Practice I.

Collection of clinical samples, sample processing.

Steps of bacteriological diagnostic procedure

1. **Sampling – clinical sample collection from the correct anatomic site of the body.**
2. **Culturing bacteria – pure culture.**
3. **Identification of bacterium species.**
   a. **morphological examination**
      microscopic morphology – characteristics of the bacterium cells
      macroscopic morphology – examine morphology of bacterial colonies
   b. **examine biochemical activities (CH-metabolism, N-metabolism, catalase test, coagulase test, etc.…)**
   c. **examine antigen structure.**
   d. **detection and identification of bacterial products.**
4. **Testing antibiotic sensitivity.**
5. **Examine the immune response of the host to the bacterial infection.**

Basic principles

- Adequate amount of samples must be collected.
- Sample must be taken before the antibiotic therapy or before the next antibiotic dose.
- Avoid contamination.
- Sample is handled as hazardous material.
- Accompanying paper must be sent with important data.

Classification of clinical specimens

Samples sterile under physiological conditions
- Blood
- Cerebrospinal fluid
- Deep tissues or biopsies

Samples sterile or with low number of non-pathogenic microbes
- Urine
- Bile

Samples with high number of normal flora microbes
- Mucous membranes
- Superficial samples from skin and wound
- Stool

Blood sampling for serological examination
- detection of antibodies specific for a given pathogen (e.g. Lyme serology)
- serum or plasma specimens
- collect serum or plasma blood samples by venipuncture or by sampling intravenous catheters
- universal precautions to reduce any potential infection risks through needle stick injuries; wear gloves when obtaining blood samples to protect yourself
- samples should reach the laboratory within 24 hrs; storage at 4°C
- storage of sera at -20°C

Blood samples for culturing bacteria
- hemoculture bottles for sample collection – enrichment medium
- different bottles for children, aerobes, anaerobes, fungi, Mycobacterium, intracellular bacteria
- venous or arterial blood samples (collected by venipuncture or from intravenous or arterial catheter devices or with the own device of the bottle)
- adolescents and adults 10 - 20 mL of blood is the minimum volume per set and 1 -2 mL for neonates and young children
- after skin disinfection
- blood must be taken 2-3 times per day from different veins
- blood should be taken **during rising fever** (cold shivers), because the highest microbe concentration can be detected in this time
- warming up of bottles to 37˚C (or at least room temperature) is essential
- transport to laboratory as soon as possible; in case of any delay, the blood cultures must be stored at room temperature
- bottles must be incubated at 37˚C for up to 7-10 days with continuous monitoring
- analysers measure the CO_2 production.

Blood Cultures

Sterile Samples

CSF, peritoneal fluid, pleural fluid, pus samples, bone marrow aspirates, tissue samples, e.g. lymph node biopsies or lung biopsies for mycobacterial culture)

- samples should be collected from sterile sites using aseptic techniques
- disinfect the skin site thoroughly prior to puncture or incision
- if necessary use sterile gloves and sterile drapes around the incision or puncture site
- aspirate the body fluid into a syringe and fill the fluid into the sterile container or into hemoculture bottles
- screw the lid back on the container and make sure that it does not leak

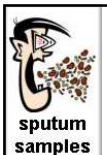
Urine

- native, mid-stream urine (taken in the morning, after cleaning the external genitalia)
- urine collected from urinary catheter port (not bag bladder washout samples)
- suprapubic punction
- sterile containers or tubes, or dip slide method
- pooled urine samples are not acceptable

- URICULT – semi quantitative examination and identification of uropathogenic bacteria
- media with different specificity
- storage at room temperature

Faeces

- sterile container with a plastic spoon
- collect pus and/or blood
- rectal swab is recommended and permissible for infants
- samples must be reach the laboratory within 2 hours
- important information must be attached to the samples (e.g. "foreign travel to...").



Sputum

- a sample collected early in the morning is preferred
- pooling of sputum samples is not recommended
- refrigerate the specimen if delay is longer than 2 hrs
- patients should rinse their mouths with water
- sample evaluation – lower respiratory tract (ratio of epithelial cells/lymphocytes)

Cotton swabs and containers with or without transport medium, cytobrush

Sampling devices

- needles and syringes – purulent material, pus
- bouillon impregnated cotton swab – dry wounds
- cotton swabs
- transport medium in sampling tubes



- **Vaginal samples**
  - with cotton swab
  - cytobrush for examination of cells and microbes
- **Mucous membranes , throat, nasopharynx, oral cavity**
  - swabs samples taken from the inflamed areas of the site
  - cytobrush: exfoliated cell collection
- **Transport:**
  - transport the sample to the laboratory as quickly as possible
  - do not refrigerate the sample
  - transport media may be used