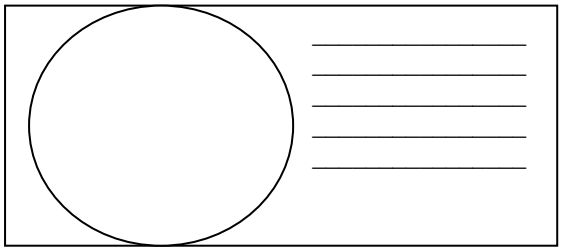
Topic P08+09: Laboratory diagnostics of tuberculosis, actinomycetes, nocardiae and spiral bacteria

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 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.

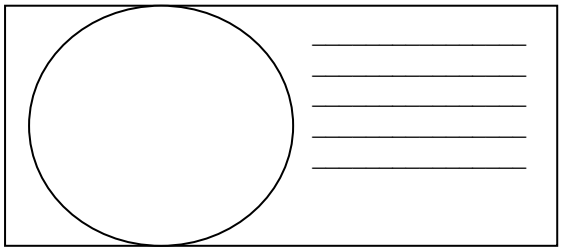
a) Staining of (negative) clinical material using Ziehl-Neelsen staining method



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.

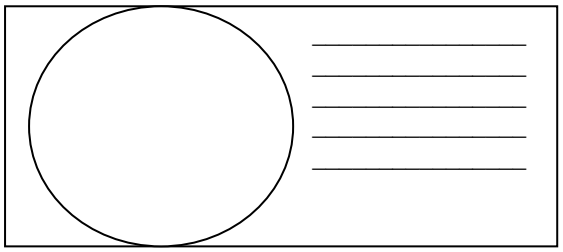
1.	During the staining the preparation is until	
2.	This reagent is made of	and
3.	Instead of this reagent, it is also possible to use	

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 08/2: 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 anaerobic box/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 08/3: 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.
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, interprete the zones as susceptible (S) resistants (R) and dubious (D).

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

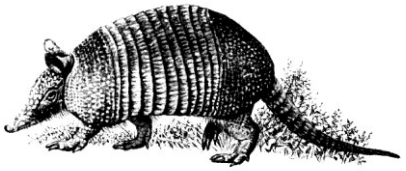
Task 08/4: PCR in diagnostics of TB

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 interprete 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 uderdevelopped states. It diagnostics is very difficult.
Fill in the following table.



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

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

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 interprete 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):		❒ K+ is OK ❒ K+ is not OK	← tick what is correct
IgM		K– absorbance level (well C1):		❒ K– is OK ❒ K– is not OK	
CAL level (well A1):		K+ absorbance level (well B1):		❒ K+ is OK ❒ K+ is not OK	← tick what is correct
IgG		K– absorbance level (well C1):		❒ K– is OK ❒ 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 rection. 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.

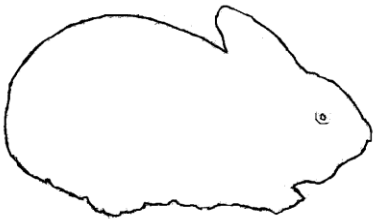
Task 09/3: Diagnostics of Lyme borreliosis using polymerase chain reaction (PCR)

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, interprete 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: _____

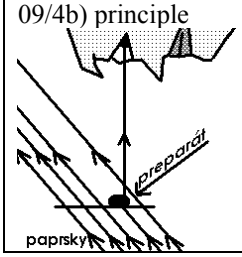
b) Darkfield microscopy

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

c) Direct immunofluorescence

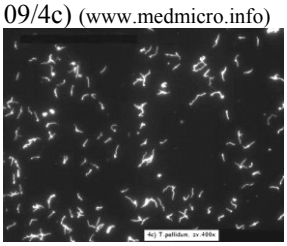
For simplification you have it already filled.

09/4b) principle



09/4b) result (www.medmicro.info)

09/4c) (www.medmicro.info)



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

Indirect diagnostics of syfilis

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	FTA-ABS	ELISA				WB		
					IgM		IgG		IgM (+/–)	IgG (+/–)	
					Absor-bance	(+/–)	Absor-bance	(+/–)			
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 who of them need to be tested using confirmation tests. Record your results directly into the table.
RRR: floculation 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 (C1 + D1) / 2		K– absorbance level (B1 value):		❒ K– is OK ❒ K– is not OK	← tick what is correct
IgM		K+ absorbance level (E1 value):		❒ K+ is OK ❒ K+ is not OK	
Cut off level (C1 + D1) / 2		K– absorbance level (B1 value):		❒ K– is OK ❒ K– is not OK	← tick what is correct
IgG		K+ absorbance level (E1 value):		❒ K+ is OK ❒ K+ is not OK	

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.