ZLLM0522c – Medical Microbiology I, practical sessions. Protocol to topic PZ09

Topic PZ09: Diagnostics of spirochetal infection

To study: *Borrelia, Leptospira, Treponema* (from textbooks, www etc **From spring term:** Microscopy, PCR, methods of antibody and antige

Lyme borreliosis

Common table for Task 1, 2 and 3. Abs. = absorbance value

Patient Letter	Short clinical		ELISA ((Task 1)		W. blott	ng (T2)	PCR	Conclusion:
	description (1–3 words characterizing the situation	IgM		IgG		IgM	IgG	(T3) (+/-)	final interpretation, recommendation
		Abs.	(+/-)	Abs.	(+/-)	(+/-)	(+/-)	('')	for future therapy
J									
Κ									
L									
Μ									
Ν									

Task 1: Detection 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 in the microtitration plate) you can see CAL level (CAL for "calibrator" – 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 the CAL level in the table below, check, whether negative control is really negative and positive control really positive. Then read and interpret ELISA results for patients J to N (write them in the main table above).

CAL level	K+ absorbance level	□ K+ is OK	\leftarrow
(well A1):	(well B1):	□ K+ is not OK	
IgM	K– absorbance level (well C1):	□ K− is OK □ K− is not OK	tick what is correct
CAL level	K+ absorbance level	□ K+ is OK	\leftarrow
(well A1):	(well B1):	□ K+ is not OK	
IgG	K– absorbance level (well C1):	□ K− is OK □ K− is not OK	tick what is correct

Task 2: Detection of antibodies to Borrelia garinii using Western blotting

In patients diagnosed in the task 1, the detection of antibodies in serum or CSF samples was performed by Western blotting. Read the results according to the instructions. Use the presented pattern for evaluation of the reaction. The 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 in the main table.

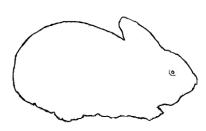
Task 3: Diagnostics of Lyme borreliosis using polymerase chain reaction (PCR)

According to the presented photos of a PCR product on the agarose gel, draw and record which of the tested samples are positive. Note, that with regard to the anamnesis, PCR reaction was performed only in two out of our five patients. After that, perform the final interpretation of all three tasks and write down a conclusion.

Syphilis

Task 4: Direct detection of syphilis

Direct detection of syphilis is only possible if suitable samples are sent to the laboratory. In some stages of the disease, however, sampling for this purpose is not possible.



a) Rabbit infectivity testing – RIT Write down the name of the rabbit stock used for the test.

(It is derived from these islands: $\rightarrow \rightarrow \rightarrow \rightarrow \rightarrow \rightarrow \rightarrow \rightarrow \rightarrow$) Exsudate from a suspect ulcer is usually evaluated with dark field microscopy and inoculated into rabbit testes. The animal starts to suffer from orchitis. Rabbit stock name:

Name

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b) Darkfield microscopy

Look at the microphotography of treponemas taken from a dark field microscope, draw the principle of dark field microscopy, and also record your observation.

c) Direct immunofluorescence

Look at the microphotography of treponemas taken from a fluorescent microscope and record your observation.

4b) principle	4b) result	4c)		

The causative agent of syphilis, Treponema pallidum, is NOT a culturable microogranism. The diagnostics depends on the stage of disease.

Indirect diagnostics of syphilis

indirect diagnostics of syphilis												
Joint table for Task 5 and 6.												
L P	organis atrices	Task 5		Task 6								
atien			Screening		Confirmation						Conclusion:	
Patient Letter		MHA-TP rrr	Z	F	ELISA WE			W	B	final interpretation,		
			1HL	FTA-ABS	IgM		IgG				recommended therapy	
			A-TP						IgM	IgG		
										(+/-)		
					-1		- >		- 	(-)		
	Shor				Absor- bance	(+/-)	Absor- bance	(+)				
	clinic charge				or-	-)	or-	$ \mathbf{J} $				
	charaeterisation											
A												
-												
B												
С												
C												
D												
E												

Task 5: Screening of syphilis – RRR and MHA-TP

Pregnant women and blood donors undergo screening performed using rapid reagin reaction (RRR) and *Treponema pallidum* microhaemagglutination (MHA-TP). Read the results of the screening in the presented group of persons and assess which of them need further tests for confirmation. Record your results directly into the table.

Positive result: RRR - flocculation in the well; MHA-TP - agglutinate formation (see Practical J07).

Task 6: Confirmation of syphilis - FTA-ABS, ELISA and Western blotting

Evaluate the results of FTA-ABS, ELISA and Western blotting (WB) in patients with suspect syphilis (see the previous task). In the ELISA reaction, count the cut-off and compare K-, K+ and patient values with it. A1 field (A1 well) represents the blank.

The field (The work) represents the ordina.									
Cut off level	K– absorbance level	🗖 K– is OK	$\boldsymbol{\leftarrow}$						
(C1 + D1) / 2	(B1 value):	🗖 K– is not OK							
IaM	K+ absorbance level	□ K+ is OK	tick what is						
IgM	(E1 value):	□ K+ is not OK	correct						
Cut off level	K– absorbance level	🗖 K– is OK	\square						
(C1 + D1) / 2	(B1 value):	🗖 K– is not OK							
IaC	K+ absorbance level	□ K+ is OK	tick what is						
IgG	(E1 value):	\Box K+ is not OK	correct						

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Leptospirosis

Task 7: Direct detection of *Leptospira* sp.

According to the presented picture, describe and draw the morphology of leptospiras cultivated in the liquid Korthoff's medium for 2 weeks. Urine of a patient with suspect leptospirosis was used for the test.

