

Introduction

Microbiology is the study of living organisms that cannot be seen with naked eye without the help of a powerful compound microscope. Thus, while studying microbiology we generally are working with unseen living forms of life. Microorganisms are present all around us and play a vital role in ecology of earth. Some are commercially used in the production of industrially important chemicals such as alcohol, organic acids and antibiotics. Microorganisms are also responsible for food fermentations thus enhancing their quality and shelf life. Microorganisms do cause several disease in animals including humans and plants and spoil food but the beneficial role they play in our daily life camouflage their harmful effects. Since the techniques employed in microbiology may be new to many of you, you ought to develop perfection. The expertise in these techniques will determine not only the success or failure in your scientific probing but these will be essential to protect you and others around you against potential hazards. You may or may not be working with pathogenic microorganisms but it is essential to observe certain protective measures while working in a microbiological laboratory. Some hints given below shall be observed faithfully for the sake of your health and safety.

- 1. Students should come in the laboratory with clean laboratory aprons. Aprons should be sterilised in an autoclave before sending for wash in the laundry.
- 2. Do not bring/leave valuables in your purses.
- 3. Food must not be eaten in the laboratory.
- 4. Do not use laboratory ware for drinking of water, tea, etc.
- 5. Keep papers, pencils, fingers and other objects out of the mouth.
- 6. Observe all possible cleanliness and neatness in the care of apparatus, microscopes and desks.
- 7. Whenever a living culture is spilled, bring to the notice of instructor incharge. Cover it with some disinfectant.
- 8. All noninfectious solid wastes, such as cotton, paper, matches, etc. should be placed in the dustbin provided for the purpose.
- 9. All the cultures should be autoclaved before discarding.
- 10. The inoculation needle used in transferring culture, should be sterilized (red hot) over the flame before and after use. Flame the wire vertically not horizontally.
- 11. In case of personal accident such as cutting or pricking of the finger or splashing of culture material in the eye, report immediately to the instructor.
- 12. At the end, put away all apparatus and clean the desk top with disinfectant.

- 13. Wash your hands thoroughly with soap and water.
- 14. Before leaving the laboratory, check that the gas and the water taps are turned off.
- 15. Keep a record of each day's work, with drawings in a laboratory notebook.

PREPARATION OF CHROMIC ACID (CLEANING SOLUTION)

Dissolve 63.0 g potassium dichromate by heating in 350 ml distilled water, cool and add concentrated H_2SO_4 into it to make the volume to 1000 ml. Sulphuric acid should be added along the side of the flask slowly. Handle the solution carefully and use rubber gloves and apron.

CLEANING OF GLASSWARE

New glassware: Prepare 1% solution of a good glass cleaner such as teepol/liquid detergent. Place new glassware like test tubes, Petri dishes, pipettes and flasks into the solution and heat to boil. Remove the glassware and rinse thoroughly with warm tap water. Allow to dry before use.

New microscope slides: Immerse new slides in alcohol containing hydrochloric acid (3%) for several hours. Remove the slides and rinse thoroughly with tap water and finally in distilled water. Wipe the slides dry with soft cotton cloth. While wiping hold the slides by their edges to prevent recontamination with grease. Keep the slides in a covered jar till use.

New coverslips: Coverslips are also cleaned free of grease by the method described for the new microscope slides.

Used glassware: The used glassware and containers should be discarded in 3% lysol or any other disinfectant. All the glassware must be sterilised in an autoclave prior to washing. This must be followed regardless of whether the glassware is contaminated with pathogenic or saprophytic organisms. After sterilisation, empty the glassware of its contents. Put melted agar into dustbin provided for the purpose. Do not put melted agar into the sink as it will solidify on cooling and shall block the drainpipes. Now wash the glassware like new glassware described earlier.

For biochemical work immerse the washed glassware in potassium dichromatesulphuric acid solution overnight, remove, wash and rinse in hot water for 3 to 4 times.

Pipettes: Place the used pipettes in 3% lysol solution. If necessary keep immersed in chromic acid overnight, wash first with tap water and than with distilled or deionized water.

Used slides and coverslips: Place slides and coverslips in a pan containing 1% solution of a good cleaner. Heat till boiling and keep for a few minutes. Remove the material, rinse thoroughly in warm tap water and finally in distilled water. Wipe dry with a soft clean cloth using the same precautions as given under new slides and coverslips. Store in a covered jar.

SPECIFIC HAZARDS

Handling of alcohol: Both ethyl as well as methyl alcohol is highly inflammable. Keep containers of alcohol covered and away from open flame.

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Breakage of glassware: In case a glass object breaks, do not pick the pieces of glass with bare hands. Sweep the pieces with a broom and dustpan and put into the containers marked for this purpose. However, be careful if a thermometer breaks and report to the instructor.

Electrical equipment: Ensure that your hands are dry when handling electrical connectors. Do not allow water or any other liquid to come in contact with electrical cords and conductors.

FIRE

- 1. If you smell a leakage of gas, open the doors and windows, ensure all gas taps are tightly closed. Do not switch on any electrical equipment or burn a matchstick until the room is free of the gas.
- 2. If gas burns from a leak, turn off the gas tap.
- 3. In case of minor fire, smoother it with a towel or book.
- 4. If a larger fire occurs, use fire extinguishers in the laboratory to put it out.
- 5. In cases of large fire involving the laboratory itself, evacuate the room after turning all gas burners off and unplugging the electrical equipment.

ACCIDENTS

- 1. Report all accidents to your instructor immediately.
- 2. For spills in or near eyes, use eyewash.
- 3. For larger spills on the body, use shower.
- 4. For heat burns, chill the affected part with ice immediately.
- 5. In case of cut or abrasion, use bandage.