

Topic P08: Laboratory diagnostics of tuberculosis, actinomycetes and nocardiae

Task 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.

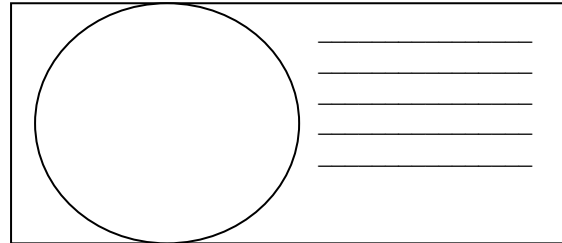
a) Staining of (negative) clinical material using Ziehl-Neelsen staining method

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 H₂SO₄). 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 to **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 _____	_____

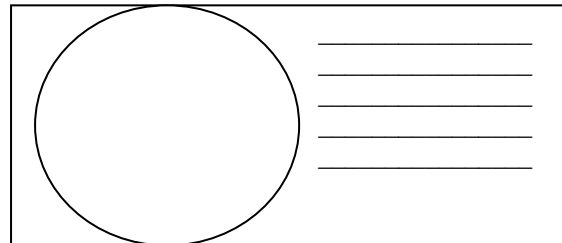
b) Microscopy of a mycobacterial culture

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.

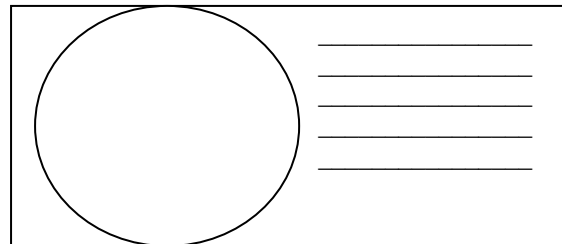
Do not forget to **describe** your picture (use lines)!



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 to **describe** your picture (use lines)!



Task 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 anaerostat/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 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

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 interpret them.

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