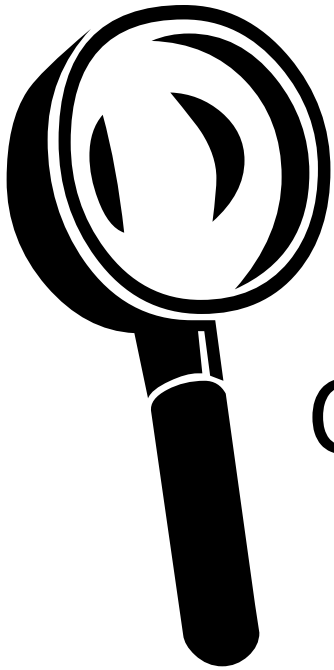


Institute for microbiology shows

TRACING THE CRIMINAL



Part thirteen:
Cooperation at investigation or
Clinical Microbiology IV

Survey

Introduction, microorganisms in blood

Diagnostics and treatment of sepsis

Wound infections: introduction, wound types

Wound infections: diagnostics and therapy

Bonus: More about sepsis

Introduction, microbes in blood

Presence of microbes in blood

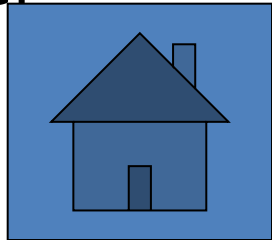
- **At normal situation** microbes are present in blood at most as transitory (e. g. during teeth cleaning). In heart tissue and vessel endothelium, of course, bacteria should not be at all.
- The term „bloodstream infection“ (BSI) is almost used for **bacterial**, eventually **mycotic** (yeast) infections
- **Viraemia** (presence of viruses in blood) is a part of various viral diseases, almost hepatitis and HIV infection (it is mentioned in the corresponding virology topic)
- Among **blood parasites** we have malaria plasmodia, trypanosomes and filariae (see parasitological topic)

Bacterial (eventually mycotic) bloodstream infections (BSI)

- **Sepsis** are infections of proper bloodstream, in the same time they are systemic infections of the complete organism. They are primary (in typhus) or secondary (catheter sepsis, urosepsis, abdominal sepsis). They are caused by bacteria or yeasts.
- **Endocarditis** are connected to previous ones, but besides presence of microbe in blood there is also a more narrow relationship to the endocardium, especially in case that it is damaged by a previous disease or treatment (rheumatoid fever, implant)

Important terms

- **Sepsis/septicaemia** is a complex situation, presence of bacteria in blood PLUS clinical symptoms (there exist clinical criteria that should be fulfilled)
- **Bacter(i)aemia** (eventually fungaemia, i. e. presence of yeasts) is just evidence of presence of bacteria (fungi) in blood without evaluation of their clinical importance.
Transient bacteraemia may be a part of spreading of bacteria in the organism, although it is not BSI (especially in pneumonia or pyelonephritis – possible use in diagnostics).
- **Pseudobacter(i)aemia** is a situation, when bacteria only seem to be present in blood (badly performed blood examination, usually skin contamination). More later



Diagnosatics and treatment of sepsis

Diagnosics of sepsis

- **blood cultures (see further)** and more microbiology examinations (changed catheter, sputum, urine according to likely original focus, lumbal puncture in suspicion for meningitis)
- **biochemical laboratory** – inflammatory markers (CRP, procalcitonin, differential blood picture)
- **Laboratory markers of disseminated intravascular coagulation (DIC):** thrombocytopenia, lower AT III etc.
- **Search for infection focuses:** RTG of heart and lung, otorhinolaryngology, ultrasound (oesophagus – focuses on heart), CT...
- Neurologic examination

Blood cultures – sampling (1)

- It means **not clotted blood**, principally very different from serological examinations (*it is neither antibody nor antigen detection, microbe should remain alive and is detected by cultivation*)
- Today we usually sample into **special vessels with transport-cultivation medium** for automatic culture (*sooner just not-clotted blood without any medium was sent*)

Blood cultures – sampling (2)

- We have to ensure **minimalisation of risk of pseudobacteraemia** (see more)
- **In adults we take 10 to 20 ml of blood, that is split into two parts (aerobic and anaerobic culture), in children usually 1–5 ml according to age** (sampling is more difficult in adults, and also in children also less bacteria are important)

Function of cultivators

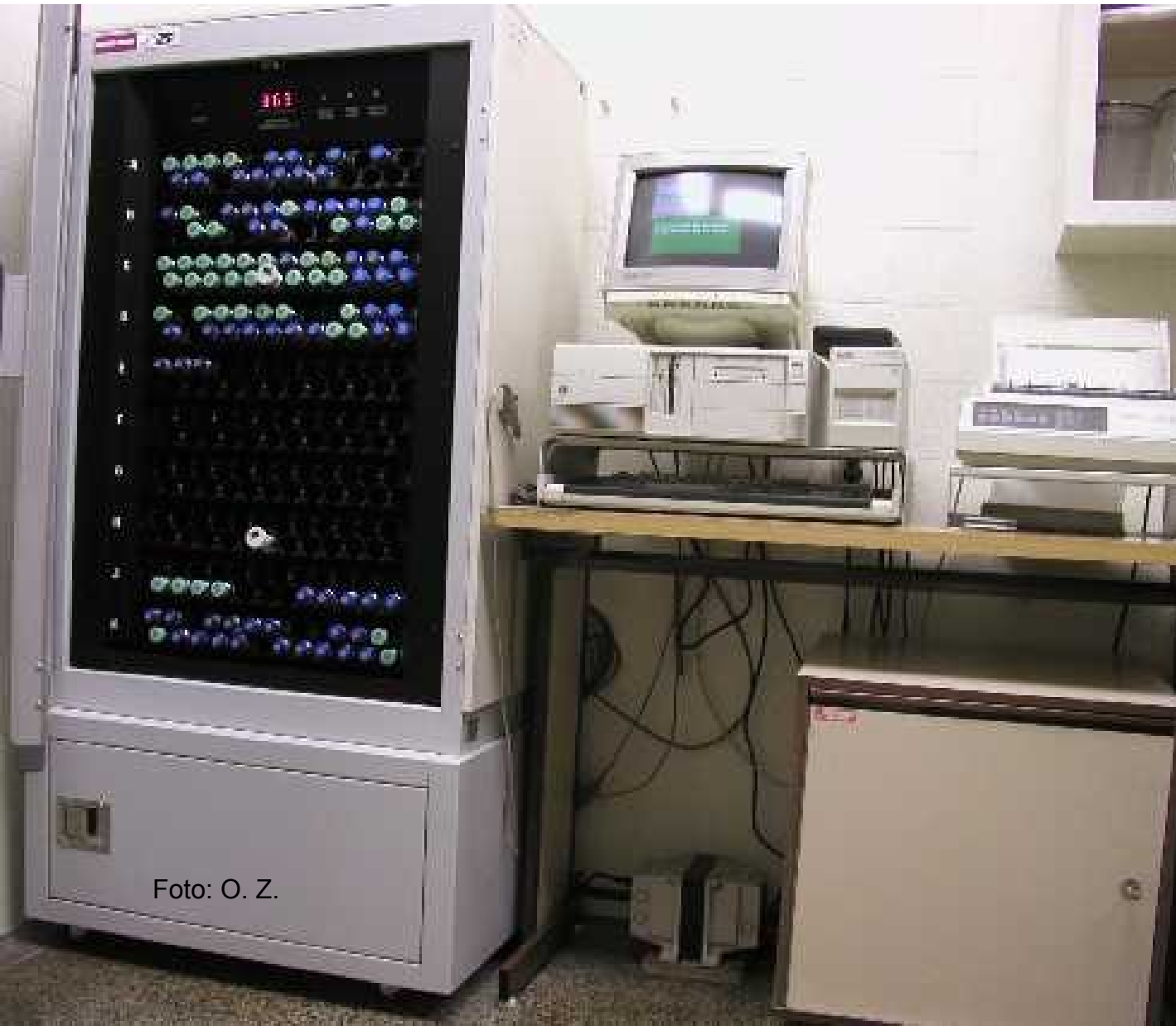
- **Cultivator, connected to a computer**, keeps automatically optimal conditions of cultivation, and also evaluates status of the vessel and indicates eventual growth (e. g. increase of carbon dioxide concentration that results in change of optical properties of the vial and is detected by a sensor)
- The growth is **signalized optically and by a sound. When nothing is growing even after a week**, the apparatus signalizes it too (it is time to give out a negative result)

Blood culture automat



Foto: O. Z.

The same automat – open



Types of blood culture vials

- There are **various types** regarding to microbes that are to be detected (aerobes, anaerobes, yeasts)
- **Some vials include charcoal or resin.** 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; charcoal or resin adsorbes the antibiotic or any chemical toxic for bacteria)
- **The most frequent types are: aerobic standard, aerobic with charcoal and anaerobic with charcoal.**

Examples of vials for blood culture – system BacT/ALERT



Standard
aerobic

Charcoal
anaerobic

Charcoal
aerobic

BacT/ALERT® SA
Aerobic. Aérobie. Aeróbico. Aeróbica.
Αερόβιος. Anaeróbica. Anaeróbica.
See instructions. Voir Notice.
Ver las Instrucciones.
Vide Instruções de Uso.
Store / Cons. 15°-30°C.
For in vitro diagnostic use.
Reg. No. 1077340225
BIO-MÉRIEUX, INC.

BacT/ALERT® FN
Anaerobic. Anaeróbica. Anaeróbica.
Αναερόβια. Anaeróbica. Anaeróbica.
See instructions. Voir Notice.
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BacT/ALERT® SA
Aerobic. Aérobie. Aeróbico.
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BIO-MÉRIEUX, INC.

Photo: archive of Institute

Examples of vials for blood culture – system BACTEC



Photo: Institute for microbiology

Pseudobacteraemia – reasons

- **Not properly done sampling**, not sufficient asepsis during taking blood
- **Sampling only from artificial catheters** (we only catch bacteria colonizing the catheter, but it is not always real cause of bacteraemia, not speaking about sepsis)

Why bacteriemia is bad? That means that the patient is treated uselessly for not-existing infection. It is also possible that the infection exists, but instead of the pathogen another microbe was found – this finding would stop the search for the real pathogen

How to avoid pseudobacteraemia – I

- To take blood **targetedly**, when presence of bacteria in blood is likely; not take it „just for sure“ when another examination is indicated
- To take **sufficient amount of specimens**: one is useless, even two are too little, three is optimum
- To take blood cultures from **suitable sites**: at least one from a new venepuncture, ideally three venepunctures plus one from venous catheter
- To take blood cultures in **suitable moment**, in septic status typically at temperature increase

How to avoid pseudobacteremia – II

- To take blood cultures **properly**, very important and frequently neglected is keeping aseptic sampling (to disinfect, not only clean skin, and to let the disinfection really dry). To send blood cultures together with **well filled in request form**; necessary is not only date, but also time and place of sampling – for result interpretation
- To send blood cultures **in the correct kit**: usually the volume is split into an aerobic and an anaerobic vial, but they are together considered to be “one blood culture”. So 3 blood cultures \times 2 vials = 6 vials! In practice, for budget reasons, it is common that there are less blood cultures, or some are only aerobic (so total number is 4 to 5 vials, sometimes even less)

How to avoid pseudobacteraemia – III

- In suspicion for **contamination of vascular catheter** the catheter is changed. The old catheter is not discarded, but sent for bacteriology examination. Today there are already methods capable to estimate whether it is a real colonisation of the catheter or a random find (see below)
- The same of course applies to **any of the implants, which are removed from the body** – the microbiological testing can bring substantial information for further treatment

How to find pseudobacteraemia when it occurred already

- Typical for pseudobacteraemia (false positivity of a blood culture) is, that
 - just **one of three blood cultures** is positive
 - or even more blood cultures are positive, but **from each one another strain would be cultured** (different susceptibility, different colony appearance) and would have **different TTD (time to detection)** – this corresponds to different quantity
 - clinical **patient problems are not corresponding to the findings**
 - Eventually **the same strain would be also found in patient's skin**

Evaluation of TTD (time to detection)

- Time **between sampling and the report of positivity from the automat** (*e. g. the automat beeps and signals positivity by a red rectangle*) is shorter in case of massive presence of bacteria in blood and longer in case of few bacteria
- In real bacteraemias the time uses to be shorter (less than 48 h) and **the same for all taken blood cultures** (plus minus two hours)
- Eventually the time might be **shorter for the blood culture from place that is source of infection** (*e. g. blood culture from central venous catheter, that represents the source of a catheter sepsis*)

Do you understand already why it is so important to write time and site of sampling to the request form?

Importance of TTD for finding interpretation

- **Example 1:** Three blood cultures were taken, all of them were positive, but one after 12 hours, the second after 36 hours, and the third after 3 days. The strains are phenotypically different → it is very likely that it is a skin contamination
- **Example 2:** Three blood cultures were taken, all of them were positive, all about the same time after the collection, the strains look similar → it is likely that the finding is the actual pathogen

When a blood culture is positive...

- The vial is **taken out from the machine**
- It is necessary to **mark the time, or TTD – period from admission to positivity**. The longer this time is, the more the contamination is likely
- 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

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 (non-standard inoculum). Usually in another step we perform **proper susceptibility testing (often using quantitative tests)**
- Exceptions are **likely contaminations** (only one of three is positive, or all three, but different strains, positivity after longer time, coagulase negative staphylococci), here we usually only perform a new qualitative test from a standard inoculum

Cooperation laboratory – ward

- Laboratory tries to **cooperate 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 useful in frame of a systematic surveillance of hospital infections
- **Details of cooperation** should be mediated individually

Microbiology examination of blood catheters

- Catheters are today usually sent in a **sterile test tube**, without being put in a liquid. In the laboratory
 - either biofilm is broken by **ultrasound** and released into the solution (so called sonication)
 - or the catheter is **rolled on the surface of** agar medium
- Both methods are **semiquantitative**, i. e. the result decides whether it is rather significant finding or contamination
- Traditional method of a catheter just placed into the broth and here let to multiply is now considered obsolete

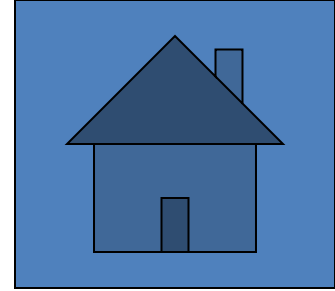
More options in examination of BSIs

- Examination of **urine, sputum, cerebrospinal fluid**, etc. is performed with regard to the suspected source of sepsis
- In some microbes **direct antigen detection** is possible in the blood without cultivation, i.e. with the possibility of almost immediate obtaining a result: mannan antigens in yeast, or antigens, the cause of typhoid fever, meningitis pathogens and the like

Therapy of sepsis

- **symptomatic therapy** – emergency or intermediary units
- Monitoring, addition of circulating liquids, oxygen, support for circulation (noradrenalin), use of peripheral and/or central venous catheters, artificial lung ventilation etc.
- **antibiotics** (initial „blind“ therapy, later targeted)
- In case of presence of abscesses their **surgical removal**
- **corticosteroids** – in initial phasis of sepsis approx. 300 mg of hydrocortisone (< 3 days)
- **anticoagulation therapy** – in case of signs of disseminated intravascular coagulation only
- change of glycaemia, level of calcium etc.

Complications and prognosis of bacterial sepsis



- **acute respiratory failure syndrome:** 40 % of patients with sepsis
- **acute renal failure** (elevated urea and creatinin)
- **circulation failure** – BP decrease (systolic BP < 90 mmHg)
- **disseminated intravascular coagulation** – Gram-negative sepsis
- **digestive tract failure** – vomiting, diarrhoea, bleeding (stress ulcer)
- **hepatic failure** – elevated bilirubin, ALT, AST etc.
- CNS damage – alteration of consciousness
- **total lethality of sepsis is approx. 40 %**
- **septic shock lethality is 70–90 %**

Wound
infections –
introduction,
wound types

Wound infections

- Infection of wounds are fairly disparate group (different origins, different location). In any case they are serious, because **microbes penetrated body surface to sites that are normally sterile.**
- Specific situation is a pyogene inflammation of surgical wounds. Its prevention and treatment is one of the important topics for the surgeons. *(Today we almost use abbreviation SSI – surgical site infection.)*
- **Purulent wound infections** only occur, when the bacterial infection of wound is accompanied by infiltration of polymorphonuclear granulocytes (due to the immune response of the host organism).

Wound classification

- **Wound classification with regard to depth:**
 - surface wound infection (skin, hypodermis)
 - deep wound infection
 - infection of organs and body spaces
- **Wound classification of with regard to the risk:**
 - 1/clean
 - 2/clean-contaminated (surgery of sites with normal microflora)
 - 3/contaminated (trauma, bacteria from outside)
 - 4/dirty-infected (inflammation in a wound)

Areal wounds

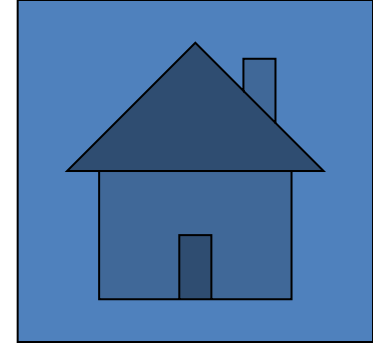
(diabetic ulcers, ulcus cruris, bedsore)

Often **mixture of various bacteria**, often participation of bacterial biofilm → treatment should be almost local (biofilm destroying) and only sometimes also supportive oral/venous antibiotic use

The most serious causative agents are *Streptococcus pyogenes* and *Staphylococcus aureus*

Bacteria that rather colonize the wound: *Escherichia coli*, *Proteus mirabilis* and other enterobacteria, *Pseudomonas aeruginosa* and yeasts

Infection × colonization of a wound



- Sometimes it is difficult to say **which microbe is responsible for an invasive wound infection** and which one only colonized it (and formed a biofilm in it)
- A typical sign of true invasive infection is **presence of causative bacteria deeper in the body (e. g. in blood culture)** and also inflammatory markers
- Pure **colonization** should not be treated with oral/venous antibiotics; topic treatment and wound debridement etc. should be performed

**Wound
infections:
diagnostics
including interpretation
and treatment**

Sampling in deep focal infections (1)

- In case of sufficient amount of pus or another liquid (exsudate, cyst contain etc.) **that liquid in a test tube should be sent** and not just swab (if possible)
- In suspicion for **anaerobic infections** (especially pus from abdominal region) it is recommended to send it directly **in a syringe. To cap a syringe** (just syringe, no needle) use **combi cap** (see picture)
- *Sending of a syringe with a needle placed to a sterile rubber cap, that was recommended sooner, is in the matter of fact forbidden for safety reasons, (needle manipulation is risky for sampling person)*

Sampling in deep focal infections (2)

- In case of impossible liquid sending (not enough liquid) we need to send **swab with transport medium**. Recently also **E-swabs** are sent (see further)
- In some cases also **smear or imprint on slide** may be effective (catching of pathogens that could not be cultured)
- **In special cases a microbiologist may be even called to an operation hall**

E-swab (1)

Transport system ESwab is sterile and consists of two parts

- Polypropylene **screw cap test tube** with liquid Amies transport medium
- **The proper sampling swab**, ended by soft nylon fibres. It is made by an advanced technology of parallel nylon fibres in electrostatic field.
- Microorganisms are **actively captured by electrostatic force of the fibbers** (in classical swab they just remain passively in the swab).



E-swab (2)

E-swab contains liquid Amies medium (without active coal, so it is not black).

The medium is slightly different from other media and **it can be also used for PCR** (in does not contain anything that could lead to reaction inhibition)

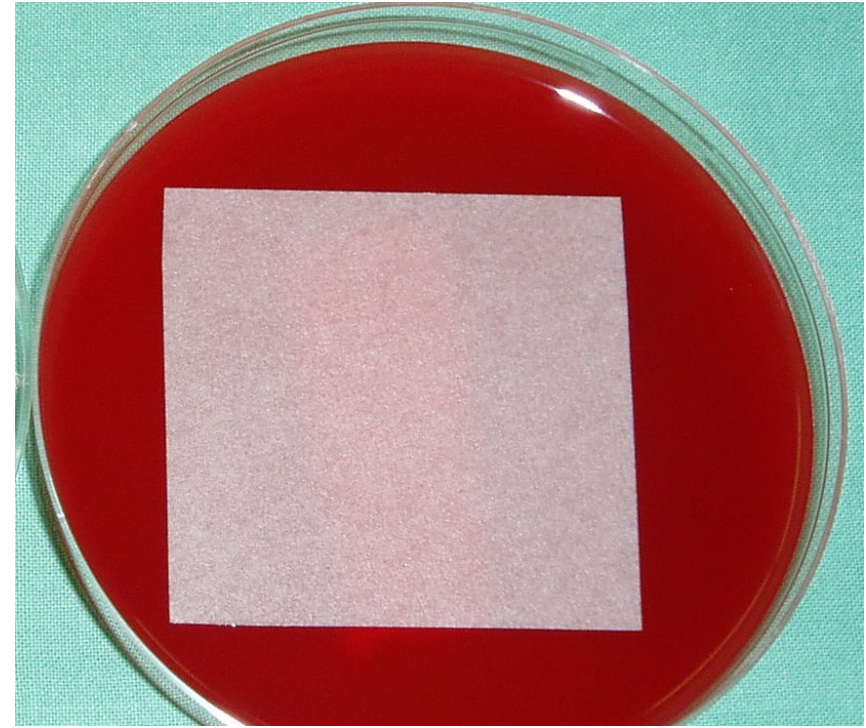
Sampling in superficial wounds

- Classical method is a **swab with transport medium**
- The swab should **catch the pathogen** (we have to reach the infection focus) and in the same time **not contaminated**, especially from skin
- It is also possible to use **indirect imprint method**: a square piece of a sterile square of filtration paper is placed on an areal wound (e. g. diabetic ulcer) and then moved to the surface of culture medium (blood agar). In the laboratory it is also placed to other media; this enables better semiquantitative evaluation of the result

Swab, or imprint?

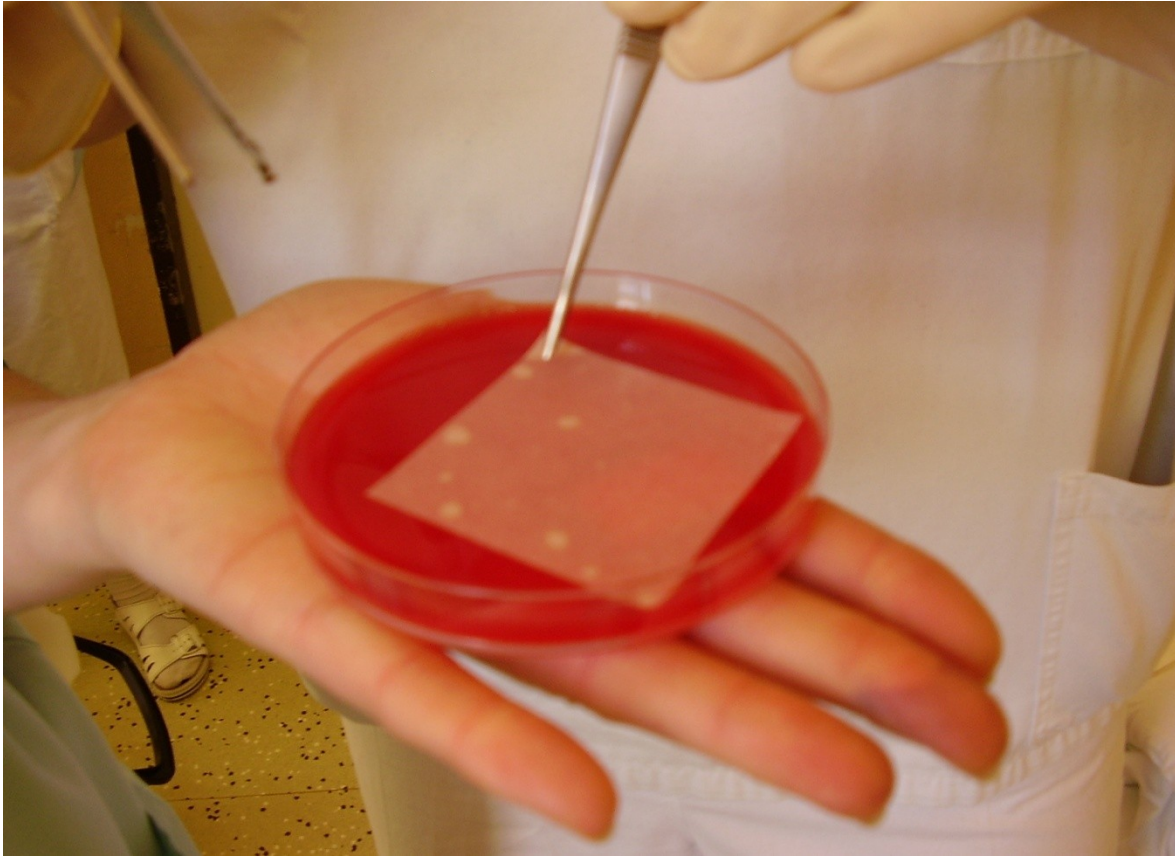
- At swab we use sterile tool with Amies transport medium. Result is **qualitative**.
- For imprint, we use 5x5 cm sterile square of filtration paper. The result is **semiquantitative**.

Swab and imprint



Pictures of imprint methods are taken from a slideshow by MUDr. Zdeněk Chovanec from I. Surgical Clinic of Medical Faculty of Masaryk University and St. Anna Faculty Hospital in Brno

Indirect imprint technique I



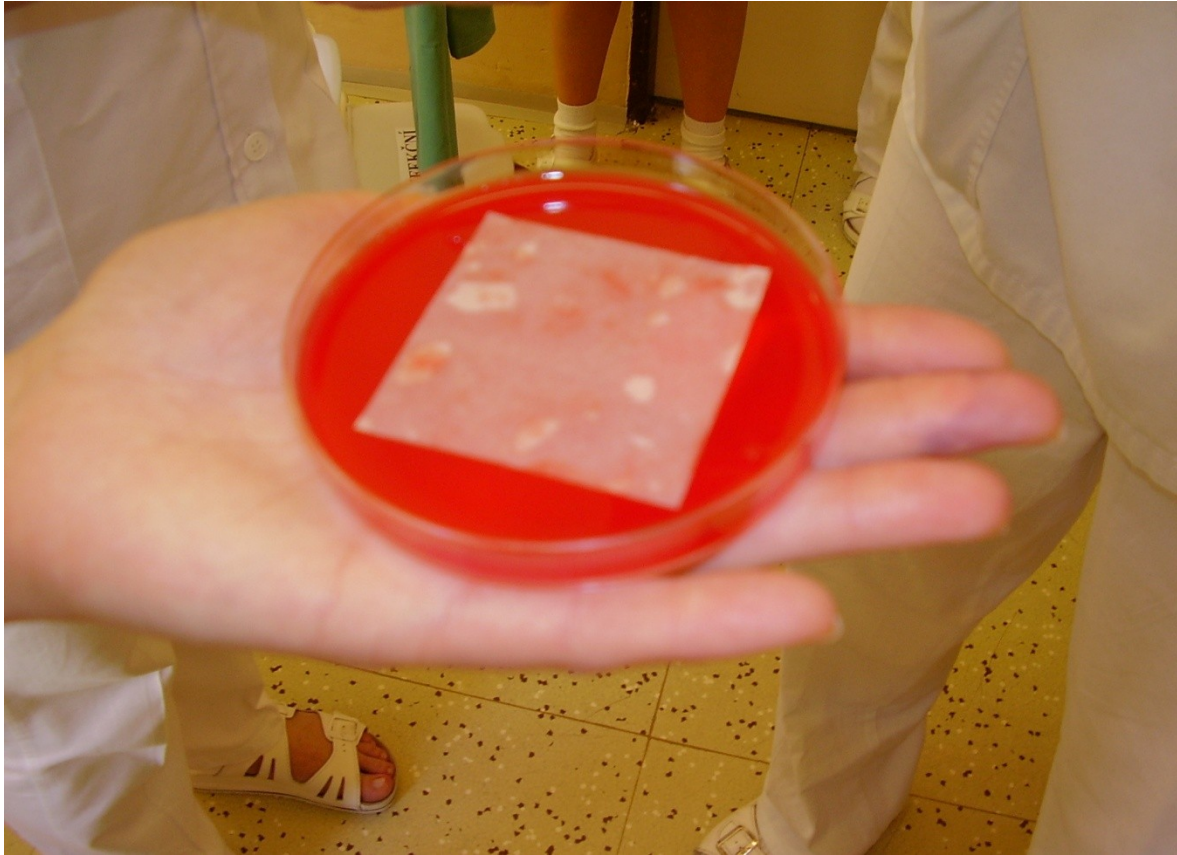
Surgeon is provided by sterile culture medium and sterile gauze or filtration paper square

Indirect imprint technique II



Now surgeon or skilled nurse moves the square to the wound so that it touches the surface and lets about a minute

Indirect imprint technique III



- Now the square is placed back to the original cultivation medium

Request form filling in in wounds

- The person taking the specimens should **fill in the request carefully**, „wound swab“ not sufficient, it is necessary to add
 - **Wound type (origin)** – surgical wound, bite, punctual wound etc.
 - **Localization of wound on body**
 - Eventually **special examinations** (although e. g. anaerobic culture for swabs from abdominal cavity is automatic)
- Also important **anamnestic data** (coming from abroad, work in agriculture) may be useful

Wound infection diagnostics

- Laboratory performs **microscopy (Gram staining)** in liquid specimens only (not swabs); always **culture, precise determination of pathogens** is done and **examination of antibiotic susceptibility**
- In microscopy, **not only microbes, but also WBCs etc.** are important
- At culture we use **liquid multiplying media** (for situations with few microbes) and also **selective media** (with NaCl for staphylococci, with amikacin or streptococci), especially in bedsores etc.

Wound swabs (without anaerobes):

Possible diagnostic scheme

- (Different in different types of wound etc.)
- Day 0: start of culture only
- Day 1: result of primary culture of specimen on blood agar (BA), Endo agar, NaCl and BA + amikacin. If all solid media are negative, broth 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 – result interpretation

- **Common flora:** none, all findings are looked as pathogen (even including microbes suspicious of being **contamination**) – so also antibiotic tests are performed
- **Pathogens:** any bacteria or *Candida* are considered possible pathogen, perhaps with the exception of coagulase-negative staphs and corynebacteria on the surface of staphylococcal skin wounds.
- **Colonisation:** in surface wounds it is questionable, whether the situation corresponds to an infection or rather to a colonisation (especially in *Pseudomonas* and *Proteus*). Antibiotic susceptibility testing is nevertheless performed even here.

Pyogene infections treatment

- **Local wound care** is important (local preparations, cleaning, debridement, eventually also larvotherapy)
- **If we do not suppose finding of anaerobic**, oxacilin (as an anti-staphylococcal drug) would be a drug of choice
- For rather **streptococcal origin**, high doses of G-penicillin should be used.
- In **hospital wound infections** targeted treatment is necessary

End

G+ cocci in chains in a blood culture

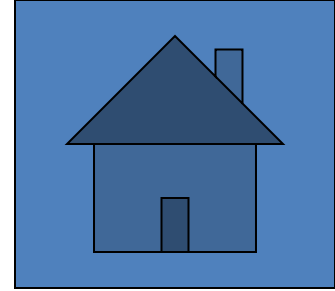


Photo: Institute of Microbiology

Bonus: 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
 - sepsis related with **pneumoniae**
 - sepsis of **abdominal origin**
 - **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/hypoglycaemia, metabolic failure, bleeding, neural symptoms etc.