

Topic J01

Safety of work. Microscopic detection of organisms – I

Wet mount, simple staining

Materials for study (from textbooks, internet etc.): Wet mount, simple staining, shape and arrangement of bacteria

Note: bring coloured pencils with you for the practical lessons

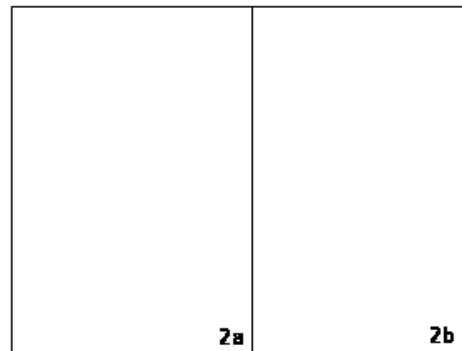
Task 1: Safety of work in the microbiological laboratory

Read carefully and put your signature on: Regulations for the Practical courses of microbiology + First Aid – Regulations for laboratory accidents (both in J00)

Task 2 Preparation of wet mount from a specimen (2a) and culture (2b)

a) Prepare and examine the wet mount of a specimen of C. A. T. swab, sent in a special transport medium. Use a slide and a coverslip. Examine magnified by 400 – 600× (use the 40× or 60× lens). When manipulating the macro screw, always watch the movement of the objective from the side so as not to break the coverslip or the frontal lens of the objective! Remark epitheliae, leucocytes, bacteria, eventually yeasts.

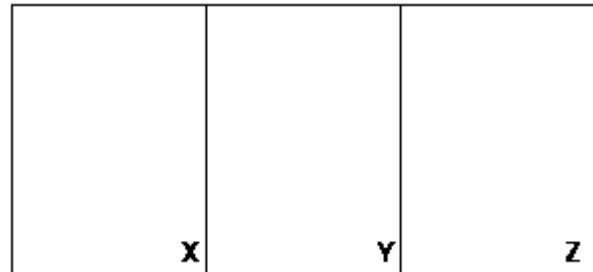
b) Prepare and examine the wet mount of yeast culture. Take a microbiological loop, sterilize it in flame of a burner and let its temperature decrease. Then take some colonies of yeasts (strain X), mix in a drop of saline and then use the same procedure of observation as in Task 2a).



Task 3: Preparation of fixed film, simple staining

The following procedure is to be done with all three strains (X = yeasts, Y = bacilli, Z = cocci). Prepare fixed film according to the assistant's instructions. Stain the presented microbial cultures with methylene blue and examine. Use the 100x immersion objective (consequent total magnification 1000x). Immersion objective is indicated with a black line (sometimes a double line). The coverslip is not used.

- Put a small droplet of immersion oil on the film. Make sure that the oil droplet does not brim over the edge of the slide and especially that it doesn't leak inside the microscope
- Watching the microscope from sidewise lower the objective with macro screw until the objective touches the oil. The side of the slide will lighten up.
- Start examining. Very (!) slowly move the objective upwards using macro screw.
- When the object appears, focus the picture with brief movements of micro screw.
- Never lower the objective with micro screw by more than one turn, rather repeat focusing from the beginning.
- After you finish your examination, carefully clean the lens with petrol.
- Draw the results of examination in your protocol.



Check-up questions

1. Where is wet mount used in microbiology?
2. Name the basic shapes of bacterial cells and their arrangement.
3. What is the term for the microscopy technique when the object is illuminated from the side and only those beams reflected by the object enter the objective – the result is a shining object in the dark?
4. Write down, step by step, the procedure of preparing fixed film, and simple staining. Don't forget about the drying. Underline the steps valid also for the wet mount. (Use the opposite side of this sheet)