

P09+10 Clinical microbiology I – general. Diagnostics of spirochetes.

To study: Sampling, specimen transport (from textbooks, www etc.). Spirochetes.

From spring term: Microscopy

Task 1: Indications for microbiological examination

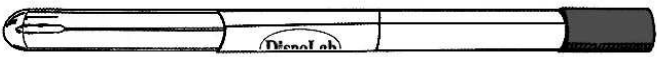
For following casuistries, fill in the table.


- ❶ Fill in always the case description (left column).
- ❷ Try to find out your solution. Try to structure your answer as follows:
 - Microbiological examination: yes/no
 - ❖ **yes** → select a specimen
 - ❖ **no** → select other steps, e. g. direct treatment – what antibiotics etc.)
- ❸ After the three minute limit, write down a correction according to the teacher's explanation.

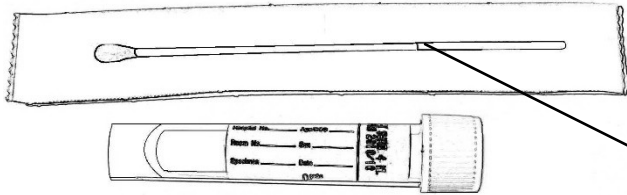
	Description of a case	Your solution (⌚ 3 minutes)	Correction according to the teacher's explanation
a			
b			
c			
d			

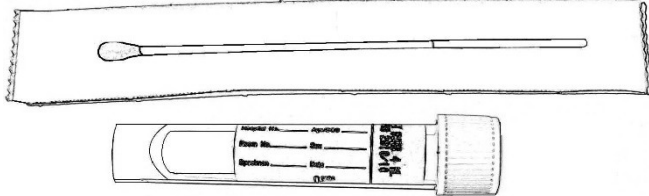
Task 2: Swabs and vessels

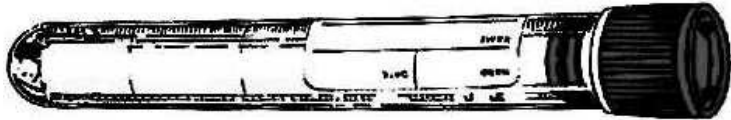
Observe the swabs in your table and fill in their "identity cards".

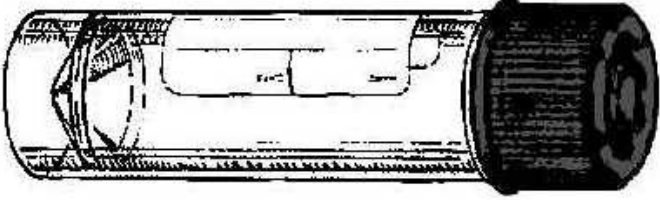
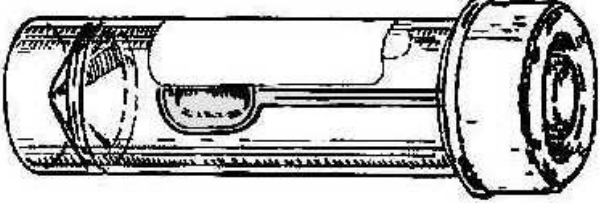


Name: Plain swab		
	Stick material	plastic, wood, aluminium
	Swab material	synthetic cotton
Practical use:		

Name: Swab with Amies transport medium		
	Stick material	plastic or aluminium
	Swab material	synthetic cotton
	Medium	Amies (Stuart, Cary Blair)
<p><i>Note: The medium may contain charcoal (then it is black); without charcoal, it would be colourless.</i></p> <p>Practical use:</p> <p>Variant with aluminium stick is used for</p>		

Name: Fungi-Quick swab		
	Stick material	plastic
	Transport medium colour	colourless
	Cap colour	
<p>Practical use:</p>		

Name: C. A. T. swab		
	Stick material	plastic
	Transport medium colour	colourless
	Cap colour	
<p>Practical use:</p>		

Name: Common test tube for microbiology		
	Sterile? (yes or no)	
	Description	made of polystyrene, 16 × 100 mm, 10 ml
<p>Practical use:</p>		

Name: Sputum test tube		
	Sterile? (yes or no)	
	Description	made of polystyrene or polypropylene, 26 × 92 mm, 30 ml
Practical use:		
Name: Stool container		
	Sterile? (yes or no)	
	Description	made of polypropylene, 26 × 82 mm, 30 ml
Practical use:		
Name:  Sampling vessel for urine		
	Sterile? (yes or no)	
	Description	made of polypropylene, 45 × 70 mm, 120 ml
Practical use:		

Task 3: Other sampling methods than swabs and vessels

Fill in the following table:

Sampling method	Typical example(s) of use
smear on a slide	
imprint with an agar	
moulage method (indirect imprint)	
uricult	

Task 4: The order form

a) Filling in the order form

Fill in the following order form with a patient name and data and the requested examination related to the disease, according to a card that has been given to you by the teacher.

Examples of a description to be written on the request form (in „Požadováno“ field):

- Throat swab for bacteriology examination
- Blood for antibodies against EB virus and cytomegalovirus
- Stool for parasites (after stay in Congo and Gabon)

Kód pojistovny 111	preznanie III A	ICP Odbornost	Datum	Čís. dokladu	Pof. č.
POUKAZ NA VYŠETRENÍ / OŠETRENÍ					
Pacient	Č. poisťovne	Základná diagnóza	ICP		
Variačný symbol	Ostatná diagnóza	Odbornost			
Odeslaný ad:	Kód náhrady	Datum	Kód	Poč.	
Požadováno:		Odbornost			
Poznámka:		Var. symbol			
Dne:		Datum			
razítko a podpis lekára		Kód			
VZP-06x/1999		Poč.			
razítko a podpis		Odbornost			

b) Order form common mistakes

To each of the following order forms write down what is wrong. There are some mistakes at filling in the order form, but you should also identify improperly requested examinations.

Kód pojistovny 111	preznanie III A	ICP Odbornost	Datum	Čís. dokladu	Pof. č.
POUKAZ NA VYŠETRENÍ / OŠETRENÍ					
Pacient: Albus DUMBLEDORE	Č. poisťovne: 22 11 22 112	Základná diagnóza: Z 0 17	ICP		
Variačný symbol:	Ostatná diagnóza:	Odbornost			
Odeslaný ad:	Kód náhrady:	Datum	Kód	Poč.	
Požadováno: wound swab		Odbornost			
Poznámka:		Var. symbol			
Dne:		Datum			
razítko a podpis lekára		Kód			
VZP-06x/1999		Poč.			
razítko a podpis		Odbornost			

Kód pojistovny 111	preznanie III A	ICP Odbornost	Datum	Čís. dokladu	Pof. č.
POUKAZ NA VYŠETRENÍ / OŠETRENÍ					
Pacient: Albus DUMBLEDORE	Č. poisťovne: 22 11 22 112	Základná diagnóza: Z 0 17	ICP		
Variačný symbol:	Ostatná diagnóza:	Odbornost			
Odeslaný ad:	Kód náhrady:	Datum	Kód	Poč.	
Požadováno: tissue for microbiology		Odbornost			
Poznámka:		Var. symbol			
Dne:		Datum			
razítko a podpis lekára		Kód			
VZP-06x/1999		Poč.			
razítko a podpis		Odbornost			

Kód pojistovny 111	preznanie III A	ICP Odbornost	Datum	Čís. dokladu	Pof. č.
POUKAZ NA VYŠETRENÍ / OŠETRENÍ					
Pacient: Albus DUMBLEDORE	Č. poisťovne: 22 11 22 112	Základná diagnóza: Z 0 17	ICP		
Variačný symbol:	Ostatná diagnóza:	Odbornost			
Odeslaný ad:	Kód náhrady:	Datum	Kód	Poč.	
Požadováno: rectal swab for parasitology		Odbornost			
Poznámka:		Var. symbol			
Dne:		Datum			
razítko a podpis lekára		Kód			
VZP-06x/1999		Poč.			
razítko a podpis		Odbornost			

Kód pojistovny 111	preznanie III A	ICP Odbornost	Datum	Čís. dokladu	Pof. č.
POUKAZ NA VYŠETRENÍ / OŠETRENÍ					
Pacient: Albus DUMBLEDORE	Č. poisťovne: 22 11 22 112	Základná diagnóza: Z 0 17	ICP		
Variačný symbol:	Ostatná diagnóza:	Odbornost			
Odeslaný ad:	Kód náhrady:	Datum	Kód	Poč.	
Požadováno: Tissue for syphilis cultivation tuberculosis		Odbornost			
Poznámka:		Var. symbol			
Dne:		Datum			
razítko a podpis lekára		Kód			
VZP-06x/1999		Poč.			
razítko a podpis		Odbornost			

c) Interpretation of Lyme borreliosis results

With help of you teacher, evaluate the results of ELISA and immunoblot to five patients. Normally, blotting is only performed in a special reason, but this time, all specimens had to be tested also by the immunoblot. On the other hand, PCR was performed just for two of them – those with suspicion for neuroborreliosis.

Patient Letter	Short clinical description	ELISA (Task 1)			Blot (Task 2)		PCR (+/-)	Conclusion: final interpretation, recommendation for future therapy
		IgM (+/-)	IgG (+/-)		IgM (+/-)	IgG (+/-)		
J	Erythema migrans							
K	Fatigue syndrome							
L	Likely neuroborreliosis							
M	“Website reader”							
N	Neuroborreliosis???							

d) Interpretation of syphilis

With help of you teacher, evaluate the results of screening and confirmatory reactions for syphilis in five patients.

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 J08).

Confirmation of syphilis – FTA-ABS, ELISA and immunoblotting

Evaluate the results of FTA-ABS, ELISA and immunoblotting 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.

Patient Letter	Short clinical characterisation	Screening		Confirmation				Conclusion: final interpretation, recommended therapy	
		RRR	MHA-TP	FTA-ABS	ELISA		Blot		
					IgM (+/-)	IgG (+/-)	IgM (+/-)		IgG (+/-)
A	Pregnant woman – screening								
B	Lesion (chancere?) two days								
C	Susp. Syphilis latens								
D	Blood donor								
E	A newborn of a mother with syphilis								

Task 6: 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: →→→→→→→→)

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:



b) Dark field 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.

<p>6b) principle</p>	<p>6b) result</p>	<p>6c)</p>
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The causative agent of syphilis, *Treponema pallidum*, is **not** a culturable microorganism. The diagnostics depends on the stage of disease.

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.

