Practical class No. 1

Laboratory safety, microbiology laboratory - facilities
Disinfection and sterilization

Hygienic hand washing.
Disinfection effectiveness control – impression method

Considerations of Laboratory Safety in Microbiological Laboratory

- Microbiological laboratory – risk workplace
- it is necessary to follow definite laboratory safety considerations!!!

2. Eating is prohibited in the laboratory

any handling of food (e.g. putting into the refrigerator, freezers, etc.)

3. Drinking is prohibited

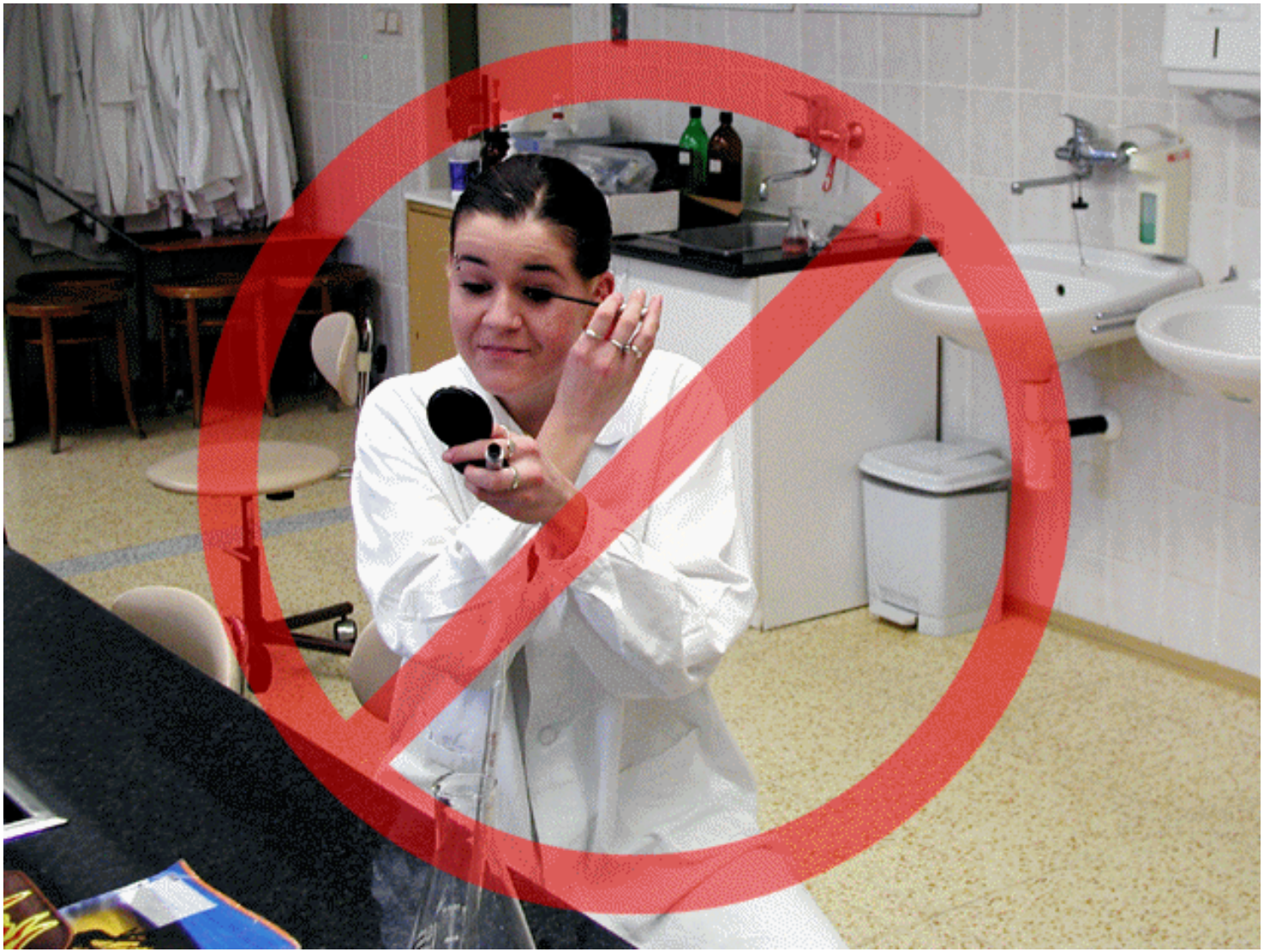
(not even PET bottles with water inside the lab)

5. It is prohibitted to bring personal things that aren´t needed for laboratory work

6. Don´t touch your face!

Don´t have a lick of the finger when turning the page in your text book or exercise book!!!!

7. It is prohibited to make-up in the lab!



8. Long hair mustn´t be loose, must be well tied together or used a tied hair cover

9. It is prohibited to put any object into the mouth

10. Prior to beginning of the practical classes it is necessary **to take off all jewellery** (rings, watch, bracelets)

Nails are **cut off short**.

11. The work with the infectious materials is prohibited to pregnant women and women up to 9 months after delivery

12. All injuries and accidents must be reported immediately

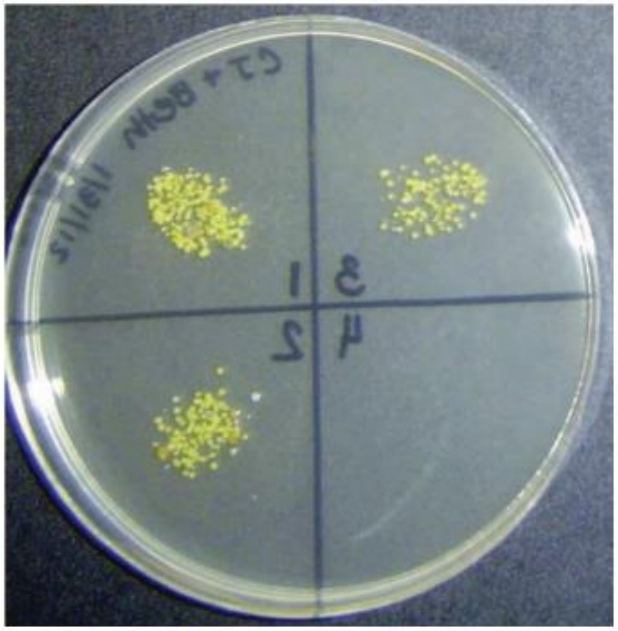
- Any injury **must be reported to the teacher**, as well as any accident of contamination with the biological materials that happens during the class!!!
- In case of any entry of infectious materials into the human body the first aid must be provided!!!

- Health care workers wear the **gloves** when working with the biological samples (Care with scratches, abrasions, agnails, eczema!)

13.Leaving the laboratory

- check the working place
- wash/disinfect the hands

- **It is prohibited to take out any infectious materials, aids or white coats from the laboratory!!!**

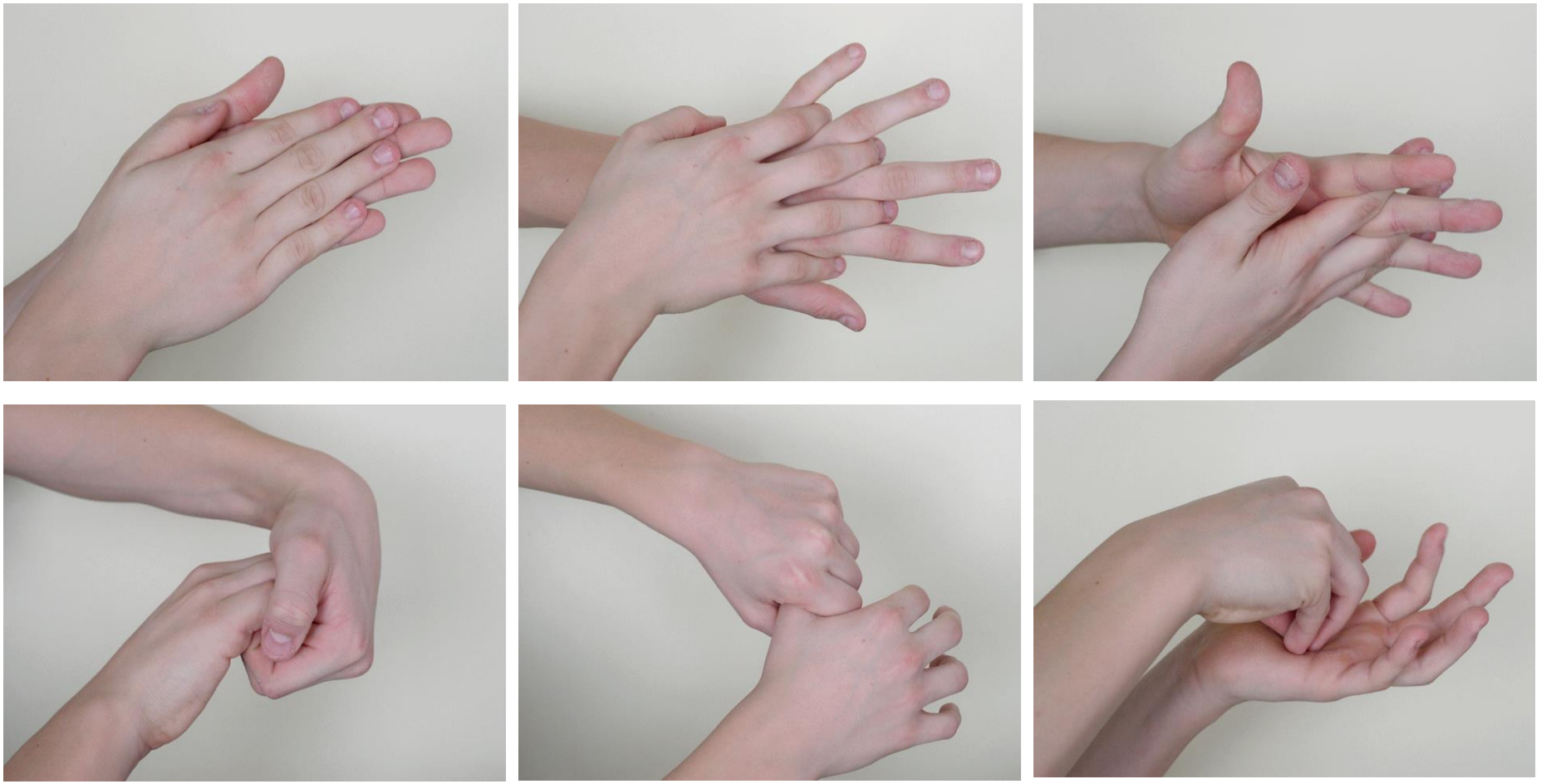


PRACTICAL PART

1. COMPARISON OF EFFECTIVENESS OF SOAP AND DISINFECTANTS USING IMPRESSION METHOD

Hand disifection – the correct procedure

- Performed according to the demonstrative instructions (every step is repeated 5x); the exposition time = 30 – 60s



Practical part

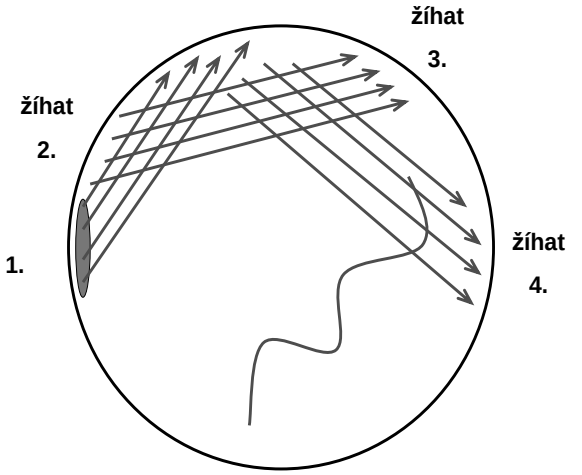
- Tools:
  - Culture medium – nutrient agar, marker, soap, vial with suspension of *St. epidermidis,* disinfectant
- Working procedure:
  - Label the nutrient agar with your name and devide it into 4 quadrants with a marker (always on the dish with agar)
  - Impress your unwashed finger on agar of quadrant No. 1
  - Contaminate the finger with suspension of *St. epidermidis*, let dry and impress the finger on agar of quadrant No. 2
  - Wash your hands with soap, let naturally dry and impress the same finger on agar of quadrant No. 3
  - Disinfect the finger with disifectant - exposition of 30 – 60s and impress the finger on agar of quadrant No.4
- Incubation: thermostat at 37°C for 24 h, aerobic conditions
- Evaluation and conclusion: Practical class No. 2

PRACTICAL PART

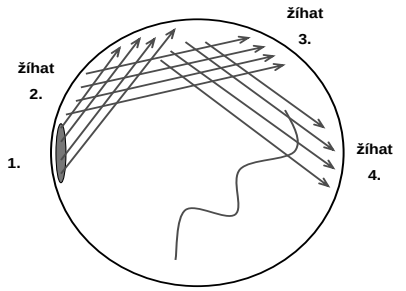
2. PREANALYTIC + ANALYTIC PHASE SWAB FROM THROAT – COLLECTION AND PROCESSING THE SWAB

Inoculation – cross streaking

- **Inoculation =** transfer of a small quantity of bacteria (pure culture) on fresh sterile nutrient agar medium
- Principle of the method: gradual dilution of originally concentrated specimen reaches single bacterial colonies growing in the end of the streaks.
- After each streak the bacteriologic loop must be sterilized in the flame or a new sterile loop must be used for each row of streaks



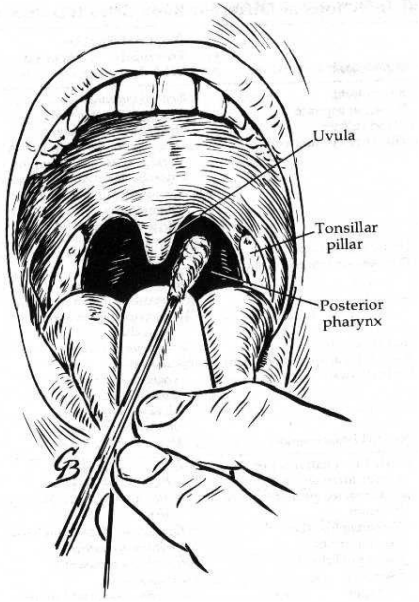
Inoculation – cross streaking

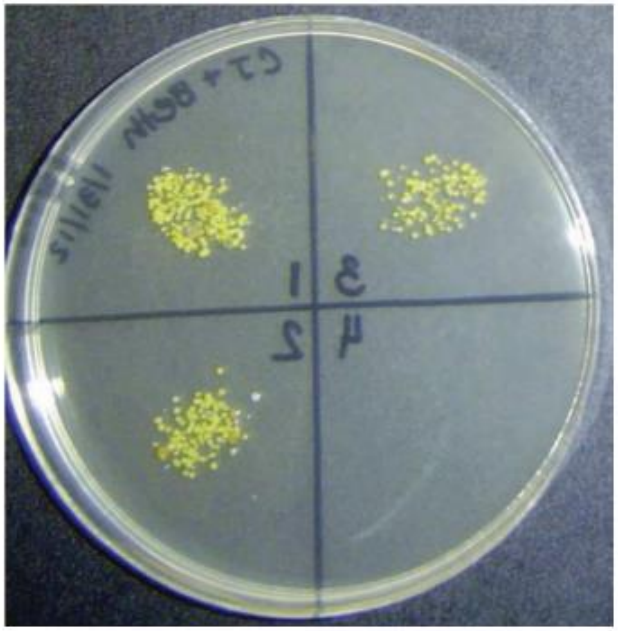


- Inoculate the bacterial colony on nutrient agar, spread well with sterile bacteriologic loop;
- Make streaks with bacteriologic loop from the inoculum in one direction;
- The second row of streaks starts at the end of previous streaks again in the same direction;
- Make the last Zig-zag streak;
- The whole procedure is performed under sterile conditions and the plate should be opened as little as possible.

Throat swab – practical performance

- **Tools:** sterile swab, (tongue depressor), blood agar, marker
- **Procedure:**
  - sterile swab is inserted behind the palatal arch – do not touch the tongue and oral cavity mucous! (tongue depressor may be used)
- Swab is inoculated on blood agar in the same way as demonstrated before
- Incubation for 18h at 37°C
- **Reading and evaluation:** 2. practical class





PRACTICAL PART

1. COMPARISON OF EFFECTIVENESS OF SOAP AND DISINFECTANTS USING IMPRESSION METHOD - EVALUATION

Morphology of bacterial colonies Growth on solid culture media

- **Bacterial colony** – growth of bacteria on nutrient agar in Petri dish
- One bacterial colony is a result of multiplication of a single bacterial cell
- **Description of bacterial colony**: shape, size, height, profile, margin, pigment, consistence, smell, hemolysis (blood agar)

Effectiveness of soap and disinfectants

- Count the number of bacterial colonies in all four quadrants
- Compare the effectiveness of soap and disinfectants – compare the results according to the number of colonies
- Put the results into the table
- Make the conclusion

PRACTICAL PART

2. THROAT SWAB - EVALUATION

Morphology of bacteria inoculation - evaluation

- Correctly performed inoculation and cross streaking detect the single bacterial colonies after cultivation in thermostat (aerobic cultivation, for 18 - 24h, at 37°C).
- Evaluate the colour, shape, profile and margin of the colonies.
- Conclude the results of throat swab cultivation into the table. Evaluate the findings.

PRACTICAL PART

3. DIRECT DETECTION - MICROSCOPY

Microscopy

- Direct detection of microorganism (from the culture, from clinical specimen)
- Microscopic morphology – shape, size, arrangement of cells
- Gram staining – basic staining in bacteriology
  - Principle: staining procedure relies on the structure of bacterial cell wall (peptidoglycan contents)

Gram staining

- **Tools**: cultures of *Staphylococcus aureus*, *Escherichia coli*, *Candida albicans*
- **Procedure:** Put 1 drop of physiological solution in the centre of the microscopic slide. Transfer a small amount of bacterial culture with the sterile bacteriologic loop and resuspend (stir) it in the solution. Make the oval inoculum of about 2 cm in diameter;
- After drying fix the slide in the methanol for 5 minutes.;
- Wash with the tap water and put the slide on the rack in the dyeing basin and cover the smear with the solution of crystal violet for 15-20 sec;
- Wash with the tap water and cover the smear with Lugol solution for 20 sec and wash with tap water;
- Decolorize the smear with alcohol as long as the dye is flowing away and then dye the smear with carbolfuchsin for 20 sec. Wash with the tap water and let the slide dry;
- Put 1 drop of immersion oil on dry smear and focuse it (without the cover slip!) using **immersion objective** (magnification 10×100).

Gram staining

- **Evaluation**:

Fill the protocol and describe the microscopy markers of stained microorganism, e.g. shape, Gram positivity, Gram negativity, arrangements of the cells