anaerobic.

**Heating may be eventually substituted by use of highly concentrated carbolfuchsin and higly 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)
Mycobacterium		
Actinomyces	blood agar	
	VL agar	
Nocardia	blood agar	
	VL agar	

Task 3: Assessment of antimicrobial drugs susceptibility

For 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 oter 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 antituberculotics

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

Antituberculotic				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 →				
Antibiotic (full name)	Zone ∅ (mm)	Interpretation	Zone ∅ (mm)	Interpretation

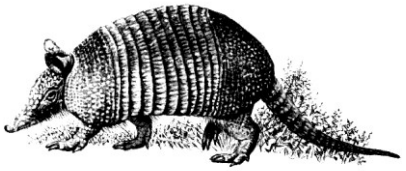
Task 4: PCR in diagnostics of TB

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 interprete 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		