Medical Chemistry and Biochemistry

PRACTICAL LESSONS

Block Course on
Molecular Biology &
Immunochemistry

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Martin Vejražka
SAFETY IN THE LABORATORY

Work in a laboratory involves certain degree of risk: accidents can and do happen. Strict adherence to the following rules is essential for preventing – or minimising – the effects of accidents.

1. Students are obliged to prepare for each practical lesson in advance. Pay your full attention to all instructions, written or spoken, before and during your work. Do not start until you are sure you have familiarised yourself with the meaning and underlying principles of the scheduled experiments. Never perform any unauthorised experiment.

2. Eating, drinking and smoking in the laboratory is strictly forbidden!!! Wash your hands before eating outside the lab.

3. Wearing laboratory coat is required.

4. Every student works at his/her working place, where he/she is obliged to keep everything clean and in order. Do not leave hot glass, used pipettes, droppers etc. on the bench. When putting the reagent bottle stoppers (plugs) on the bench, always take care to avoid contamination of bench with the reagent. When using some reagent, always return the bottle to its place immediately after use.

5. In case of spillage of some combustible liquid, gas burners must be immediately put out, electricity switched off, the room provided with good ventilation, and the combustible removed by absorption into a porous material, which is then disposed to a safe place.

6. Spilled acid is immediately diluted with water or sodium carbonate, and washed out with water. Spilled alkali is washed out with water.

7. Glass fragments and any other trash with sharp edges must be disposed into cans marked and designated for this purpose.

8. Most compounds used in the laboratory are toxic. Concentrated solutions of acids and alkali are extremely corrosive. Work with concentrated acids, alkali and some organic solvents requires maximal possible care. Use the plastic shield (eye protection) for experiments with concentrated acids. Regular glasses are not adequate substitute. Contact lenses should never be used in the laboratory, because many volatile substances can react with them and ultimately damage the eyes.

9. Wear latex gloves for work with biological material. Students will be always notified in advance if the scheduled experiments include work with biological material.

10. Always use a bulb or a pump to draw liquids into a glass pipette.

11. Any work with volatile, fuming, irritating, or highly toxic substances is to be restricted only to the fume chamber.

12. When trying to smell gaseous reaction products, always waft it to your nose by a hand movement above the container. Avoid any straight inhalation of toxic gases.

13. Be extremely careful when working with the gas burner (open flame) and objects that have been heated (tubes, asbestos grid etc.). It is forbidden to leave any burner flame unattended. If the flame jumps into the burner, or suddenly goes out, close the gas inlet immediately and readjust the burner.

14. When heating a test tube solution in the flame, direct the tube mouth always out from your face, as well as other persons around.

15. Dispose all solutions and chemicals as instructed. Solutions that can be disposed to the sink must be always thoroughly diluted: solvents miscible with water about 1:10, aqueous acids or alkali about 1:30.

   Solvents immiscible with water, explosive substances, concentrated acids and bases, and substances that with water, acids or alkali generate toxic or irritating gases, are not allowed to be discharged into the sink. Dispose them according to instructions from your teacher.

16. Notify your instructor immediately in case of any injury or any other unexpected event.
WORK WITH ETHIDIUM BROMIDE

Ethidium bromide is an intercalating dye used for detection of DNA in electrophoretic gels. It wedges into the double helix of DNA and may interfere with its function. Ethidium bromide is a suspected cancerogen, mutagen and teratogen. Observe the following instructions when working with this compound:

1. Work carefully, think over the procedure.
2. Avoid accidental spilling of solutions. Let the test tubes with ethidium bromide open for as a short time as possible.
3. All solutions and gels containing ethidium bromide are treated as a hazardous waste and collected in special containers. This applies even for electrophoresis buffer in which the stained gel was immersed.
4. Use gloves. Soiled gloves are to be changed immediately, avoid spreading ethidium bromide by touching other things!
5. Keep the working place clean – it allows you to notice immediately that the desk was spoiled with a solution containing ethidium bromide.
6. Objects contaminated with ethidium bromide are to be cleaned with acidic solution of potassium permanganate or chloramine.
7. Inform immediately your assistant professor about any accident, for example contamination of hands, spilling solution of ethidium bromide etc. Teaching staff has special guidelines on procedures for these situations.

WORK WITH ACRYLAMIDE

Acrylamide is used to prepare polyacrylamide gels. It can be absorbed by skin or by inhalation and may cumulate in the body. Acrylamide acts as a neurotoxic and cardiotoxic substance. Working with acrylamide:

1. Use gloves. The gloves must be immediately changed in case they are soiled – acrylamide can penetrate them while exposed for a longer time.
2. Remnants of the acrylamide solution are to be treated as a toxic waste. On the other hand, solid polyacrylamide gel is non-toxic.
3. Avoid accidental spilling of the solution. Close the vials with acrylamide immediately after use. Eventually spilled solution should be absorbed with tissue cloth and discarded into toxic waste.
4. Inform immediately your assistant professor about any accident, for example contamination of hands, spilling solution of acrylamide etc. Teaching staff has special guidelines on procedures for these situations.

WORK SAFETY WITH ELECTROPHORESIS

Instrumentation for electrophoresis is constructed to minimalise the risk of electric shock, even in case of an unaware or incorrect manipulation. Despite it, use of electrophoresis and accessories requires special attention and observing some security rules.

1. Follow the procedure and instructions of teacher. Check that the apparatus is in a good condition and inform the teacher about any defect (or even a suspicion on a defect). It is forbidden to work with a damaged instrument.
2. Do not overfill the electrophoretic chamber above the limit. Outer surface of the chamber, the lid and the surroundings of the instrument must be dry.
3. When switching on, first close the lid, then connect cables into the outlet of power supply. Note the colour coding: red cable to red outlet (+), black to black (–). Turn the voltage regulator to the left stop (i.e. 0 V on the output) and switch on the power supply. Finally, set the required voltage.
4. When switching off the electrophoresis, the procedure is exactly the reverse: first, set the voltage to zero, then switch off the power supply, disconnect both cables and finally remove the lid.
5. Do not touch the electrophoresis, power supply or cables with wet hands.
6. Do not move the electrophoresis under voltage in order to prevent splashing buffer over edges of the chamber.
7. Power supply must be connected directly to the electrophoretic chamber. Do not try any unauthorised connection (e.g. serial interconnecting of several power supplies).
LABELLING DANGEROUS REAGENTS

International signs are used for labelling dangerous stuffs. A simplified description of these signs is provided here. Symbols listed here are used throughout the text, it is however not possible to mark the reagents in the student lab with them – micro-test tubes are too tiny for such labels.

<table>
<thead>
<tr>
<th>Symbol</th>
<th>Code</th>
<th>Description</th>
</tr>
</thead>
<tbody>
<tr>
<td>F+</td>
<td>Extremely flammable. Vapours blaze up immediately when in contact with fire under ambient conditions.</td>
<td></td>
</tr>
<tr>
<td>F</td>
<td>Very flammable. Compounds that blaze up after a short contact with incendiary source.</td>
<td></td>
</tr>
<tr>
<td>O</td>
<td>Oxidising. May ignite flammable materials, support fire.</td>
<td></td>
</tr>
<tr>
<td>T+</td>
<td>Very toxic. Substances that may cause death or harm to health when inhaled, swallowed or penetrated into skin in a very small amount.</td>
<td></td>
</tr>
<tr>
<td>T</td>
<td>Toxic. Substances that may cause death or harm to health when inhaled, swallowed or penetrated into skin in a small amount.</td>
<td></td>
</tr>
<tr>
<td>C</td>
<td>Corrosive.</td>
<td></td>
</tr>
<tr>
<td>Xi</td>
<td>Irritating. Substances that do not have properties of corrosives, nevertheless upon prolonged or repetitive contact may damage skin.</td>
<td></td>
</tr>
<tr>
<td>Xn</td>
<td>Harmful. Substances that do not have properties of poisons but may cause harm to health or death. Carcinogenic, mutagenic and similar substances are also marked with this sign.</td>
<td></td>
</tr>
<tr>
<td>N</td>
<td>Dangerous to environment.</td>
<td></td>
</tr>
<tr>
<td>E</td>
<td>Explosives.</td>
<td></td>
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</table>

FIRST AID IN LABORATORY ACCIDENTS

Corrosion of the eye: If an acidic or alkali solution gets into the eye, immediately wash the eye with plenty of tap water and seek medical help. Do not use any neutralising solutions.

Corrosion of the skin: Remove the stained clothes, and wash with plenty of tap water. Then neutralise:
- for acid burns use cca 6% sodium carbonate
- for alkali burns use cca 2% citric or acetic acid
Cover the damaged skin with a sterile bandage.

Burns: As soon as possible cool the affected area of the skin with a lot of cold tap water. Then, do not apply anything else but sterile bandage. Only very light burns can be treated with a liniment and covered.

Open wound: Stop bleeding and prevent wound infection. Small wounds (cuts by pieces of glass) should be washed with stream of water; and sterile cover provided.

Inhalation of harmful substances: Move the victim to fresh air, loosen the clothes, and start resuscitation if necessary. Even after seemingly successful treatment seek medical help.

Ingestion of harmful substances: Drink about 0.5 litre of water, or equal amount of 2% sodium carbonate (ingestion of acid) or 1% acetic or tartaric acid (ingestion of alkali). Seek medical help.
INFORMED CONSENSUS

I, the undersigned

name

agree with determination of my genotype and phenotype of the blood group system AB0 during practical laboratory course hold at the Institute of Medical Biochemistry, 1st Faculty of Medicine, Charles University in Prague. For this purpose I agree with taking a sample of capillary blood from ball of a thumb and smear of buccal mucosa. Genetic examination of DNA will be performed to assess the genotype.

I understand that if I know or learn in the future the blood group of my relatives the result of DNA test may be informative with regard to determination of paternity.

I am acquainted in detail with the protocol that will be used for tests, comprehend it and have no challenges against it.

I am informed that I may refuse the tests at any time, even in the course of the experiment. It would not be necessary to substantiate such a repeal and no consequences would follow for me.

The Ethical Committee of the General Faculty Hospital, Ref. No. 465/04 S, approved the protocol of experiment for purposes of lessons at the Institute of Medical Biochemistry.

Prague, (date)

Signature