P13 Clinical microbiology IV – examination of wound and bloodstream infections

To study: Your own protocols (especially Special bacteriology)

Wound infections

Task 1: Specimens in wound infections

Try to fill in the following table:

Type of wound	Superficial wound	Deep wound with amount of pus sufficient for being sent as a liquid	Deep wound with not sufficient amount of pus	Wound with pus, possibly containing anaerobic bacteria
Sampling method				
When a specimen from a wound is send to the laboratory, it is very important to fill in the request form, especially to write 1) _____ and 2) _____				

Task 2: Indirect imprint method for superficial wound examination

a) Indirect imprint method – performing

Perform the indirect imprint method in pairs. Place a sterile filtration paper on your mate‘s forearm (instead of a superficial wound). Let it 10 seconds here, then using tweezers, transport it carefully to a Petri dish with nutrient agar. After that, remove it and throw it away.

In practice, the filtration paper is not discarded, but sent together with the agar plate to the laboratory. In the laboratory the filtration paper is placed to two or three more media: agar with 10 % NaCl, chromogenic URI medium etc. After that, all media are cultivated overnight.

b) Indirect imprint method – reading of results

Try to read the preliminary result of imprint method on URIchrom chromogenic medium using recounting scheme on your table and with the help of the key of colours of individual bacteria on the chromogenic medium. Attention! You have real results from real patients. Your result is not supposed to be the same as the result of your neighbour with another agar plate. Even the number of strains may be different. More precise determination and antibiotic susceptibility test would not be performed in this task.

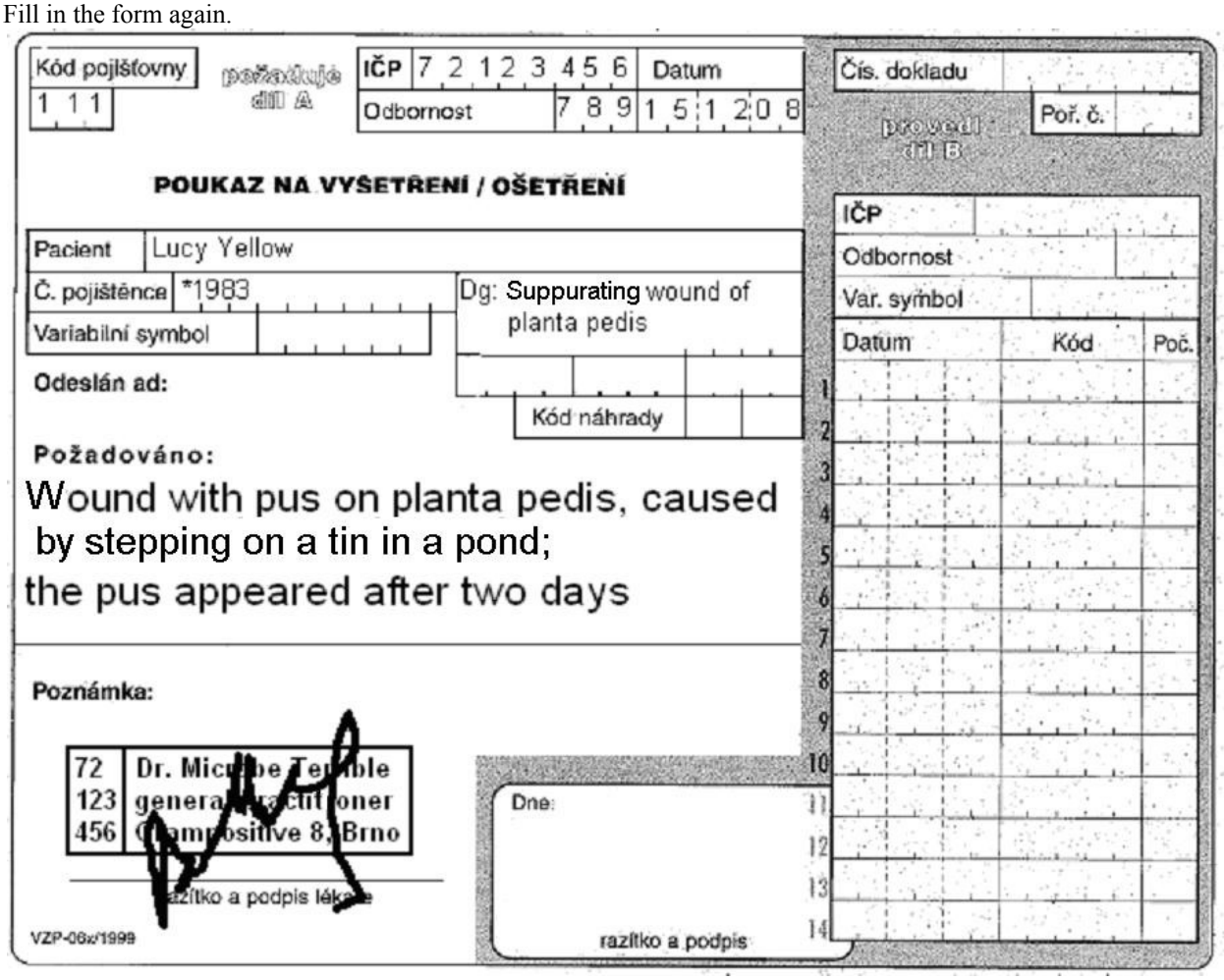
The cultivation result of my imprint contained:

Likely group or genus of bacterium	Quantity (approx. number of colonies per 25 cm^2)
1.	
(2.)	
(3.)	

Clue for preliminary diagnostics: Staphylococci – white on URI, growing also on NACL, white colonies on blood agar; **Haemolytic streptococci** – haemolytic colonies on blood agar, not growing on NACL, on URI not growing or *(S. agalactiae)* pale turquoise blue. **Enterococci** have greyish colonies on blood agar and small, but rich turquoise colonies on URI. **Enterobacteriaceae and G- non-fermenters** – growing on Endo agar. ***Escherichia*** is pink on URI, ***Klebsiella*** is blue on URI, ***Proteus*** is yellow on URI, ***Pseudomonas*** is white or slightly green (because of its own pigmentation) on URI. *All this is only preliminary, the algorithms from previous practicals are valid!*

Task 3: Deeper wound swab result

In the case of a wound swab, there is no "common flora". That is the main difference between wound swab and e. g. swabs from respiratory ways: it is not necessary to search for a pathogen among the normal flora.
On the other hand, we mostly use more culture media to detect all possible pathogens, even if they would be in a mix of them. Besides blood agar and Endo (or McConkey) agar we usually use also blood agar with 10 % NaCl and blood agar with amikacin in order to search for streptococci and enterococci (but none of these media is used in our task). In other situations there is one pathogen only, and even in small amounts, so we have to multiply it in a liquid medium (broth). Also this medium is not present in our task.
Fill in the form again.



Patient: Amanda Red *1984 Dg.: wound of planta pedis					
Specimen: wound swab* Ordered by: Dr. Microbe Terrible					
*note: pyogene wound on planta pedis, swimming in a pond					
Growth on blood a. (incl. smell)	Endo agar:	MH agar:	Oxidase:	Conclusion:	Interpretation

Antibiotic susceptibility test

Piperacillin+tazobactam (TZP)	S ≥ 18 R < 18		Ciprofloxacin (CIP)	S ≥ 25 R < 22	
Gentamicin (CN)	S ≥ 15 R < 15		Ceftazidime (CAZ)	S ≥ 16 R < 16	
Ofloxacin (OFL)	S ≥ 16 R < 13		Colistin (CT)	S ≥ 11 R < 11	

write S = susceptible, R = resistant, eventually I = intermediary
*result of this test is also valid for doxycycline
Final conclusion and recommendation for treatment: _____

Bloodstream infections

Task 4: Blood cultures – processing

Describe the use of three types of blood culture vessels.

Fill in which data should not be missing on the order form in the case of blood culture (only "material type/examination type" field)

Explain:

Why is absolute sterility in blood culture samples more necessary than in any other blood specimens (e. g. those sent for biochemical examination)?
How many blood cultures should be taken and why?

Fill in the missing fields in the description of blood culture processing and examination according to the video clip and the teacher's explanation.

A blood culture vessel arrives in the laboratory. Here it is put into a _____.

The positive result is demonstrated by _____ and _____.

When the cultivation is positive, a smear is prepared and the content of the vessel is _____

onto the blood and Endo agar. Also, a preliminary _____ test is performed directly

from the specimen; as the inoculum is not standardized here, its results are only _____.

Task 5: Blood cultures – microscopy of a positive specimen

The cultivator for blood cultures revealed a positive result. For preliminary treatment, a Gram stained smear is performed from the contain of the vessel. Observe the result and write it. **Attention!** The slides have origin in real blood cultures of different patients. Therefore your result may be simply different from that of your neighbour with a different slide.

Blood culture contained gram-positive – gram-negative* cocci – bacilli* arranged in _____**
** delete as appropriate **only for cocci (pairs, chains, clusters…) or G+ bacilli in palisades*

Task 6: Blood cultures – cultivation result

Observe cultivation result of a positive blood cultures inoculated on solid media. Suggest more methods for detailed diagnostics of bacteria. Try to assess preliminary antibiotic susceptibility. Also here you are not supposed to have the same results as your neighbour.

Name of medium			
Growth Y/N, appearance of colonies			

More tests of more detailed determination: _____

_____

Preliminary name of the microbe: _____

Preliminary antibiotic susceptibility testing

Name of the set of antibiotics:

Antibiotic	Susceptibility Interpretation	Measured size	Result (encircle)	Antibiotic	Susceptibility Interpretation	Measured size	Result (encircle)
1.	R < S ≥		S–I–R	4.	R < S ≥		S–I–R
2.	R < S ≥		S–I–R	5.	R < S ≥		S–I–R
3.	R < S ≥		S–I–R	6.	R < S ≥		S–I–R

Task 7: Blood cultures – interpretation

Find suitable interpretation for results of two different patients.

John White, *1942, elevated temperature and inflammatory markers, three blood culture specimens sent to the laboratory	Joe Black, *1945, elevated temperature and inflammatory markers, three blood culture specimens sent to the laboratory
I Central venous catheter. Time to detection 10 hours, finding: *Staphylococcus hominis*, susceptible to oxacilin, tetracycline, vankomycin, resistant to erythromycin, klindamycin, co-trimoxazole.	I Central venous catheter. Time to detection 8 hours, finding: *Staphylococcus epidermidis*, susceptible to oxacilin, resistant to tetracycline, vankomycin, erythromycin, klindamycin, co-trimoxazole.
II Peripheral catheter. Time to detection 13 hours, finding: *Staphylococcus hominis*, susceptible to oxacilin, tetracycline, vankomycin, resistant to erythromycin, clindamycin, co-trimoxazole.	II Peripheral catheter. Time to detection 26 hours, finding: *Staphylococcus hominis*, susceptible to oxacilin, tetracycline, vankomycin, erythromycin, clindamycin, co-trimoxazole, no resistance observed
III Venipuncture. Time to detection 13.5 hours, finding: *Staphylococcus hominis*, susceptible to oxacilin, tetracycline, vankomycin, resistant to erythromycin, clindamycin, co-trimoxazole.	III Venipuncture. Time to detection 38 hours, finding: *Staphylococcus epidermidis*, susceptible to oxacilin, co-trimoxazole, vankomycin, resistant to tetracycline, erythromycin, clindamycin.
Likely interpretation:	Likely interpretation: