

#### Institute for Microbiology shows

#### TRACING THE PATHOGEN

Part twelve:

Cooperation at investigation or Clinical Microbiology III

#### List of topics

Infections of urinary tract – survey

Urine sampling

Possibilities for urine specimen processing

Result interpretation, prevention and treatment of UTI

Infections of genital tract – introduction

Sampling and examination of genital infections

## Urinary tract infections – survey

### Importance of urinary tract infections (UTI)

- Besides respiratory infections, this is another very important group of infections with economic losses and inconvenience for patients
- Dangerous is risk of complications for example, from cystitis and pyelonephritis may become the bearing may become the emergence of urosepsis, i. e. infection of the bloodstream
- IMC are very common, especially in women
- Causative agents are usually bacterial, antibiotic therapy is therefore often (though not always) indicated

#### Urinary tract of a healthy person

- Kidney without microbes normally
- Renal pelvis without microbes normally
- **Ureters** without microbes normally
- Bladder of young and middle aged people without microbes normally
- **Bladder of seniors** even under normal circumstances it can be settled in microbes, which does not make problems and becomes a "normal flora"
- **Urethra** normally without the microbes with the exception of the final part (bacteria from the skin + partly specific flora: viridans Streptococcus, aerococci, etc.)

#### Anatomic classification of UTI – 1

- Urethritis cases are rather connected with genital infections and are discussed in the context of this issue
- Cystitis are the most common UTIs, especially for women (they have a shorter urethra). They are often associated with situations where a stream of urine as a natural protection system is weaker, for example:
  - the pelvic floor disorders (typically in women after childbirth)
  - for hyperplasia of prostate (that is, by contrast, for women rare;-))
- Complications of cystitis may be pyelonephritis

### Clinical description of cystitis and pyelonephritis

- Burning during urination
- Frequent urination, small amounts of urine
- Sometimes urine is bloody and turbid
- Orientation strip test may show leucocytes and eventually also pH elevation
- In case of back pain and fever, it is usually not only cystitis, but pyelonephritis
- In case of pyelonephritis, the strip test would show also presence of proteins in the urine

#### Attention – cystitis symptoms are not specific

- Diseased urination (frequent urination, incontinence, burning) may have **another cause than cystitis**, which should be revealed or excluded
- It can be a **sexually transmitted disease** (*Chlamydia* or *Mycoplasma* infection, gonorrhoea)
- It can be a **non-infectious inflammation** (mechanical irritation of catheterization, etc.) or other non-infectious cause (even incipient tumour!)
- It could also be an inflammation of the walls of the bladder (e.g., infectious, parasitic/Schistosoma, as well as non-infectious)

In all these cases is finding in the urine culture is negative

#### Pyelonephritis

- Pyelonephritis is an inflammation of the renal pelvis, unlike glomerulonephritis.
  - Glomerulonephritis affects the glomeruli and is usually non-infectious; however, it may be an autoimmune origin after a streptococcal infection.
- It is more serious than cystitis, it does not only affect the lumen of the urinary tract, but kidney tissue, so antibiotic therapy should respect it
- Usually arises as a complication of cystitis, but the origin can also be haematogenous
- Recurrent pyelonefritis may also be complicated by urolithiasis (bladder stones)

# Urine sampling

#### **UTI** diagnostics

- Anamnesis, that might also include sexual life (gonorrhoea and other urethritis)
- Clinical examination
- Orientation diagnostic strip examination (the presence of bacteria in urine)
- Biochemical examination the presence of bacteria, proteins, etc.
- Microbiological testing is recommended for uncomplicated and necessary for complicated one (not speaking about pyelonephritis)

#### Sampling and transport of urine

- The most reliable urine is obtained by suprapubic puncture. In practice, however, rarely used
- Quite good is also catheterized urine (catheterization performed due to sampling)
- Commonly collected urine sample may not be bad, if it is properly sampled and transported
- Urine from permanent catheter is the worst of possible samples, sometimes, of course, there is nothing for us. Sometimes also urine from nephrostomy is used.

There is no reason to use cathethrized urine globally. It only makes sense when the outcome is repeatedly doubtful.

#### Permanent catheter urine sample

- If it is possible to wait a few days e. g. for the exchange of the catheter, it is better – the result of the newly exchanged a catheter will be far better (but it is advisable to wait after replacing some time until the colonizing bacteria from the old catheter are washed away)
- If we cannot wait, we sample the urine, but we must reckon with the fact that the interpretation is ambiguous (inflammation and not just the colonization is likely in case of leucocytes present in the urine)
- We must think whether we are considering treatment with antibiotics at all; If not (asymptomatic bacteriuria) the examination looks to be useless
- Microbiological examination of the catheter itself, sent to a laboratory, is not recommended (nearly impossible interpretation), although some laboratories perform it

#### Sampling of spontaneously urinated urine

- It is the sampling from the middle stream of urine spontaneously urinated (routine type with secondary risk of contamination during sampling)
- Technique: The container for the collection of urine must be sterile, wide-neck (e.g. a beaker\*), knowledgeable patient thoroughly washed prior to the collection's external genitals with soap and water and (possibly) wipe off the outer estuary of the urethra's swab moistened with disinfectant in solution (especially in children, however, the use of disinfectant is not recommended)

\*this is as it is written in the official recommendations, in practice, it depends on the situation; If the patient urinates directly into a test tube, it is better

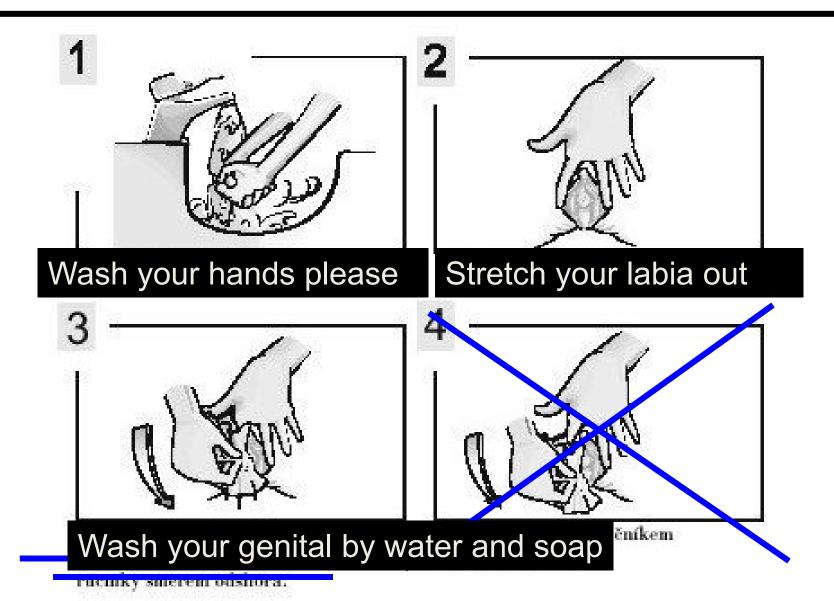
#### Sampling in males and females

- Males by one circulating movement
- Females should be in wide position over the toilet, they stretch out the labia by one hand and they wash the genital by a tampon using the other hand from front to back.
- •After that, the patient urinates the first part of urine and the midstream is taken into sterile vessel without breaking the urination. Sampled urine is placed into a sterile container for transport.

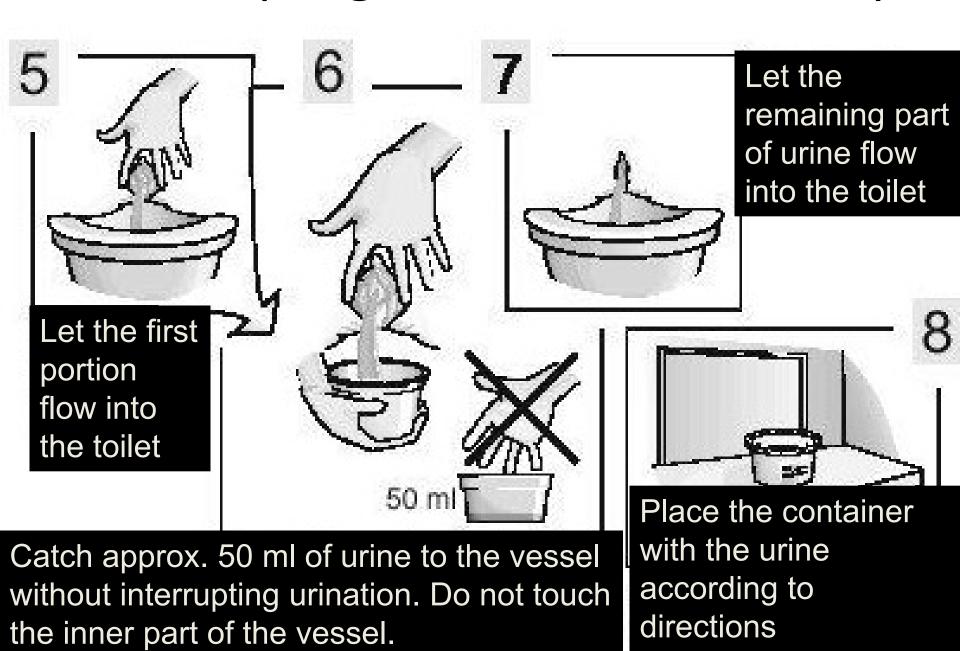
(Czech Medical Society of John Evangelist Purkyně, RECOMMENDED GUIDLINES FOR GENERAL PRACTICIONERS, Project of Healthcare Ministry of Czechia by help of a grant IGA MZ ČR 5390-3)

#### Urine sampling in a female – technique

http://www.lab-turnov.ic.cz/schema\_1.php

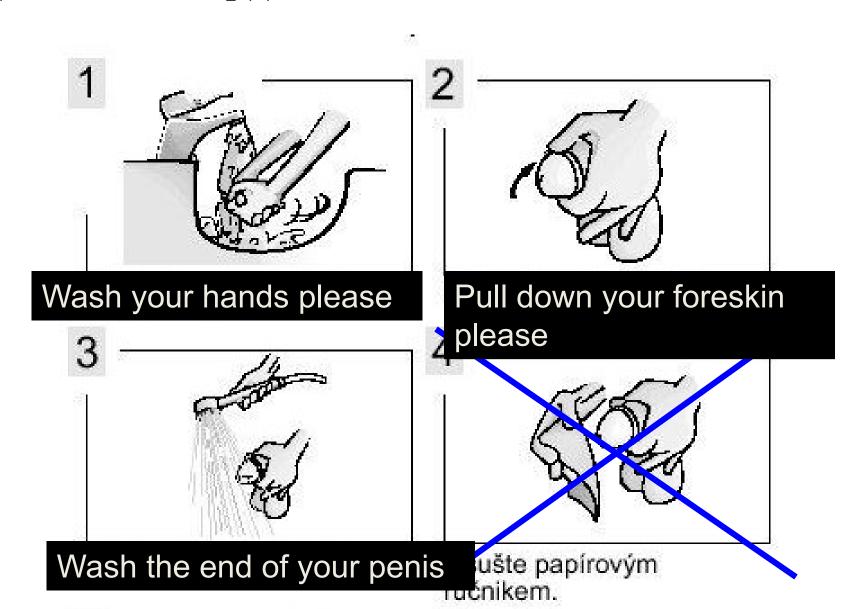


#### Urine sampling in a female – technique



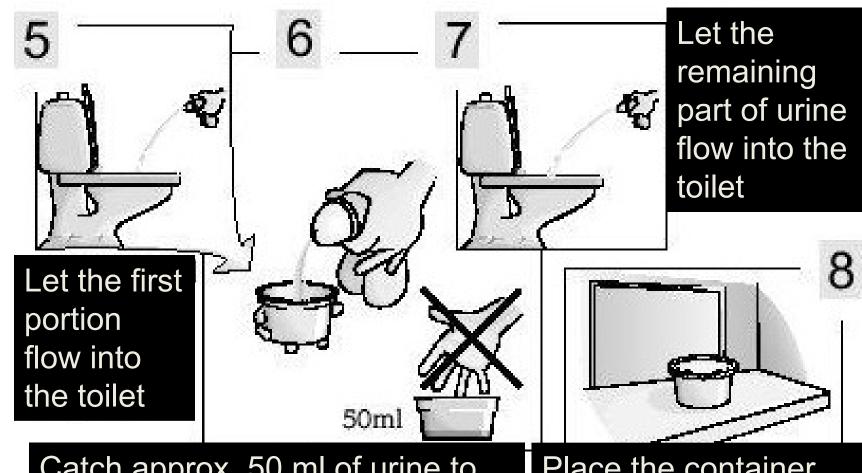
#### Urine sampling in a male – technique

http://www.lab-turnov.ic.cz/schema\_2.php



#### Urine sampling in a female – technique

http://www.lab-turnov.ic.cz/schema\_2.php



Catch approx. 50 ml of urine to the vessel without interrupting urination. Do not touch the inner part of the vessel. Place the container with the urine according to directions

#### Exceptions of urine sampling rules

- In suspicion for urethritis we take first portion of urine (we wash out the microbes from urethra).
- In prostatitis we rather use last portion of urine
- For schistosomosis we collect last portions of urine several time, at least 20 ml needed. (In the laboratory, the urine will let settle and then we look for the eggs of the parasite in the sediment on the bottom). Transport should be quick.

### Sampling in small children and catheterized patients

- In children
  - Urine is obtained by collecting the bags tightly
  - Method is burdened with a relatively high risk of secondary contamination
  - The bag should not be attached more than 30 minutes
  - It should be removed immediately after the pee
- In catheterized patients we should reckon with the fact that any result is indicative for the colonization of the catheter, rather than for the infection. The sampling must be carried out so as to minimize the risk of further contamination

#### Urine transport

- For evaluation of an UTI, the quantity is important see further. The quantity can only be evaluate if the microbes would not multiply in the urine during the transport. If they do, the quantity is changed
- Therefore, it is essential to return to the urine laboratory within two hours after sampling (or even faster)
- If, exceptionally, this cannot be fully met, storage in a refrigerator should be used (unlike for other specimens)

#### **URICULT** type devices

- The purpose of these units is to totally eliminate the time lag between the collection of urine and the beginning of cultivation. The urine is sampled and a special tool with cultivation media is placed in it. Then the urine is removed and the media start to be cultured (sometimes without sending to the lab, if a small incubator is available for it)
- On these plates, however, the microbes are difficult to diagnose. This method is therefore doesn't apply, as it was originally expected. In its use of large regional differences.
- If it is used, it is necessary to strictly follow the correct procedure

#### How to use an URICULT



- The cap with culture media should be screwed out carefully (to let the cap in the air during sampling)
- Urine midstream is used for filling in the Uricult container to 3/4 (directly or from a sterile container).
- The device with cultivation media is placed into the urine in the container
- After several seconds the device is removed
- Excess urine is left to drain on the bottom of the plates, then sucked out with filter paper (do not touch the media)
- The urine is removed from the vessel including the remaining drops
- Exceptionally it is possible to perform the sampling by placing the both sides of the media in the stream of urine

# Urine specimen processing

### Qualitative and semiquantitative urine examination

- When a quantitative examination of the urine is diluted and given on a few of the culture media.
- At semiquantitative examination, urine is not diluted, but a calibrated single use loop on is used. The examination is less laborious, but also less accurate.
- Of course, not only quantity is assessed, but also the normal way to diagnose the microbe is used.

#### Semiquantitative processing 1

- A calibrated plastic loop for 1 μl is used
- That means that when it is placed into the water or a liquid with similar surface tension just one microlitre is kept in the "eye" of the loop
- This microlitre is inoculated to one half of blood agar plate (in practical session: on a total plate)
- After that it is normally incubated (24 h, 37 °C)
- The other day colonies are counted. According to the number of colonies the result is interpreted
- In our laboratory we use now chromogenic medium instead of formerly used Endo or McConkey agar

#### Bacteria on a chromogenic medium



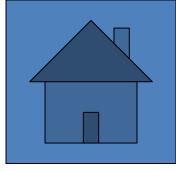
#### Semiquantitative processing I

- Number of colonies after incubation corresponds to the number of CFUs in 1 µl of original urine
  - CFU = colony forming unit: one microbe, a pair, a short chain, a small group. In practice we neglect the difference between a microbe and a CFU, so we say that we count microbes when we really count CFUs
- If the number of colonies approximately corresponds the number of microbes in 1 μl of original urine, then the number of colonies × 1000 corresponds the number of microbes in 1 ml of original urine. 10 colonies 10<sup>4</sup> microbes in one millilitre, 100 colonies 10<sup>5</sup> microbes/ml

#### Automated culture systems

- Some companies now offer automated culture systems, which detect positivity already after four hours and they even refer the antibiotic sensitivity (Italian system UroQuick). Some, especially the private laboratory systems welcome it and base their microbiological examination of urine on this system.
- However, this approach is very risky, because the determination of the antibiotic susceptibility testing without specifying the type of bacteria is very treacherous. If there is such a system combined with the classic diagnosis, the damage is not necessary. However, it is unacceptable to use such a system without its results being interpreted the microbiologist (e.g. location of the instrument into biochemical laboratories).

### Urine Basic diagnostic schedule



- Day 0: start of culture only
- Day 1: result of primary culture of specimen on BA, EA/URI, expedition of all negative results, pathogen testing
- Day 2: expedition of positive results, if bacterial susceptibility is sufficient (if not, → more tests)
- Day 3: expedition of remaining results

### Result interpretation, prevention and treatment of UTIs

#### Urine – result interpretation

- There is no common flora, nevertheless, in elderly often asymptomatic bacteriuria (ABU), it is not necessary to treat it
- Differentiation of contamination, but also colonisation (especially in cathethrized patients) is often very difficult, often possible only based on clinical situation (the microbiology finding itself is not sufficient)
- Among pathogens, the most common is Escherichia coli, more Enterobacteriaceae, yeasts, enterococci, Streptococcus agalactiae, Staphylococcus saprophyticus etc

#### Interpretation of urine examination I

- When we find one microbe, it is valid that
- Quantity over 10<sup>5</sup> microbes in 1 ml is considered likely uroinfection. In elderly it nevertheless might be a colonization
- Quantity 10⁴-10⁵ is borderline. It is not sure whether the specimen was taken properly (e. g. in babies) it is rather considered a contamination. It is rather important in men and children. Antibiotic susceptibility is nevertheless tested
- Quantity < 10<sup>4</sup> is considered a contamination
- Not valid for punctured and cathethrized urine

### Semiquantitative urine evaluation at finding one microbe

Number of colonies	Number of CFU (bacteria) in 1 µl of urine	Number of CFU (bacteria) in 1 ml of urine	Evaluation (valid for 1 bacterium)
Less than 10	Less than 10	Less than 10 <sup>4</sup>	Contamination
10–100	10–100	10 <sup>4</sup> –10 <sup>5</sup>	Borderline
More than 100	More than 100	More than 10 <sup>5</sup>	Infection

#### Interpretation of urine examination II

- In case of finding two microbial species approx.:
- Quantity < 10<sup>5</sup> is evident contamination
- Quantity > 10<sup>5</sup> is borderline (unsure)
- In case of finding three microbial species:
- Nearly always we take it as contamination
- Exception: one microbe in quantity >  $10^5$ , two other microbes <  $10^4$  → first microbe is considered pathogen, the other two contamination
- In practice we also take into account what species of microbes do we have (staphylococci use to be taken less seriously)

#### Asymptomatic bacteriuria (ABU)

- Only real actual infection that is causing the problem should be treated – not the mere presence of bacteria in urine (particularly in older people),
- However, there may be exceptions:
  - pregnant women we treat even ABU because urinary infection can become a focus of a vaginal infection → infection during delivery
  - or some other risk situations, e. g. a person with immunodeficiency; here also that bacteria might be the source of infection of other organs

#### Treatment of UTI

- For uncomplicated community (= not hospital) cystitis sometimes plant extracts (cranberries) are sufficient.
- As to antibiotics, for cystitis suitable nitrofurantoin is suitable (does not concentrate in the blood, but in the urine). Another option is to co-trimoxazole, amoxicillin, second generation cephalosporins, doxycycline, and more
- For hospital cystitis treatment should be chosen according to the susceptibility (but this is useful even for outpatients)
- For pyelonefritis (inflammation of the renal pelvis) the antibiotics must penetrate not only into the urine, but also into the renal tissue. Nitrofurantoin or norfloxacin is therefore not applicable. Targeted treatment for the causative agent is used.

#### Prevention of UTIs

#### Very effective preventive techniques:

- To urinate after sexual intercourse (especially in women)
- To prefer hormonal contraception to barrier methods
- Change frequently menstrual devices
- Do not use spermicide gels, creams, gels and perfumed napkins

#### Completely wrong and dangerous techniques:

- Excessive hygiene
- Overuse of so called disinfection gels and soaps
- Frequent bathing in bathing foams

According to the "Recommended guideline for antibiotic treatment of community infections of kidneys and urinary tract infections in the primary care"



# Infection of reproductive

organs –

introduction

#### Importance of this group of infections

- Infections of sexual organs are also quite frequent infections
- The problem is that it is difficult to assess how frequent they really are. Ill people often try selftreatment and remain hidden to the healthcare, because they are ashamed and they have shame to speak about it (including with a doctor)
- Another problem is difficult implementation of effective measures for treatment and prevention.
   Also in diseases where sexual intercourse does not play the main role (e. g. vaginal mycoses) it is useful to treat both (all) partners

#### Normal situation of genital organs

- In normal situation there are no microbes:
  - In females in uterus, tubas, ovarias
  - In males in prostate, ducts, testes
- Specific normal flora is in vagina (lactobacilli, some more aerobic and anaerobic microbes).
   Also microflora of distal part of urethra is partially specific
- Vulva is the borderline between vaginal and skin flora
- In males also prepuce bag is specific is specific, besides skin flora there are also e. g. nonpathogenic mycobacteria etc.

#### Classification of sexual infections

- The classic sexual diseases are transmitted almost exclusively by sexual way. They are a subject of registration and reporting under the special laws. For us, this includes primarily gonorrhoea and syphilis
- Other infections of genital organs are those that affect the sexual organs, but sexual transmission is not the only or even the most important
- There also exist infections transmitted sexually, but not affecting directly sexual organs (hepatitis B, AIDS, etc.)

There exists the term "sexually transmitted infections" – STI (formerly STD – sexually transmitted diseases). The content of the term is rather changeable by its user.

#### Classical sexual diseases

Gonorrhoea  Syphilis (lues)	Neisseria gonorrhoeae ("gonococcus") Treponema pallidum	Common also in Europe
Lymfogranuloma venereum	Chlamydia trachomatis serotypes L <sub>1</sub> , L <sub>2</sub> , L <sub>3</sub>	Exceptionally also in Europe
Chancroid (ulcus molle)	Haemophilus ducreyi	In Europe
Granuloma inguinale	Klebsiella (ex: Calymmatobacterium) granulomatis	rare, from other countries

## Other agents of sexual infections – 1

- Human papillomavirus (related to cervical carcinoma – almost types 16 and 18, other types – causing condyllomata acuminata etc.)
- Herpes simplex virus type 2, eventually also type 1
- Virus of molluscum contagiosum
- Chlamydia trachomatis serotypes D to K
- *Ureaplasma urealyticum, Mycoplasma hominis* and more urogenital mycoplasms
- Gardnerella vaginalis, Mobilluncus mulieris,
   anaerobic bacteria (bacterial vaginosis more later)

## Other agents of sexual infections— 2

- Enterobacteriaceae, streptococci, enterococci, staphylococci and more agents of so called areobic vaginitis
- Yeasts especially of genus Candida
- Trichomonas vaginalis
- Pubic lice also maybe classified here, although directly reproductive organs are not attacked

#### Interpretation of "positive" findings

- Like the other places with normal microflora (intestine, the oral cavity) vagina can also be considered an ecosystem. Its stability is influenced both by microbes and the host-side factors
- In many cases the culture positivity itself is no reason to treatment, what's important is the clinical context. This concerns in particular the anaerobic bacteria, gardnerel, urogenital species of *Mycoplasma* and *Chlamydia*
- Microscopy is often useful for the interpretation.
   Unlike the culture we see ratios of bacteria

#### Bacterial vaginosis (BV)

- Bacterial vaginosis is a condition where the normal vaginal flora in the vagina is diseased and vagina contents larger amounts of Gardnerella, Mobiluncus, and anaerobic bacteria. All of them may be in the vagina also normally, but usually not so many
- We can't determine a unique causative agent
- Almost no leucocytes are present. Some bacteria blocate their migration to the inflammation site. On the other hand, In microscopy we see epithelial cells covered with bacteria – clue cells
- Treatment: metronidazole, probiotics

#### Nugent scoring system

- Some laboratories use microscopy of vaginal smear for counting of so called Nugent score. Score over 7 is nearly sure for vaginosis
- Nugent score is based on "morphotypes" = "bacteria that look like…"
  - there exist three typical morphotypes that are counted:
    - Gardnerella/Bacteroides morphotype (slender, rather Gram-negative or Gram-variable straight rods)
    - Mobilluncus morphotype (slender curved rods)
    - Lactobacillus morphotype (long, robust, rather Gram-positive rods) unlike the previous two, the presence of this morphotype speaks against and not for the diagnosis of bacterial vaginosis

Note: Nugent scoring systém can only show us cases of classical bacterial vaginosis. Nevertheless, there also exist cases of a simillar disorder, but just different clones of lactobacilli (and not Gardnerella etc.) present. Obviously, in these cases the Nugent score is low, and such a case cannot be distinguished by microscopy.

## Nugent score counting in the original article

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Nugent, R. P., M. A. Krohn, and S. L. Hillier. 1991. Reliability of diagnosing bacterial vaginosis is improved by a standardized method of Gram stain interpretation. J. Clin. Microbiol. 29:297-301

TABLE 1. Scoring system (0 to 10) for Gram-stained vaginal smears<sup>a</sup>

Score*	Lactobacillus morphotypes	Gardnerella and Bacteroides spp. morphotypes	Curved gram- variable rods
0	4+	0	0
1	3+	1+	1+ or 2+
2	2+	2+	3+ or 4+
3	1+	3+	
4	0	4+	

<sup>&</sup>quot;Morphotypes are scored as the average number seen per oil immersion field. Note that less weight is given to curved gram-variable rods. Total score = lactobacilli + G. vaginalis and Bacteroides spp. + curved rods.

was unaware of the clinical or microbiological findings for

<sup>&</sup>quot;0, No morphotypes present; 1, <1 morphotype present; 2, 1 to 4 morphotypes present; 3, 5 to 30 morphotypes present; 4, 30 or more morphotypes present.

#### Simplified counting "+" to "++++"

- + = not in every field
- ++ = in every field, but not frequent
- +++ = in every field, frequent
- ++++ = very frequent, first object you
   see when looking to the microscope

#### Nugent score counting

Due to the fact that it is a microscopic and not the culture proof, we work with so-called morphotypes. For example, bacteria belonging to the "morphotype Lactobacillus" may no Lactobacillus, but it is very likely

- Morphotype Gardnerella/Bacteroides: not present = zero point, + = one point, ++ = two points, +++ = three points, ++++ = four points
- Morphotype Lactobacillus: the contrary: not present = four points, positivity ++++ = zero points
- Curvet Gram-labile rods: none = 0 points, + or ++ =
   one point, +++ or ++++ = two points

#### Aerobic vaginitis (AV)



- Besides bacterial vaginosis there also exists classical (i.e., containing leucocytes) bacterial vaginitis (colpitis; however, the concept of vaginitis, an incorrect combination of Latin and Greek, unfortunately took and used)
- However, it is very difficult to distinguish the agent of the inflammation from the accidental discovery or colonization of the vagina
- Most commonly we find Enterobacteriaceae, enterococci,
   Streptococcus agalactiae, Staphylococcus aureus
- Treatment depends on the presence of symptoms, with the exception of Streptococcus agalactiae (outside of pregnancy is recommended rather woman healing due to the transfer to the newborn; in pregnancy itself we do not treat, but the delivery is protected)

## Sampling and examination in genital infections

### Possible specimens in genital infections – anatomic classification

- Vaginal swab usually from the rear side of vagina, using gynaecologic mirrors, must not be contaminated by the microbial flora of the vulva
- Cervical swab also using gynaecological mirrors
- Urethral swabs in both genders
- Swab from penis, prepuce, glans in men
- Ejaculate (or swab from ejaculate)
- Swab from labia in females
- Invasive specimens (content of cyst etc.)

#### Possible specimens in genital infections

- according to the causative agents
- Amies swab for aerobic bacteria, *Gardnerella*, anaerobic bacteria, event. also urogenital mycoplasms (some laboratories use special media for mycoplasms)
- Dry swab is almost used for non-cultivation detection of antigens and DNA, i. e. in chlamydias, papillomaviruses etc.; if we wish to have a specimen from deeper layers of the mucosa, we would use a brush
- E-swab may eventually replace both previous swabs (as the producer says, it enables both culture and PCR)
- C. A. T. swab is for yeasts and *Trichomonas*
- Smears may be sometimes very useful
- Clotted blood is used for antibody detections (e. g. syphilis)

#### Smears from vagina or urethra

- It is a situation where the clinician directly makes a smear of secretions on the slide. Caution – If the slide is not sterile, the swab should not be used for culture
- We evaluate both quantity of individual formations, and also final appearance of the preparation. According to communication, it is eventually possible to coung Nugent score of BV
- In gonorrhoea we rather send urethral and cervical swabs, an usually only one slide to Gram stain.
   More in the material to Neisseria

## Classical "mikrobial appearance of vaginal microflora" (MAVM)

- A classic, recently already less commonly used variant of microscopy in gynaecology used to be microbial appearance of vaginal microflora
  - They used to send two slides with vaginal smears
  - On was Gram stained for bacteria, but also epithelial cells, WBC-s, yeast cells etc., the other one was Giemsa stained (mainly for *Trichomonas*)
  - Indivitual formations were evaluated (+ to ++++) and finally all smear was graded as a Roman number I to VI (sometimes with sub-types, e. g. IIa<sub>2</sub>)
- Following pictures show some possibilities of vaginal smear results

