

## PZ12 Clinical microbiology III – examination in urogenital infections

To study: special bacteriology from your own protocols

### Urinary tract infections

#### Task 1: Sampling and transport of urine

According to the teacher's explanation, tick which sentences concerning urine sampling and transportation are true/false.

Urine examination is recommended in non-complicated and necessary in complicated cystitis. <input type="checkbox"/> true <input type="checkbox"/> false
Microbiologists recommend the use of catheterized urine as a routine way of sampling the urine for bacteriology. <input type="checkbox"/> true <input type="checkbox"/> false
It is not important whether foreskin (prepuce – in men) or labia minora (in women) are in the way of urine stream during sampling the urine for bacteriology. <input type="checkbox"/> true <input type="checkbox"/> false
External orifice of urethra should be carefully washed and eventually also disinfected before sampling the urine for bacteriology. <input type="checkbox"/> true <input type="checkbox"/> false
The vessel into which the patient urinates should be sterile. <input type="checkbox"/> true <input type="checkbox"/> false
The test tube used for urine transportation to the laboratory should have a yellow cap. <input type="checkbox"/> true <input type="checkbox"/> false
If urine is not "routinely taken", the order form should contain information whether it has been catheterized, punctured, or whether it is a specimen taken from a permanent catheter. <input type="checkbox"/> true <input type="checkbox"/> false
Urine from a permanent catheter is equally important for bacteriological diagnostics as the catheterized urine (just for examination). <input type="checkbox"/> true <input type="checkbox"/> false
Urine specimen should be delivered to the laboratory within 2 hours after sampling; if this is impossible, it should be kept in a refrigerator. <input type="checkbox"/> true <input type="checkbox"/> false
Urine sample is better than urethral swab in gonorrhoea diagnostics. <input type="checkbox"/> true <input type="checkbox"/> false

#### Task 2: Inoculation of sample of urine

Observe your teacher demonstrating for you inoculation of sample of urine (or video with the same topic, if available). Fill in empty places in the following text:

Urine sample is inoculated using calibrated loop, made of \_\_\_\_\_. The term "calibrated" means that it is set to specific volume, usually \_\_\_\_\_. The specimen of urine is inoculated to two media: \_\_\_\_\_ and \_\_\_\_\_. Instead of the second medium we could also use \_\_\_\_\_ or \_\_\_\_\_. After inoculation, the specimen is incubated overnight in a thermostat at \_\_\_\_\_°C.

#### Task 3: Evaluation of semiquantitative cultivation of urine

After inoculation and incubation (see Task 2), the agar plates with result of urine specimen cultivation are evaluated. The number of colonies is counted (of estimated approximately) and recounted to number of bacteria in a millilitre of the original specimen of urine.

Number of colonies on agar	Number of bacteria in one microlitre of the original urine (µl)	Number of bacteria in one millilitre of the original urine (ml)	Interpretation
<10			
10–100			
>100			

Kód pojišťovny 1 1 1	pacientů dipl A	IČP 7 2 1 2 3 4 5 6	Datum	Čís. dokladu	Prof. č.
		Odbornost 7 8 9	1 5 1 2 0 8		
<b>POUKAZ NA VYŠETŘENÍ / OŠETŘENÍ</b>					
Pacient	Carolina Red				
Č. pojištěnce	*1952				
Variabilní symbol	Dg.: acute cystitis				
Odeslán ad:	Kód náhrady				
<b>Požadováno:</b>					
urine (commonly sampled) for bacteriological examination					
<b>Poznámka:</b>					
72 123 456	Dr. Microbe Terrible generální praktičer Kompostová 8, Brno				
razítko a podpis lékaře					
Dne:					
razítko a podpis					

Form for results of Enterotest 16:

ONPG	1H	1G	1F	1E	1D	1C	1B	1A	2H	2G	2F	2E	2D	2C	2B	2A
+	black	blue	red	blue	red	green	black	blue	blue	yellow	yellow	yellow	yellow	yellow	yellow	yellow
-	colourless	green	yellow	green	yellow	yellow	colourless	yellow	yellow	green	green	green	green	green	green	green
?																
1	2	4	1	2	4	1	2	4	1	2	4	1	2	4	1	2
Code:			Identification						Probability %			T index				

Patient: Carolina Red *1952 Dg.: acute cystitis			
Specimen: normal urine Ordered by: Dr. Microbe Terrible			
Growth on Blood agar:	Growth on Chromotest URI medium	Conclusion:	Interpretation
Quantity:	Enterotest 16 result:		

Antibiotic susceptibility test

Ampicillin AMP	R < 14 S ≥ 14		Tetracycline* TE	R < 12 S ≥ 15	
Cephazolin KZ	R < 14 S ≥ 18		Cefuroxime CXM	R < 18 S ≥ 18	
Co-trimoxazole SXT	R < 13 S ≥ 16		Norfloracin NOR	R < 19 S ≥ 22	
Nitrofurantoin F	R < 11 S ≥ 11				

write S = susceptible, R = resistant, eventually I = intermediary

\*result of this test is also valid for doxycycline

Final conclusion and recommendation for treatment: \_\_\_\_\_

**Task 4: Interpretation and treatment of UTI**

In following table in each cell (except cells in the first column) **one term is wrong**. Add a dot to all terms you consider wrong. After that, check your choice with your teacher, and **strike through all the really false terms**.

Clinical situation	Most likely pathogens	Drug of choice for initial therapy	Alternative drugs (allergy etc.)
Asymptomatic bacteriuria (ABU) pregnant women	<i>Escherichia coli</i> <i>Klebsiella pneumoniae</i>	nitrofurantoin* ofloxacin	amoxicillin linezolid
Asymptomatic bacteriuria (ABU) other situations	<i>Streptococcus pyogenes</i> <i>Enterococcus sp.</i>	no therapy nitrofurantoin	no therapy cefuroxime
Acute non-complicated cystitis (community cystitis, that means „not-nosokomial“ one)	<i>Clostridium sp.</i> <i>Escherichia coli</i> <i>Staphylococcus saprophyticus</i> <i>Klebsiella pneumoniae</i>	ciprofloxacin nitrofurantoin	co-trimoxazole (co-)amoxicillin vankomycin cefuroxime
Accute pyelonephritis	<i>Escherichia coli</i> <i>Bacteroides fragilis</i> <i>Klebsiella pneumoniae</i> <i>Proteus sp.</i>	(co-)amoxicilin cefuroxime nitrofurantoin	co-trimoxazole norfloxacin imipenem

\*except first trimester and the second haft of the third trimester

**Infections of genital system**

**Task 5: Sampling methods in STIs and other infections of reproductive organs**

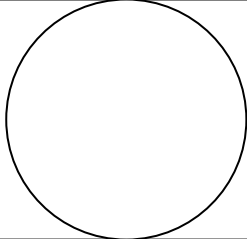
Find suitable swabs or other sampling methods for following clinical situations (suspicions for individual diseases). For some of them more than one sampling method is suitable. Use numbers 1 to 6 and mark your choice to individual situations. Correct yourself with help of your teacher.

- |                     |                          |
|---------------------|--------------------------|
| Bacterial vaginosis | Syphilis                 |
| Aerobic vaginitis   | Mycoplasma infection     |
| Vaginal mycosis     | Chlamydia infection      |
| Gonorrhoea          | Papillomavirus infection |

Numbers: 1 – Amies swab 2 – C. A. T. swab 3 – plain (dry) swab 4 – a smear on a slide 5 – clotted blood for indirect examination 6 – ulcer durum scraping for dark-field microscopy and PCR

**Task 6: Evaluation of vaginal smears**

In diagnostics of vaginal infections, one very important method is microscopy. Cultivation results may be positive even when the amount of bacteria (e. g. *Gardnerella* sp.) is not significant. Therefore, microscopy is better, because we can see the ratio between various *morphotypes* of bacteria, and also other structures (epithelial cells including those with adhered bacteria – so called “clue cells”; white blood cells; yeast cells etc.). Sometimes, two smears are sent to the laboratory: one is stained by Giemsa staining (almost because of *Trichomonas vaginalis* diagnostics, as *T. vaginalis* cannot be Gram stained very well) and the other by Gram (especially for bacteriology).



\_\_\_\_\_

\_\_\_\_\_

\_\_\_\_\_

Nugent score for BV:

Observe a result of a vaginal smear and draw your result in the laboratory report. Try to count the **Nugent score of bacterial vaginosis** with help of following guide:

**A. Morphotypes**

- **Morphotype *Lactobacillus*** = **robust** and long G+ rods
- **Morphotype *Gardnerella/Bacteroides*** = **subtle** Gram-negative or Gram-variable **straight** rods
- **Morphotype *Mobiluncus*** = **subtle** Gram-negative **curved** rods.

Other objects (cocci, human origin cells, yeast cells) are **not counted**

**Note:** the term *morphotype* means “bacteria that look in the microscope the same as”, so not all bacteria of *Gardnerella/Bacteroides* morphotype are really either *Gardnerella* or *Bacteroides*.)

**B. The counting system (+ to +++) – simplified**

Bacteria are <b>extremely frequent</b> , they may be seen in the first moment of looking to the field	++++
Bacteria are <b>very frequent</b> , each field contains lots of them	+++
Bacteria are <b>present in each field</b> , but they are not frequent	++
Bacteria are not very frequent, there are <b>fields with no bacteria at all</b>	+
Bacteria are <b>completely absent</b>	–

Note: Similar system can be also used for other microscopies, e. g. sputum evaluation

**C. The proper Nugent scoring system (simplified):**

Points added	<i>Lactobacillus</i> morphotype presence	<i>Gardnerella/Bacteroides</i> morphotype presence	<i>Mobiluncus</i> morphotype presence
0	++++	–	–
1	+++	+	+ or ++
2	++	++	+++ or ++++
3	+	+++	
4	–	++++	

So each smear may get 0 to 4 points for *Lactobacillus* morphotype (the more bacteria of this morphotype, the **less** points), 0 to 4 points for *Gardnerella/Bacteroides* morphotype presence morphotype (the more bacteria of this morphotype, the **more** points) 0 to 2 points for *Mobiluncus* morphotype presence morphotype (the more bacteria of this morphotype, the **more** points)

The criterion for bacterial vaginosis according to Nugent's criteria is a total score of 7 or more is labeled as Bacterial Vaginosis a score of 4 to 6 is called intermediate, and a score of 0 to 3 is considered normal.

*Reliability of diagnosing bacterial vaginosis is improved by a standardized method of gram stain interpretation. R P Nugent, M A Krohn, and S L Hillier, J Clin Microbiol. 1991 February; 29(2): 297–301.*

**Task 7: Evaluation of vaginal swabs**

Vaginal swabs are usually cultured on blood agar, Endo agar, agar with 10 % NaCl, special blood agar for *Gardnerella vaginalis*, eventually also VL agar (anaerobic culture). As a normal flora, we can observe lactobacilli: very tiny colonies with viridation. There exist many species of lactobacilli, with different relations with oxygen, although they use to be microaerophilic. Nevertheless, sometimes they are able to grow on blood agar in normal atmosphere, sometimes in *Gardnerella* agar in an incubator with elevated CO<sub>2</sub> concentration, and sometimes under anaerobic conditions only. Besides lactobacilli, normal finding may contain small amounts of staphylococci, *Enterobacteriaceae* and some other bacteria. Sometimes lactobacilli are absent, especially in swabs taken from women after climacterium.

Dental students do not perform this task practically.