

## P12 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<br>1 1 1  | požaduje<br>díl A   | IČP 7 2 1 2 3 4 5 6<br>Odbornost 7 8 9 | Datum<br>1 5 1 2 0 8 | Čís. dokladu     | Poř. č.  |
| <b>POUKAZ NA VYŠETŘENÍ / OŠETŘENÍ</b>  |                     |  |                      | IČP              |          |
| Pacient<br>Carolina Red  | Dg.: acute cystitis |  |                      | Odbornost        |          |
| Č. pojištěnce<br>*1952   | Variabilní symbol   |  |                      | Var. symbol      |          |
| Odeslán ad:  | Kód náhrady         |  |                      | Datum            | Kód Poč. |
| <b>Požadováno:</b>   |                     |  |                      | 1                |          |
| urine (commonly sampled) for bacteriological examination                       |                     |  |                      | 2                |          |
|  |                     |  |                      | 3                |          |
|  |                     |  |                      | 4                |          |
|  |                     |  |                      | 5                |          |
|  |                     |  |                      | 6                |          |
|  |                     |  |                      | 7                |          |
|  |                     |  |                      | 8                |          |
|  |                     |  |                      | 9                |          |
|  |                     |  |                      | 10               |          |
|  |                     |  |                      | 11               |          |
|  |                     |  |                      | 12               |          |
|  |                     |  |                      | 13               |          |
|  |                     |  |                      | 14               |          |
| <b>Poznámka:</b>   |                     |  |                      |                  |          |
| 72 Dr. Microbe Terrible<br>123 general practitioner<br>456 Compositive 8, Brno |                     |  |                      | Dne:             |          |
| VZP-06x1999  |                     |  |                      | 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 |        |        |        |

|  |                       |             |                |
|--|-----------------------|-------------|----------------|
| <b>Patient: Carolina Red *1952 Dg.: acute cystitis</b>         |                       |             |                |
| <b>Specimen: normal urine Ordered by: Dr. Microbe Terrible</b> |                       |             |                |
| Growth on Blood agar:  | Growth on Endo agar:  | Conclusion: | Interpretation |
| Quantity:  | Enterotest 16 result: |             |                |

Antibiotic susceptibility test

|                       |                  |  |                     |                  |  |
|-----------------------|------------------|--|---------------------|------------------|--|
| Ampicillin<br>AMP     | R < 14<br>S ≥ 14 |  | Tetracycline*<br>TE | R < 12<br>S ≥ 15 |  |
| Cephazolin<br>KZ      | R < 14<br>S ≥ 18 |  | Cefuroxime<br>CXM   | R < 18<br>S ≥ 18 |  |
| Co-trimoxazole<br>SXT | R < 13<br>S ≥ 16 |  | Norfloxacin<br>NOR  | R < 19<br>S ≥ 22 |  |
| Nitrofurantoin<br>F   | R < 11<br>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><br><i>Klebsiella pneumoniae</i>  | nitrofurantoin*<br>ofloxacin                    | amoxicillin<br>linezolid                                       |
| Asymptomatic bacteriuria (ABU) other situations                                      | <i>Streptococcus pyogenes</i><br><i>Enterococcus sp.</i>   | no therapy<br>nitrofurantoin                    | no therapy<br>cefuroxime                                       |
| Acute non-complicated cystitis (community cystitis, that means „not-nosokomial“ one) | <i>Clostridium sp.</i><br><i>Escherichia coli</i><br><i>Staphylococcus saprophyticus</i><br><i>Klebsiella pneumoniae</i> | ciprofloxacin<br>nitrofurantoin                 | co-trimoxazole<br>(co-)amoxicillin<br>vankomycin<br>cefuroxime |
| Accute pyelonephritis  | <i>Escherichia coli</i><br><i>Bacteroides fragilis</i><br><i>Klebsiella pneumoniae</i><br><i>Proteus sp.</i>             | (co-)amoxicilin<br>cefuroxime<br>nitrofurantoin | co-trimoxazole<br>ofloxacin<br>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**

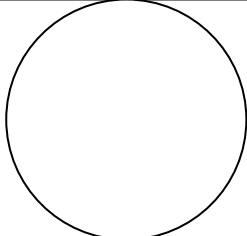
Find suitable swabs or other sampling methods for following clinical situations (suspicions for individual diseases). For some of them more than one sampling method is suitable. Use numbers 1 to 6 and mark your choice to individual situations. Correct yourself with help of your teacher.

- |                     |                          |
|---------------------|--------------------------|
| 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 following media:

- **blood agar** (for common bacterial pathogens)
- **Endo agar** (or McConkey agar)
- **Agar with 10 % NaCl** (for staphylococci)
- **Special blood agar variant for *Gardnerella vaginalis*** (GVA agar)
- **WCHA agar** (anaerobic culture) – only sometimes

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. In practice, it is possible to see three variants of the growth of these microbes:

- sometimes they are able to grow even on blood agar in the **normal atmosphere**
- sometimes they do not grow in aerobic conditions, but they grow on *Gardnerella* agar in an incubator with **elevated CO<sub>2</sub> concentration**
- sometimes they only grow on WCHA **in anaerobic conditions.**

So, be prepared to all possibilities and do not be surprised.

Besides lactobacilli, some other findings can be still considered “normal”, namely

- small amounts of coagulase-negative staphylococci
- small amounts of *Enterobacteriaceae*
- small amounts of anaerobic bacteria (if they are not very frequent and they smell is not that of big intensity, they are mostly considered “normal finding”)

On the other hand, sometimes lactobacilli are absent, especially in swabs taken from women after climacterium, or as a result of previous antibiotic treatment.

Write your findings and try to make a conclusion.

| Medium                             | Normal finding   | Possible pathogens   | My finding |
|------------------------------------|--|--|------------|
| Blood agar                         | Lactobacilli, small amounts of coagulase negative staphylococci, very small amounts of <i>Enterobacteriaceae</i> | <i>Staphylococcus aureus</i> , <i>Enterobacteriaceae</i> , <i>Streptococcus agalactiae</i> and many others |            |
| Endo agar                          | No growth, or just very small amounts of <i>Enterobacteriaceae</i>   | Mostly <i>Enterobacteriaceae</i>   |            |
| NaCl agar                          | No growth, or a staphylococcus later proven to be coagulase-negative species                                     | Mostly <i>Staphylococcus aureus</i>  |            |
| GVA agar                           | Like on blood agar (maybe different morphology of colonies)  | <i>Gardnerella</i> seen as very small colonies with partial haemolysis*                                    |            |
| WCHA agar                          | Like on blood agar, + even small amounts of anaerobic bacteria   | Anaerobic bacteria in huge amounts presenting unpleasant scent   |            |
| More tests<br>(only if performed): |  |  |            |

\*Compare with a positive control, if available

**Final conclusion:**

**In my “red box team” of letter \_\_\_\_\*, we have found**

- Normal flora only
- Normal flora with a patogen, namely \_\_\_\_\_

\*A, B, C, D, E, F, G or H