

Institute for microbiology shows

TRACING THE CRIMINAL



Part twelve:

Cooperation at investigation or
Clinical Microbiology I

Institute of Microbiology shows

TRACING THE CRIMINAL

Part thirteen:

Cooperation at searching, or
Clinical microbiology II



Introduction (material comes on Christmass, too 😊)



Survey of topics

Introduction to clinical microbiology

1 Indication

2 Sampling + 3 Transportation

2A Sampling: Blood cultures

2B Sampling: Urine samples

2C Sampling: Other examples

4 Decision how to process 5 Proper processing

4A Processing and „reading“ in respiratory specimens

4B Processing and „reading“ in wounds & urine spec.

6 Result sending 7 Interpretation

Introduction to clinical microbiology

Story One – 1

- Peter was coughing all the time, so he visited a doctor. The doctor wanted to prescribe antibiotics directly, but then he remembered, that microbiologists told him to perform examinations. So he performed a throat swab. In the swab, *Haemophilus influenzae* was found, susceptible to cefuroxim. Peter started to use ZINNAT (a drug that contains cefuroxim).

Story One – 2

- Peter, though, was no better. He became angry and visited a doctor on a pulmonary clinic. Here, serology of respiratory viruses was performed and high titers of antibodies against *Mycoplasma pneumoniae* found. Peter started to use SUMAMED (Azithromycin) and soon his status became much better.

The problem was caused not only by *Mycoplasma pneumoniae*, but

- also by GP X. Y., because:
 - he was right in remembering, that it is mostly useful to know the pathogen and antibiotic susceptibility before treatment
 - nevertheless, he made a mistake in decision what microbiological method is indicated in this case
 - a specimen of sputum had to be sent, and at negativity of culture (or at indicia showing rather an atypical pneumonia than a classic one) eventually clotted blood for serology of respiratory viruses (this examination contains also some non-viral agents, namely *Mycoplasma* and *Chlamydia*)
- Remark, that sometimes macrolides are the good solution (although usually I am rather fighter against their abuse)

Story Two

- Nicol felt sore throat, and so she visited a doctor. Throat swab was performed, but **only common flora was found**. Doctor was surprised, elevated polymorphonuclears and CRP **showed that it should be a bacterial pyogene infection**.
- Doctor knew Nicol and knew that she had more sexual partners. After a direct question, she admitted that she had performed oral sex with a risky partner. **A new swab was performed, now with notice „gonorrhoea examination“**. And he was not mistaken.

Who was guilty? Only *Neisseria gonorrhoeae*

- General practitioner worked very good; gonococcal pharyngitis is not so common that it would be routinely examined. But he was good, that he found it after the primary negative examination.
- Doctor was clever – he knew that **each specimen type has its routine laboratory schedule**. This schedule is used always when there are no special requests. Special requests should be written on laboratory request.

Clinical microbiology – what is it?

- **Clinical microbiology „sensu lato“** is medical microbiology – so the part of microbiology, that describes microbial flora of human and human pathogens
- **Clinical microbiology „sensu stricto“** describes proper processes between clinical workplace and the laboratory, including organisation of proper laboratory examination

Process of clinical examination – everything matters!!!

CLINICIAN

Indication: to do it? what type?

Proper sampling of material

Material transport

P 13

Result sending

Interpretation in context of other results and patient status (to treat **patient**, not lab finding)

LABORATORY

P 12

Decision how to elaborate

Proper material elaboration



1 Indication

1A Indication – WHETHER to do anything

- The main key to success is to ask how will be my action changed in relation with examination result.
- When i see that not regarding the result my further relation to the patient will be the same, the examination is probably useless
- This is not valid in epidemiologic indications and in prophylactic indications (like screening of microbial colonisation in serious patients)

1B Indication – WHAT to do

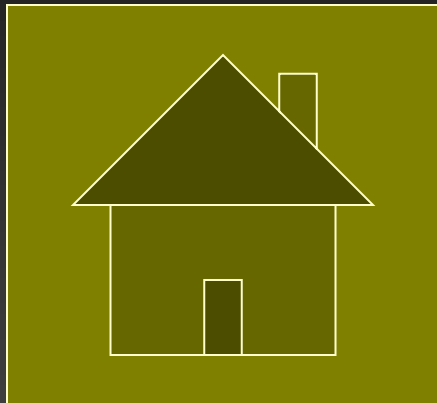
- Decision that „I want to perform an examination“ is not the end of everything. I have to think about **what examination should be done.**
- I have to know **pathogens spectre and methods of their examination**
- One part of that is also decision about how to perform sampling technically (including: what vessel or sampling kit should be used)

Three types of pathogens (1)

- Pathogen type *Streptococcus pyogenes*. It is not necessary to know that I mean just THIS pathogen, but I have to know pretty well where it is supposed to be localised (throat, lungs...)
- Pathogen type *Mycobacterium tuberculosis*. I have to know where the pathogen is localised, but also to know what group of pathogen is searched – so I have to write it to the request form
- Pathogen type *Toxoplasma gondii*. It is not necessary to know where in the body the pathogen is placed, but I have to know that I search for THIS pathogen.

Three types of pathogens(2)

- Pathogen type *Streptococcus pyogenes*. Bacteria and yeasts that can be cultivated, so majority of microbes from P01 to P06 and partially also P10
- Pathogen type *Mycobacterium tuberculosis*. It is still direct diagnosis, but special methods, the agent cannot be caught at normal culture. Mostly microbes from P07, P08, J13, part of P06 (gonorrhoea).
- Pathogen type *Toxoplasma gondii*. Indirect diagnostics, eventually direct diagnostics of viral antigen. Spirochets of P09, viruses from J11 + J12, but also many others (for example just *Toxoplasma*)



2 Sampling

(including order form)

3 Transportation

2 Proper sampling

3 Sample transport to the laboratory

- These phases cannot be divided – sampling should be performed with regard to material transport to the laboratory
- There are three types of samples:
 - Cotton swabs on a plastic stick or wire
 - Liquid and solid specimens sent in vessels (mostly sterile vessels)
 - Other and special cases, see later
- Proper filling of the request, sent together with the sample, is very important too!

Swabs



Other types of material than „swabs“ and „vessels“

- **smear on a slide:** gonorrhoea, actinomycosis, directly sent thick drop and thin smear etc.
- **in dermatology and epidemiology** just the culture medium that is filled to the margin of the dish; **in surgery moulage with a filtration paper**
- **uricult** – special way of urine sending just cultured on a medium; for many reasons, it is not very common.
- **quick diagnostic sets**, mostly based on direct antigen detection; simple manipulation, available even for non-microbiological personel. In case of doubts about the result it is necessary to use classical sending to the lab.

Election: How to sample?

Liquid sample, or swab?

- Usually, sending solid/liquid sample is preferred in comparison with sending swab
- Though, there are **many exceptions**, e. g.
 - in bacteriology usually **rectal swab is sent and not stool** (although it is not a mistake to send stool)
 - **urethral swab** in gonorrhoea is recommended rather than urine sending

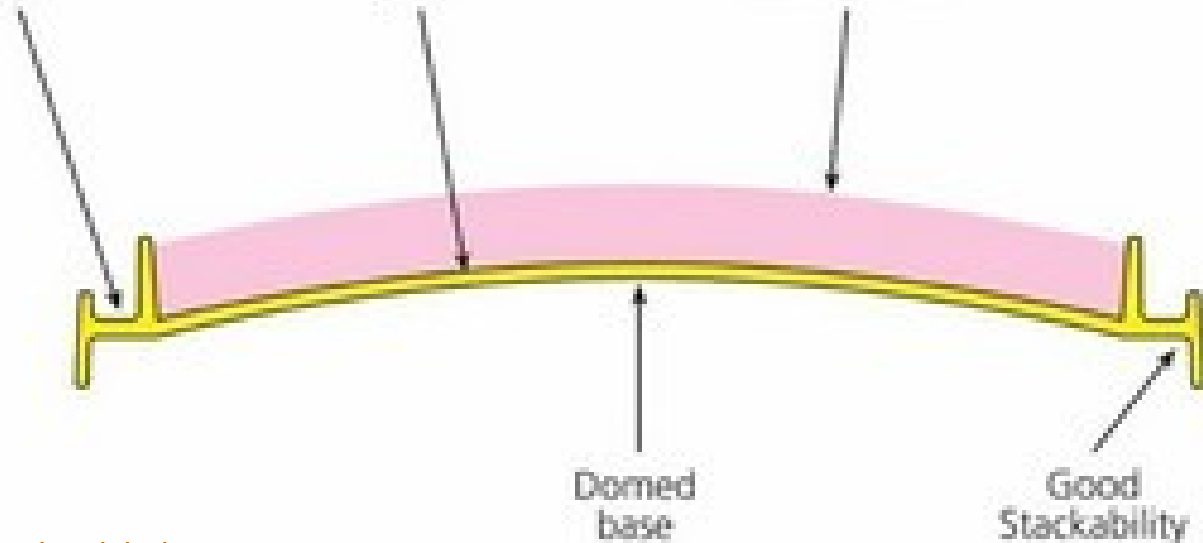
Contact plate



Triple vented

Numbered grid

25cm² surface area



Uricult

www.mediost.com



Some types of swabs



Plain (dry) swab www.calgarylabservices.com

Today its use is for PCR and antigene detection only, not for culture!

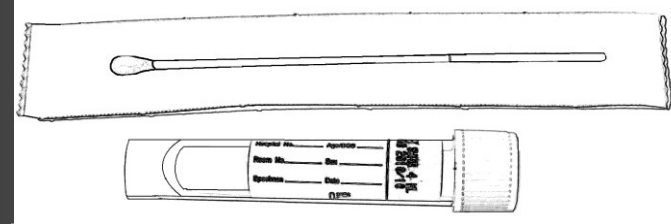


Amies medium with charcoal www.herenz.de

Universal transport medium for bacteriology (all types of swabs). The wire variant important, if we want to go „behind the corner“

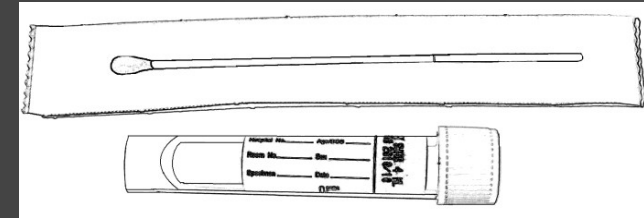
More swabs

Fungi Quick (for yeast and molds)

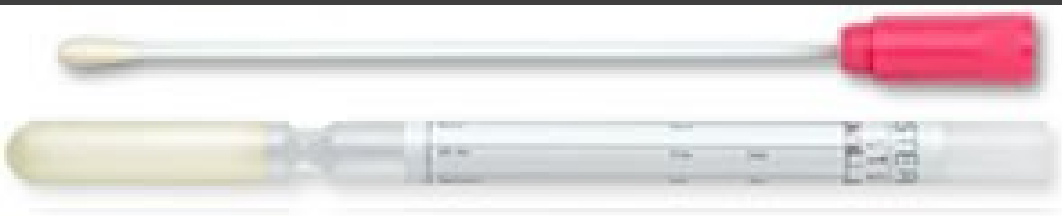


www.copanswabs.com

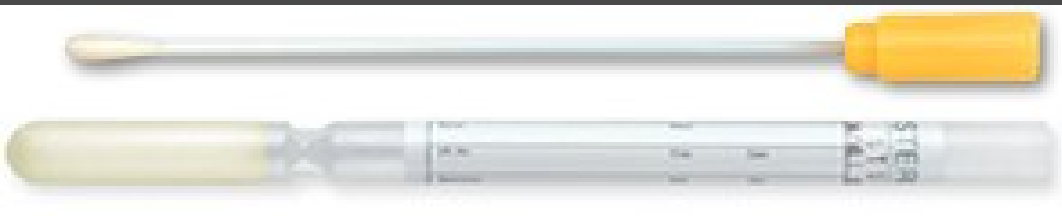
C. A. T. swab (for Candida And Trichomonas,
from genitals only)



www.copanswabs.com



Virus swab www.copanswabs.com



Chlamydia swab

www.copanswabs.com

Survey of swabs

Dry swab on a stick:
search for antigen and
DNA

Dry swab on a wire: the
same, if I need to get to
an inaccessible place

Swab in Amies medium
on a stick: universal for
bakteriological culture
(incl. anaerobes,
gonorrhoea, campylob.)

Swab in Amies medium
on a wire: the same, if I
need to get to an
inaccessible place

Fungiquick – fungi

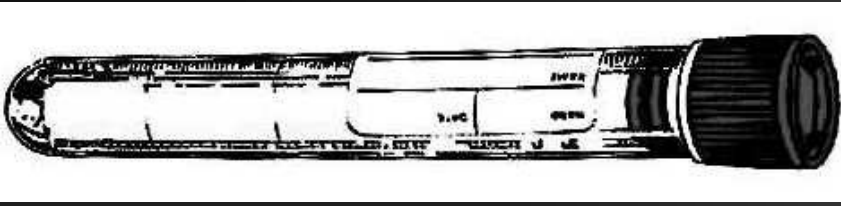
C. A. T. – fungi and trichomonas (genital swabs)

Samples with medium for viruses, event. chlamydiae

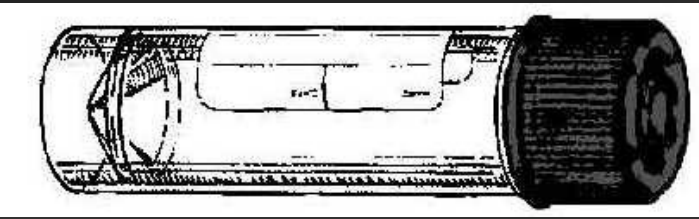
Vessels

- Sampling vessels are used for **solid and liquid samples**. In fact, size is not so important, also colour of the cap has no real importance. Nevertheless, sometimes laboratory wants e. g. yellow cap for urine, red cap for blood (to simplify sample classification); if so, it is necessary to accept it
- In anaerobic culture it is better to send just a syringe with needle stucked into a sterile rubber cap
- Specimens should be transported to the laboratory **as soon as possible**, but the most important situation is in urine sampling; here, 2 h is maximum transport time!

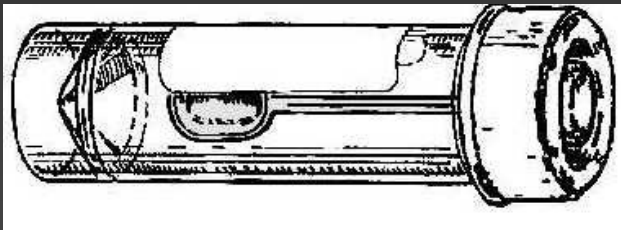
Vessels



Common test tube. Universal use: clotted blood (serology), urine, CSF, pus, punctate etc.; blood and urinary catheters, parts of tissue...



Sputum vessel. Not only for sputum, but also larger parts of tissue etc.



Stool vessel, for parasitology. Only this one does not have to be sterile!



Vessel for urine sampling. It is better, if the patient urinates just into a test-tube, but especially for women this is difficult (except if they are in shower). So they can urinate into this vessel, and then a nurse removes the urine into a test-tube.

Various vessels



Order form 1

- Properly filled order form is very important!
- Identification data: for identification, payment, for knowing, whom to send the result etc.
- Precise description of material and requested examination
 - do not write only „swab“ without adding more
 - even „wound swab“ is not enough (what type of wound, where is its localisation)
 - Catheterized urine × urine from permanent catheter
 - write, whether e. g. anaerobes are requested
 - not to request examination that is not available or is useless (e. g. cultivation examination of syphilis)

Order form 2 – what to write in

- **real diagnosis**, in case of more diagnoses, write all of them, or the one related with examination /e. g. (1) diabetes mellitus, (2) vaginal discharge/
- **acute / chronical status / control after treatment**
- to add present or planned **antibiotic therapy**, eventually allergy to antibiotics

Order form 3 – what to add more

- **traveller anamnesis** – tropical countries etc.
- **professional anamnesis** – job in agriculture etc.
- in **serological examination** date of first symptoms, first / second specimen
- in **gynecological materials** phase of menstruation cycle (and rather not to sample during menses)
- in case of **irregular samples** to consult it telephonically

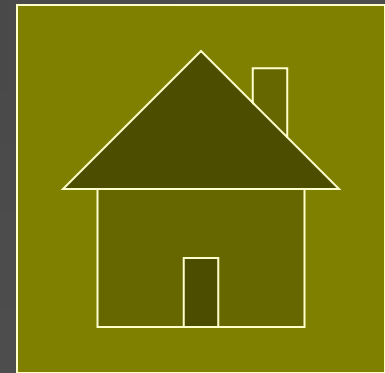
Order form filling – conclusion

We should not forget to fill in all important parts of the order form:

- **fields describing the patient** (name, date of birth/birth number, insurance, ward, diagnosis...)
- **fields describing the sample** (type of specimen, localisation, important circumstances)
- and all **other important parts** (especially anamnesis)

Mistakes in order form filling

- A common type of mistake is an insufficient description of sample type
- It is also bad to order examinations that are not suitable for the given situation (for example, search for antibodies in a pathogen, where cellular immunity is leading and antibody search does not have any importance)



2A Sampling: blood cultures

Basic terms concerning septicaemiae

- **Sepsis/septicaemia** is a status, where bacteria caused bloodstream infection with fever, metabolic failure and other **clinical symptoms**
- **Bacter(i)aemia** is any presence of bacteria in blood, even a transitory one, that has no meaning for the organism
- **Pseudobacter(i)aemia** is a situation, when bacteria only seem to be present in blood (badly performed blood examination, usually skin contamination).

Types of septicaemia

- **Primary sepsis** – some bacteria do sepsis „normally“, e. g. typhoid fever salmonellae or partially also meningococci
- **Secondary sepsis** – sepsis coming after failure of an organ
- **Special types of sepsis**
 - urosepsis – sepsis in kidney failure
 - catheter sepsis as hospital disease

Sepsis – clinical picture

- instable body temperature
- decreased muscle tonus
- intolerance of food, diarrhoea
- respiratory problems – frequent, irregular breathing, breath pause, failure
- blood circulation problems – pulse more or less frequent, blood pressure decrease
- common icterus, hyper/hypoglykaemia, metabolic failure, bleeding, neural symptoms etc.

Definition of a blood culture


- It means **not clotted blood**, principally very different from serological examinations
- Today we usually sample into **special vessels** for automatic culture
- We need to take **two, but better three blood cultures** at rise of temperature
- Ideal is **to use a new puncture every time**, or at least one venepuncture + central venous catheter + peripheral venous catheter (bacteraemia × colonisation of entry)

How to take blood

- **To work aseptically!** Not only because of the patient, but also because of the sample. It is not sufficient to clean skin by petrol, it is necessary to perform really disinfection.
- **The disinfection should be let to act long enough**, in alcohol agents to drying (to let the disinfectant really dry)
- **The best is to use 3 blood culture vessels of the same type**. Eventually to add e. g. one anaerobic
- **To fill the order form**, not forgetting the time of taking blood and site of sampling (CVK/peripheral catheter/venepuncture)

Types of culture vessels

- There are **various types** regarding to microbes that are to be detected (aerobes, anaerobes)
- **Some vessels („FAN“)** include charcoal. They are designed for culturing blood of patients already treated by antibiotics (classical vessel could give a false negative result – the antibiotic would suppress the growth)



Standard
aerobic

Charcoal
anaerobic

Charcoal
aerobic

Bact/ALERT[®] SA

Aerobic. Aérobic. Aeróbica. Aerobisch.
Αερόβιος. Aeroba. Aerobisch.
See instructions. Voir Notice.
Ver las Instrucciones.
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Store / Cons. 15°-30°C.
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Function of cultivators

- Cultivator, connected to a computer, keeps automatically optimal conditions of cultivation, and also evaluates status of the vessel and indicates eventual growth (e. g. change of CO₂ tension)
- The growth is signaled optically and by a sound. When nothing is growing even after a week, the apparatus signals it too (it is time to give out a negative result)

Haemocultivator



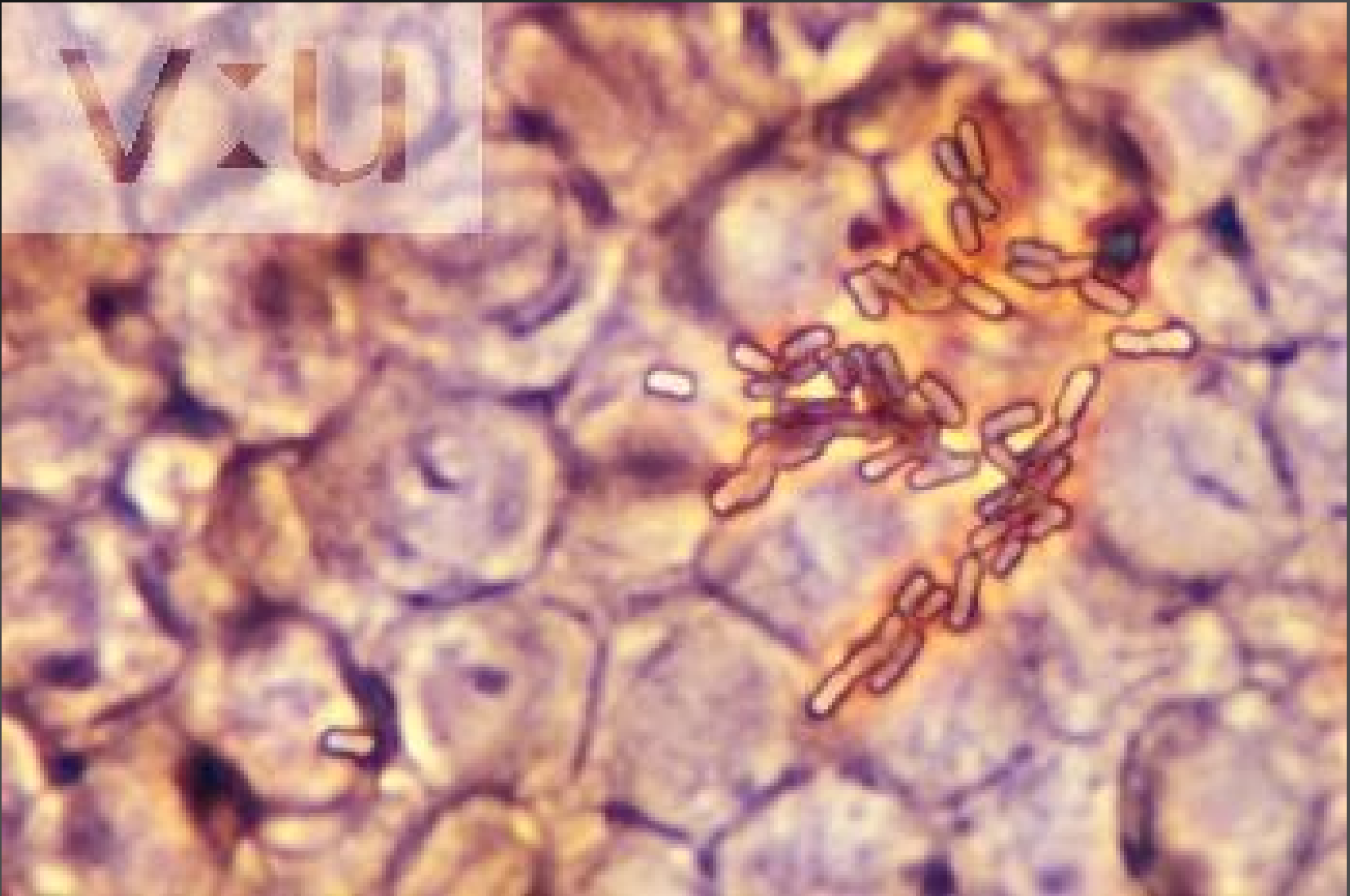
When a blood culture is positive...

- The vessel is brought from the apparatus
- It is necessary to mark the time, or period from admission to positivity (more on the next slide)
- We perform inoculation to solid media, Gram stained smear and according to its result „directly“ orientation disc test of susceptibility; instead of standard suspension just fluid from vessel is used
→ the result is unsure

Why it is so important to write the timing of sampling

- **Example 1:** Three blood cultures taken, all of them positive, but one after 12 hours, another after 36 hours and the third after 3 days. The strains are phenotypically different → it is very likely that those are skin contaminants
- **Example 2:** Three blood cultures taken, all of them positive, all of them after about the same time after sampling, strains look like the same → it is likely that it is a true pathogen

E. coli in blood culture, phase contrast

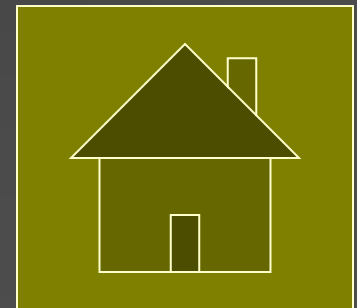


What to do after that

- We have to count that **direct tests are only orientation tests**, for not standard content of bacteria in individual blood samples. Usually in another step we perform **proper susceptibility testing (often using quantitative tests)**
- Exceptions are **likely contaminations** (especially in coagulase negative staphylococci)

Cooperation laboratory – ward

- Laboratory tries to **coopetate with clinicians** already during blood culture, mostly in form of telephonic report, sending preliminary results (even in negative blood cultures) etc.
- Also **long term evidence of positive findings** is usefull in frame of a systematic surveillance of hospital infections
- **Details of cooperation** should be mediated individually



2B Sampling:
urine samples

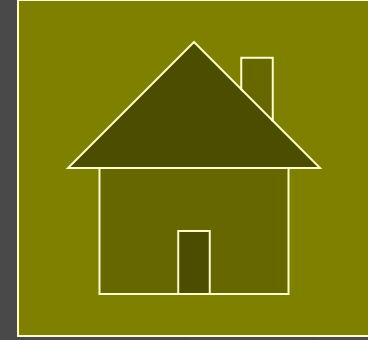
Urine examination (Part One)

- Urine examination is recommended in non-complicated and necessary in complicated cystitis: **true**
- Microbiologists recommend use of catheterized urine as a routine way of sampling urine for bacteriology: **false, normally taken urine is usually sufficient, but it should be sampled properly**
- It is not important, whether prepuce (in men) or labia minora (in women) is in the way of urine stream when sampling urine for bacteriology: **false, we should avoid contamination as much as possible**

Urine examination (Part Two)

- External orifice of urethra should be carefully washed and eventually also disinfected before taking sampling urine for bacteriology **true**
- The vessel, that the patient urinates in, should be sterile **true (and important!)**
- The test tube used for urine transportation to the laboratory should have yellow cap **false, it depends on individual organization**
- The order form should contain information whether urine is „routinely taken“, cathetrized, punctated, or whether it is a specimen taken from a permanent catheter **true**

Urine examination (Part Three)



- Urine from permanent catheter has the same value for bacteriological diagnostics as cathetrized urine (just for examination) **false** (urine from permanent catheter is worse, cathetrized urine is better than "normal" urine)
- Urine specimen should be delivered to the laboratory in 2 hours after sampling, in impossible, it should be kept in refrigerator **true**
- Urine sample is better than urethral swab in gonorrhoea diagnostics **false**

2C Sampling:
more examples

Stool sampling

- **Bacteria** – in Amies transport medium
- **Yeasts** – the same, but better in FungiQuick medium
- **Viruses (isolation)** – sample sized like a hazelnut; when viral isolation is to be performed, it is necessary to send it at 0 °C
- **Viruses (antigen)** – sample sized like a hazelnut; no influence of the temperature
- **Parasites** – again hazelnut sized, not necessarily sterile. Mark traveller anamnesis! Usually three specimens.
- **Pinworms** – Graham method – perianal imprint on special sticky tape, to be microscopied
- **Clostridium difficile toxin** – as for parasitology

Moulage method in flat wounds

- This method is used to **better quantify microorganisms, especially in wounds.**
Using square filtration papers, we can better differentiate between a real pathogen and an accidentally found contaminant
- You will use this method not for wounds, you will only try it to your own skin.

Smears – Vaginal/urethral smear

- **Smears** are useful for actinomycosis, anaerobes, etc. Smears are also often used together with **vaginal swabs**.
 - **Two slides** are sent.
 - One is Gram stained (for examination of bacteria, yeasts, but also epithelial cells and WBCs)
 - The second smear is Giemsa stained (mostly because of *Trichomonas*)
 - We evaluate both **quantity of individual objects** and **entire appearance** of the preparation
- In case of **suspicion for gonorrhoea**, urethral swabs are taken. We see white blood cells and intracellular diplococci.

Normal picture: epithelial cells, lactobacilli (Döderlein bacillus)

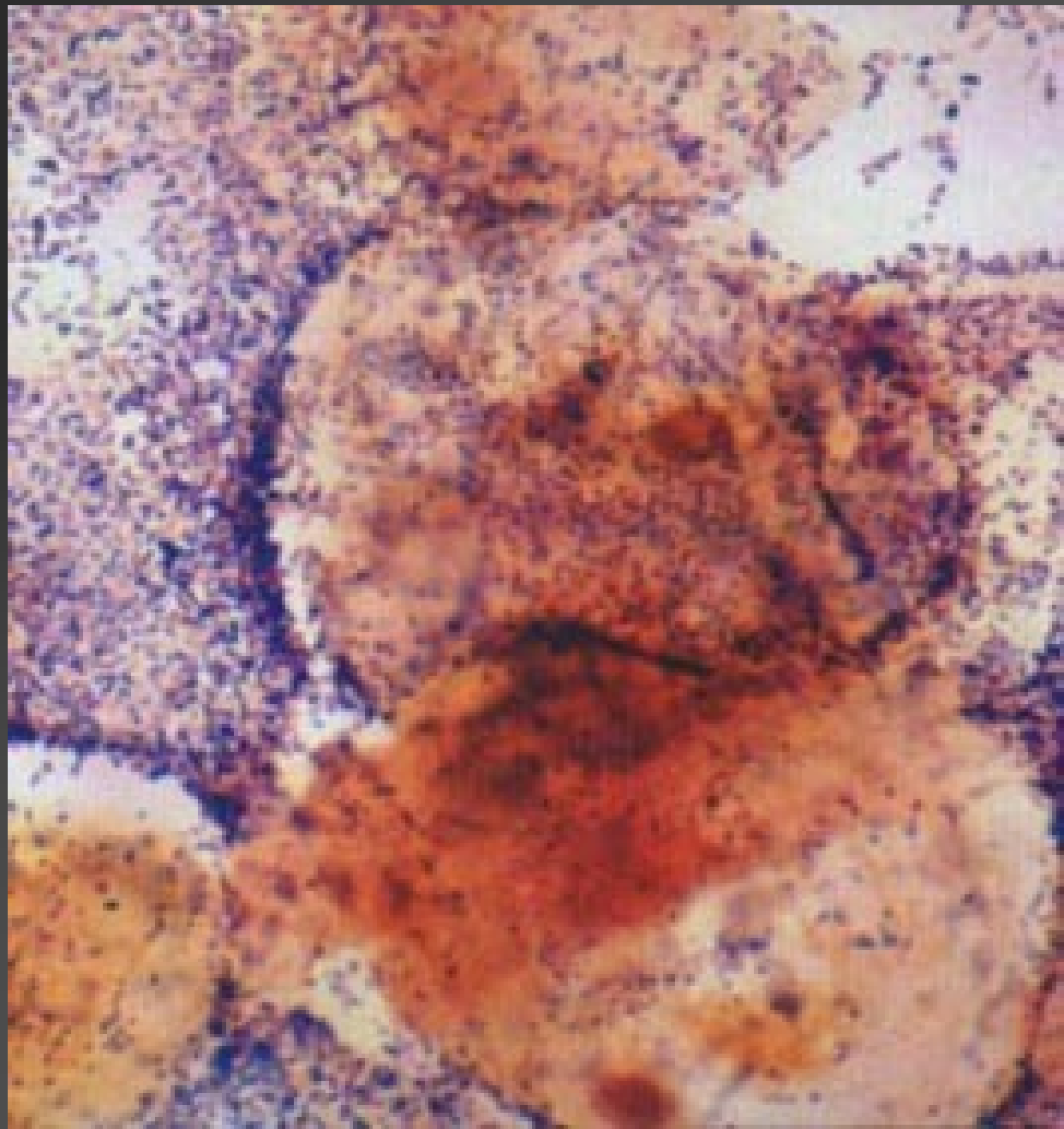


Giemsa

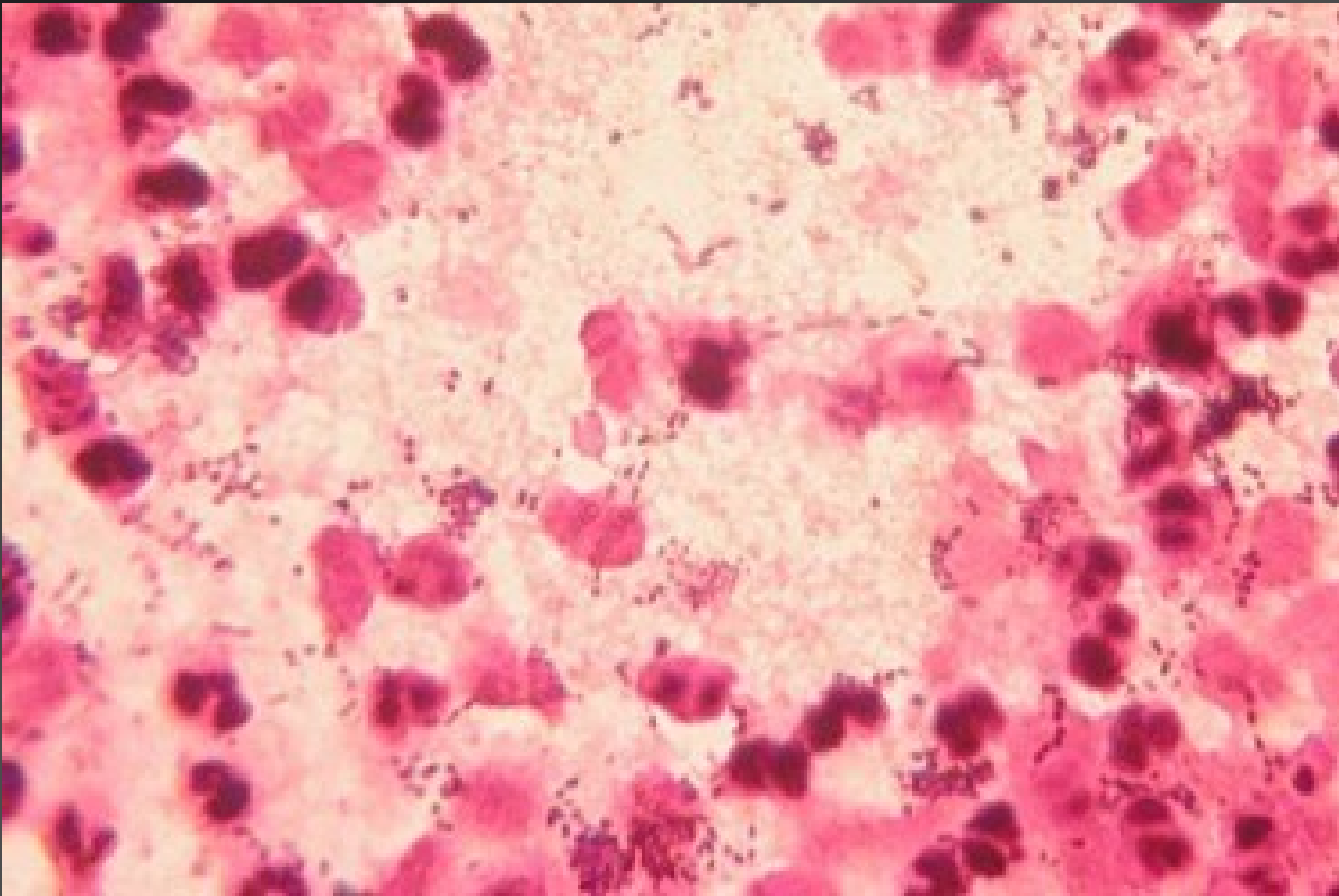
Gram

Picture of bacterial vaginosis

(lactobacilli
replaced by
gardnerellae,
event. mobilunc
and other
bacteria,
common clue
cells – bacteria
adhered on
epitheliae)



Aerobic vaginitis (unlike vaginosis, here leucocytes are present)

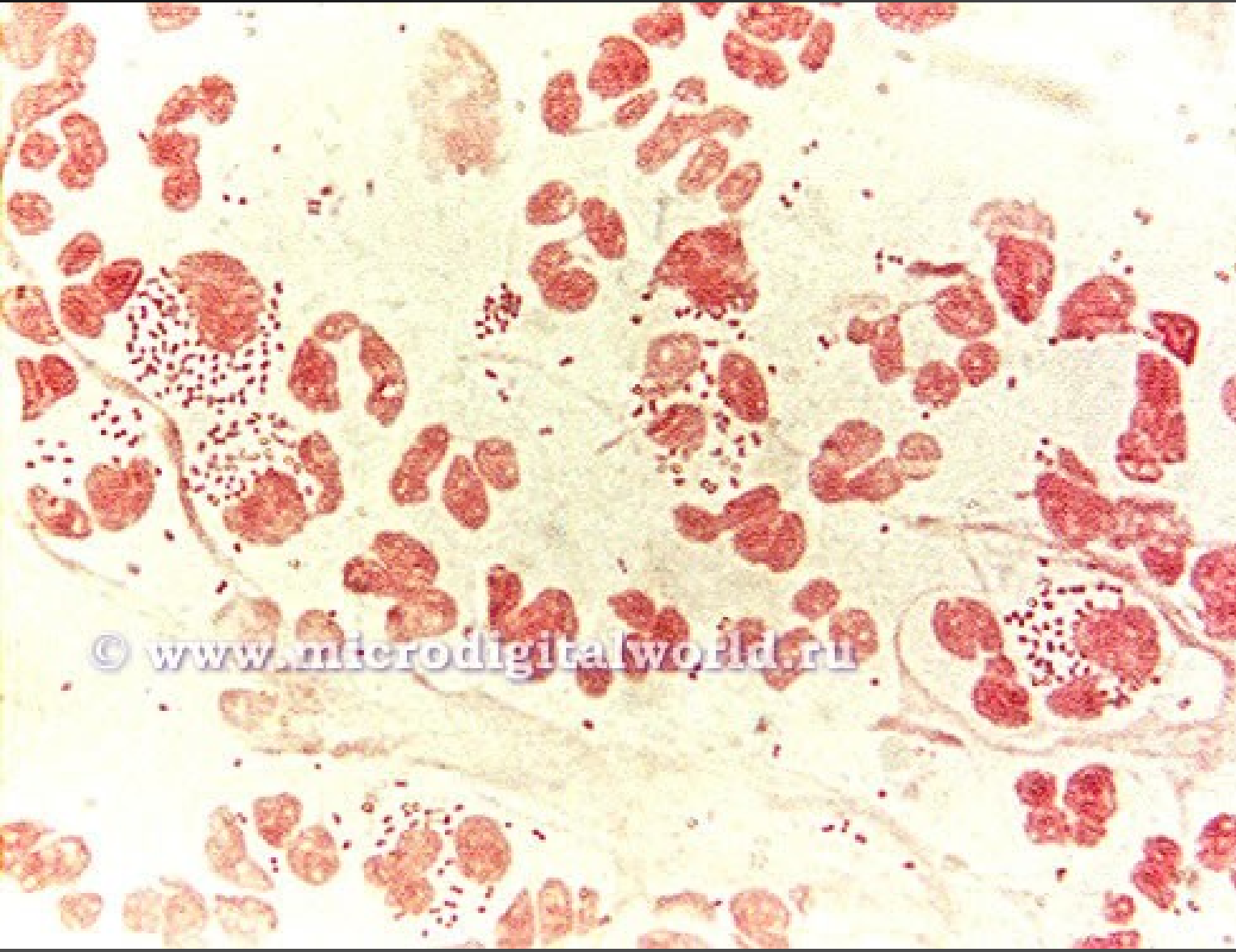


Gram

<http://en.microdi-gitalworld.ru>

Gonorrhoea

<http://en.microdigitalworld.ru>



© www.microdigitalworld.ru

Gram

Trichomonosis

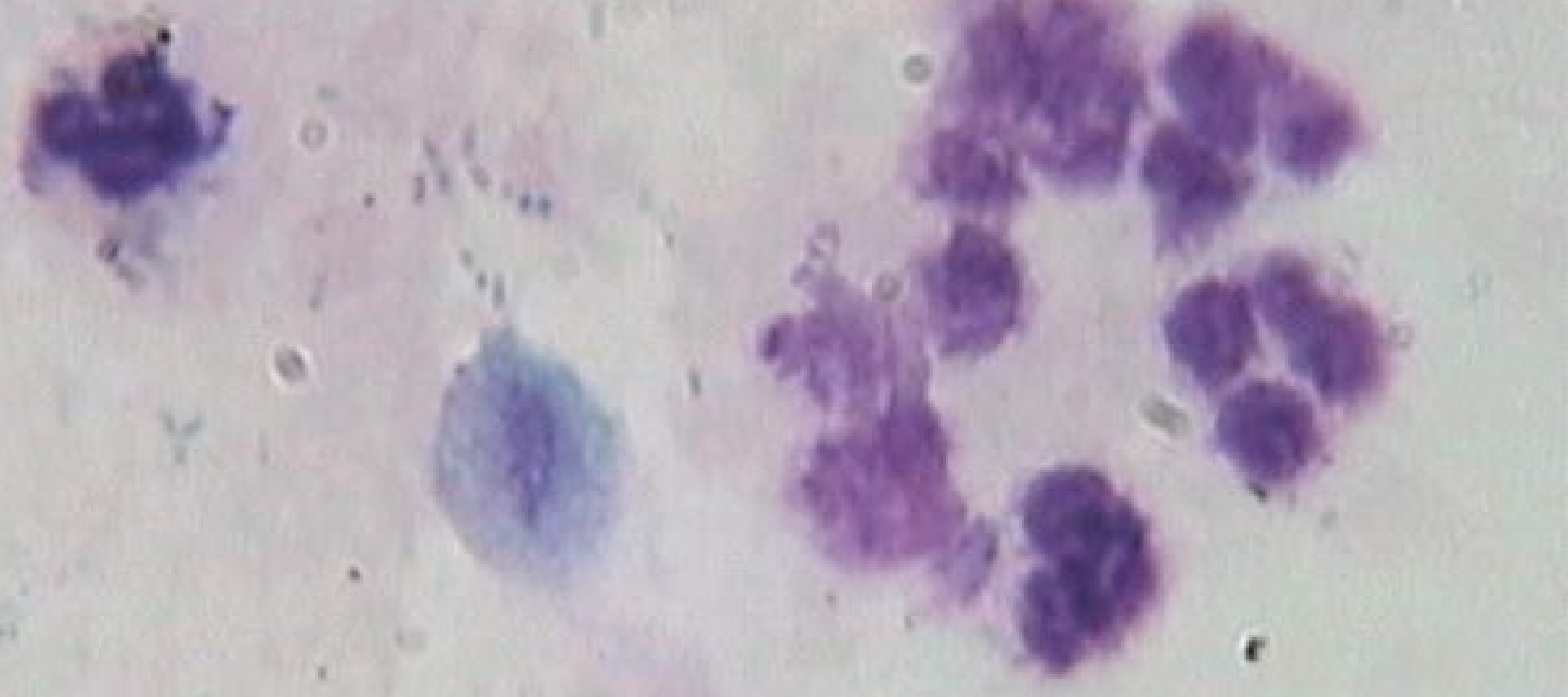


Photo by: Dr S.M. Sadjjadi
parasito@sums.ac.ir

Giemsa

Vaginal mycosis

<http://en.microdigitalworld.ru>



PMN leukocyte - 1
spherical form of Candida - 2
hyphal form of candida - 3



4 Decision how
to process

5 Proper
processing

4 Decision, how to process the specimen

- It is described in operation standards (OS). For each sample type the OS says, what methods should be used for it, and what methods should be applied.
- Nevertheless, everything is not in OS. Especially in extraordinary cases it is on **decision of experienced microbiologist**, how to process the specimen
- In important cases **is no mistake to phone to the laboratory and ask for an advice.**

5 Proper specimen processing (1)

- Proper processing is usually done by laboratory assistants, formerly with secondary education, today with bachelor's degree or equivalent
- The procedure should be aseptic, to avoid risk of laboratory contamination. Work in a biohazard box is also a good prevention of hospital infections

www.medmicro.info



5 Proper specimen processing (2)

- Processing of bacteriological specimens usually contains following:
 - before proper processing, some specimens are homogenized, centrifuged etc.
 - in some specimen types **quick methods** – microscopy, eventually direct antigen examin.
 - nearly always it is based on **culture on several solid media**
 - sometimes also **multiplication in liquid media** (in conjunctival swab: ONLY this point)
- Processing of other specimens (serology, PCR, mycology, parasitology) is special and related with examination type and specimen type

Laboratory of clinical bacteriology

Assistant 2 „does repeatings“: in positive specimens susceptibility test and identification tests are prepared

Microbiologist „reads the laboratory“ – observes the results of the culture

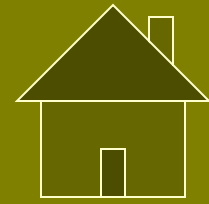
Assistant 1 writes the results



What may a pathogen be confused with

- **With a contaminant:** mostly bacteria of genera *Bacillus*, *Micrococcus*, *Kocuria*, but also small amounts of staphylococci, fungi etc.
- **With an accidental finding:** in throat swabs a microb that came there with food
- **With common flora:** only in sites, where some normal microflora is present

Survey of common flora



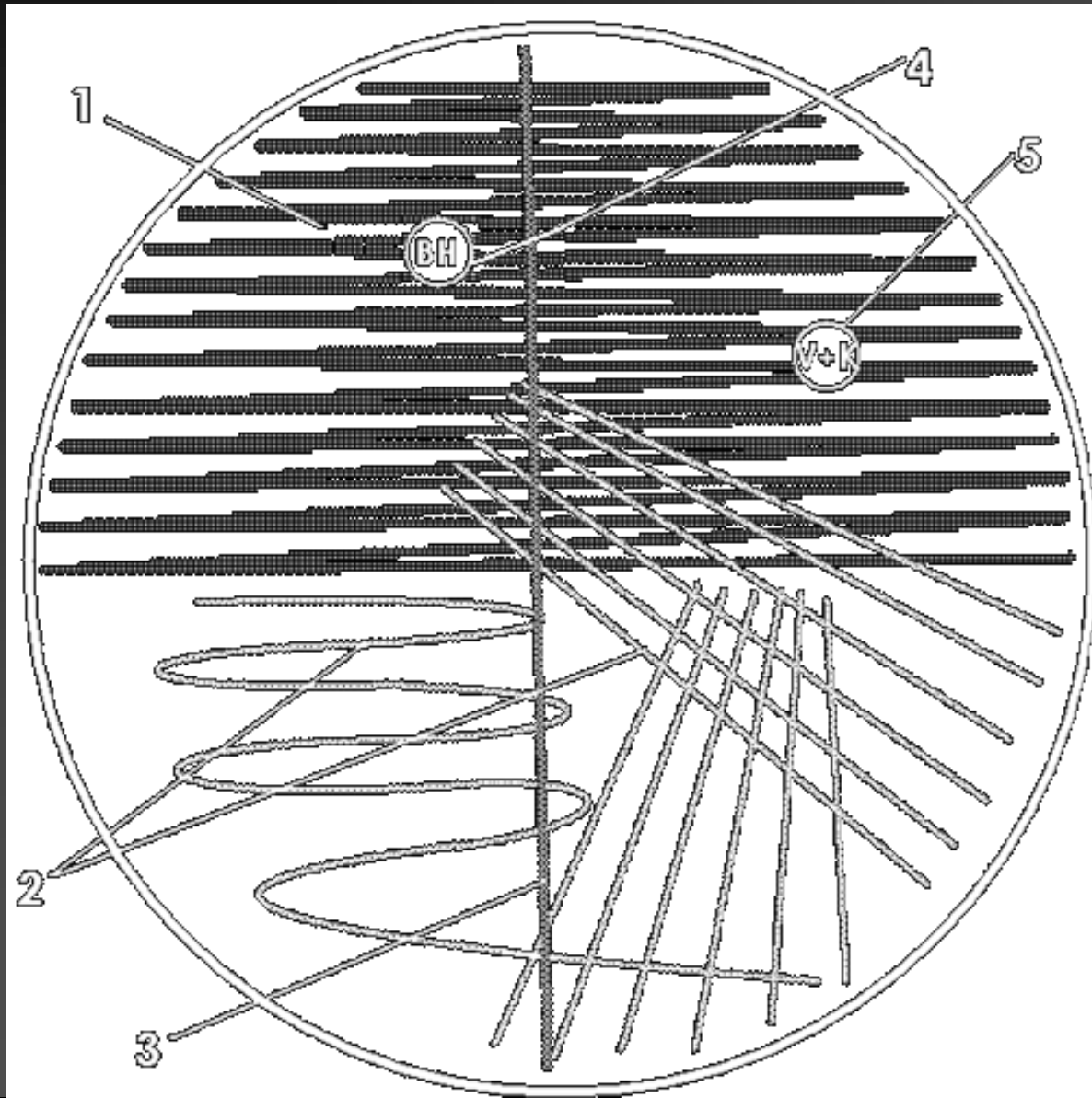
Skin, nose, external ear, skin and axilla	Staphylococci (incl. <i>Staph. aureus</i>), coryneforms, yeasts
Pharynx & oral cavity	Oral streptococci and neisseriae. Hemophili, small amounts of pneumococci, meningococci, anaerobes, non pathogenic treponema
Large (and small) bowel	Anaerobes, enterobacteria, enterococci, <i>Entamoeba coli</i>
Vagina	Lactobacilli, small amounts of other bacteria
Margins (lips etc.)	Mixture from both sides

4A Processing and „reading“ in respiratory specimens

How to find a pathogen among common oropharyngeal flora

- Normal flora consists of greyish, viridating colonies (oral streptococci) and yellowish, usually a-haemolytical colonies (oral neisseriae). They use to make a dense „carpet“ on the surface of agar medium and they make search for pathogens quite difficult, nevertheless possible:
 - Haemolytic streptococci (and also *Staphylococcus aureus*) are visible by a strong haemolysis on blood agar
 - For haemophili detection we use antibiotic disc with bacitracine – higher concentrations than in bacitracine test (to decline the normal microflora)
 - For meningococcal detection we use another disk, with mixture of vancomycin and colistine

Detection of pathogen in throat/sputum



1 swab inoculation

2 loop inoculation

3 staphylococcus line

4 bacitracin disc (for hemophili)

5 V + K disc (colistine and vancomycine) for meningococci

In all parts of inoculated area we search for colonies with haemolysis. They could be streptococci (rather colourless) or goldish

Cultivation result of throat swab with common flora

The bacitracin disk may be placed either on the *Staphylococcus* line, or approx. 1 cm far from it, both ways are used.

In these sites we search for haemophili



Explanations to following screens

- **BA** – blood agar
- **EA** – Endo agar; usually, McConkey agar may be used as an alternative
- **BA+AMIK** – blood agar with amikacin, selective for streptococci a enterococci
- **NaCl** – BA with 10 % NaCl, selective for stafylococci
- **B** – broth
- **URI** – urichrome, a chromogenic medium for the most important pahogens from urine

Sputum examination

A white plastic sputum cup is shown, filled with a thick, yellowish, opaque sputum sample. The sputum has a uniform, creamy texture and fills most of the cup. The cup is set against a dark background.

Sputum examination

Diagnostic schedule (1)

- **Day 0:** microscopy (Gram staining)
- **Day 1:** result of primary culture on BA, EA and NaCl. If only common flora is present, EA is discarded and BA and NaCl is prolonged to another day. An eventual pathogen is identified and its antimicrobial susceptibility assessed. If there is a small amount of a pathogen, isolation is performed (colony is carefully picked by a loop and reinoculated to a new agar plate to obtain a pure culture)

Sputum examination

Diagnostic schedule (2)

- **Day 2:** expedition of negative results (observation of prolonged BA cultivation). Expedition of majority of positive results, if identification is finished antibiotic test result is OK. If not (too many resistances, more atb needed), or if only isolation is done, it is necessary to continue.
- **Day 3:** expedition of majority of remaining positive results (resistant, difficult detection...)
- **Day 4:** extraordinarily expedition of remaining results (combination of several problems)

Sputum – possible findings

- **Common flora:** There is no flora in LRW, but always a contamination from URW is present: oral streptococci and neisseriae
- **Pathogens:** pneumococci, pyogenous streptococci, haemophili (typical pneumoniae). Causative agents of atypic pneumoniae are mostly non-culturable. **If you would find *Staphylococcus aureus*, you can use treatment using oxacilin, eventually, if oral oxacillin would not be available, to use 1st generation cephalosporins.**

Practical note

- Small, greyish, nearly colourless, viridating, are **oral streptococci**.
- Small, yellowish, without viridation, without haemolysis (or a slight partial haemolysis), oxidase positive are **oral neisseriae**
- If there is something more on our plate, and especially if this „something“ has a strong haemolysis, it is probably the **expected pathogen**.

„Reading“ of bacteriology



Throat swab

biology.clc.uc.edu



Throat swab

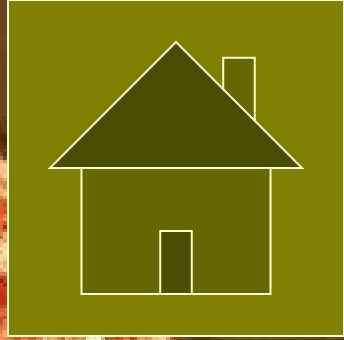
Diagnostic schedule

- **Day 0:** only start of the cultures
- **Day 1:** result of primary culture of specimen on BA and EA. NaCl is not used here. Here, too, BA cultures with common flora are prolonged
- **Day 2:** expedition of all negative and majority positive results
- **Day 3:** expedition of mostly all remaining results

Pharynx – possible findings

- **Common flora:** Oral streptococci and neisseriae; haemophili (mostly *H. parainfluenzae*), but normal are also small amounts of *S. aureus*, pneumococci, meningococci, moraxellae etc. More components of common flora (anaerobes, spirochets) are not found in normal culture
- **Pathogens:** pyogenic streptococci, arcanobacteria; often nothing is found and it is viral origin (EB viruses and others)
- **Treatment:** In case of *Streptococcus pyogenes* found to be a pathogen, V-penicillin is used.

Throat swab – common
flora and pyrogenous
streptococcus



4B Processing
and „reading“ in
wound and
urine specimens

Wound swab



OCT 13 2004

Wound swab

Basic diagnostic schedule

- (Different in different types of wound etc.)
- **Day 0:** start of culture only
- **Day 1:** result of primary culture of specimen on BA, EA, NaCl and BA+AMI. If all solid media are negative, B is observed; if turbid, a subcultivation to solid media is performed
- **Day 2:** expedition of negative and some positive results; too resistant bacteria → more tests
- **Days 3, 4:** expedition of remaining results

Wound swab

- **Common flora:** none, all findings are looked as pathogen (we test even microbes suspicious of being contamination – better treat a contamination than not treat a pathogen)
- **Pathogens:** wide spectrum of bacteria, from staphylococci through streptococci and pseudomonads to anaerobes in abdominal wounds and pasterurellae in wounds after being bitten by a dog. It is recommended not to use antibiotic before atb susceptibility result.
- Recommendation for treatment: e. g. ciprofloxacin in our case; but **local care of the wound** is more important than antibiotics!

Urine



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

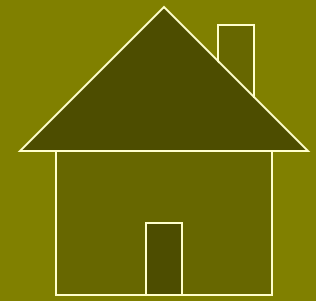
Urine

- There is **no common flora**, nevertheless, in elderly often asymptomatic bacteriuria, it is not necessary to treat it
- As **likely contamination** (or accidental finding) is counted everything below 10^4 / ml, everything below 10^5 / ml in finding of two various bacteria and everything in three/more bacterial strains
- **Among pathogens**, the most common are enterobacteria, enterococci, *S. agalactiae*, staphylococci etc.

Semiquantitative processing

- A **plastic loop** is used – the „eye“ of the loop catches always 1 μl of urine
- This microliter is inoculated to one **halfth of blood agar plate** (you have it on a **total plate**)
- Further **we inoculate Endo agar or URchrom**, here we assess it only qualitatively
- Of course, besides quantity examination we also examine genus and species of the bacterium as usually
- **In our case, we would recommend nitrofurantoin for treatment.**

Semiquantitative urine evaluation



Number of colonies	Number of CFU (bacteria) in 1 μ l of urine	Number of CFU (bacteria) in 1 ml of urine	Evaluation (valid for 1 bacterium)
Less than 10	Less than 10	Less than 10^4	Contamination
10–100	10–100	10^4 – 10^5	Borderline
More than 100	More than 100	More than 10^5	Infection

6 Result
sending

7 Interpretation

6 Result sending

- Result is **sent after finishing of the diagnostic process**. Sometimes a preliminary result is sent after finishing the basic aerobic culture, and the remaining part (yeast culture, anaerobic culture etc.) is sent later
- Result contains **a partial interpretation**: a microbiologist comments clear contaminations, accident findings, common flora, comments the findings in a note

7 Interpretation

- **Definitive interpretation of finding should be done by the clinician.** Only the clinician, not the microbiologist, has the microbiology result together with the biochemical, rtg, ultrasound result. And only he has done the anamnesis and clinical examination of the patient.
- Of course, **consultation of a clinician and a microbiologist is very useful in serious cases.** On the other hand, it is not possible to consult each case.

A picture of laboratory

www.medmicro.info



The End

