Lab Skills: Biochemistry

Code: SKI2086

2017/2018

Period 2

Contents

General information

Research-based learning is the core of the lab skills. Students are required to find the theory linked to a practical assignment and to come up with their own protocols. The goal of this set-up is that students learn to think about the background of experiments and the practical execution.
The practical sessions take place at the Randwyck campus: Universiteitssingel 40 and 50, 6229 ER Maastricht.
The appendix in this course manual contains information on how to work safely in the lab. Read this information prior to each practical and make sure you understand every detail and work according to the safety rules and regulations.

Aims of the skills training

The aim of this skills training is to familiarize students with skills and knowledge concerning biochemistry. The course starts with an introduction into Good Laboratory Practice (GLP) and Safe Laboratory Practice (SLP). Students perform biochemical experiments on several different topics.

Attendance requirement

The attendance requirement for the 7 meetings (opening lecture, tutorial meeting and practical sessions) is 100%. There is no possibility to make up for a missed meeting by means of an additional assignment. If you don't meet the attendance requirement, you are not eligible for a resit.

Assessment

In this course there are several elements of assessment:
1. written protocol proposals (in groups of two students) and lab journal entries for each practical (⅓) – for more information, see below;
2. written lab report (in groups of two students) for 1 practical (⅓) – for more information, see below;
3. a protocol that is written individually at home and has to be executed in the lab. This practical is finished with a question list (1 per individual student) (⅓)

Resit

Students who initially fail the course, but who have complied with the compulsory attendance requirement and took part in all of the assessment during the course, are eligible for one resit.
The overall grade for this course consists of a grade for the written protocol proposals (⅓), a grade for one written lab report (⅓) and a grade for the final practical and question list (⅓). The course coordinators will decide on the format of the resit. The resit serves the purpose of lifting your overall grade to sufficient/above 5.5. It does not replace the overall grade.
In order to receive a grade for the assessment you will have to do a serious attempt at passing the assessment. If it is not deemed a serious attempt you will not receive a grade and you will not qualify for a resit.

Evaluation

At the end of each course and skills training you are given an evaluation form. It is of the utmost importance that you fill out the evaluation forms. Although it might not be visible to you, the courses undergo regular revision based on your comments on the evaluation forms. As an example, the workload in SKI2086 Lab Skills Biochemistry has been diminished, the amount of deadlines have been decreased, the assessments of the lab reports has been changed and a session on how to write a proper lab report has been incorporated in the schedule, all based on last year evaluations.
So please remember to fill out and hand in the evaluation forms that you will be given; not only for this skills training, but also for other courses.

Writing Assignments

Protocol proposals

Each practical team needs to hand in a protocol proposal (preferably about three/four A4 pages) for each practical. The proposal should include a short section on the theory behind the experiment, the aim, and a clear and concise practical protocol containing all steps taken during the experiment (for deadlines, see schedule). All students receive feedback on their proposal. Students, who fail the proposal, are allowed a second chance (for deadlines, see schedule). In case of a pass, students are allowed to join the practical. However, if students fail the second proposal, they are not allowed to take part in the practical. Exclusion of one practical can be compensated by an additional assignment.

Lab Journal

All scientists working in a laboratory keep a lab journal which will be provided. This is a book in which all experimental details and (raw) data and results are written down. In this Lab Skills a lab journal will have to be kept by each pair of students. The lab journal should be kept tidy and organized. Most scientists keep their journal in chronological order, which seems most suited in this case.

Practical reports

Two of the practical sessions are concluded by a practical report written by each practical team. The first report is a so-called practice report, on the practical session 'Electrophoresis and MALDI-TOF spectrometry'. This report is discussed during a tutorial group meeting to indicate possible improvements and corrections. The second report is on the practical session 'Enzymes'. This report is marked and counts towards ⅓rd of your final grade.
The practical descriptions in the course manual contain some guidelines as to what should be included in the reports. In general, a report should contain the following sections:

- Front page: *name, ID#, experiment, experimental date, report date, tutor*
- Title
- Abstract/summary: *a brief summary of the experiment, including the hypothesis and the main findings*
- Introduction; *includes the theory behind the experiment, the aim of the experiment, the hypothesis, the (theoretical) result that you expect to find*
- Experiment; *includes the used materials and apparatus (specifications, brand, manufacturer, serial or registration number - when a strange result is found in an experiment, the used apparatus can be traced, and it can be checked whether the problem lies in the apparatus.), the methods used (the protocol in (academic) scientific language)*
- Results: *includes observations, data, calculations, error calculations, etc.*
- Discussion & Conclusion: *includes an explanation of the results in the light of the hypothesis (Do the results meet the expectations of the hypothesis? How can the difference between hypothesis and actual findings be explained?)*
- Literature: *includes all used sources (when you use information from a scientific article, university textbook or internet source, always include the reference. Not doing so, is a form of plagiary.)*

For more information and tips and tricks on how to write a proper lab report, take a look at: http://labwrite.ncsu.edu/index_labwrite.htm

Course coordinators

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Schedule (preliminary, please check for changes on EleUM)

Day	time	activity	Topic	Location
Wed 1 Nov	8:30-13:00	Lecture + introduction 1st practical topic	Lab safety and general information; Introduction and practice session: how to use a pipet / how to calculate and pipet dilutions / protein determination and quantification	G6.204 UNS50
Fri 3 Nov	17:00	Deadline protocol	Cholesterol	
Mon 6 Nov	12:00	Feedback protocol	Cholesterol	
Tue 7 Nov	12:00	Deadline protocol 2nd chance	Cholesterol	
Wed 8 Nov	8:30-13:00	Practical session	Cholesterol	C4.556 UNS40
	12:30-13:00	New topic	Electrophoresis and MALDI-TOF	
Fri 10 Nov	17:00	Deadline protocol	Electrophoresis and MALDI-TOF	
Mon 13 Nov	12:00	Feedback protocol	Electrophoresis and MALDI-TOF	
Tue 14 Nov	12:00	Deadline protocol 2nd chance	Electrophoresis and MALDI-TOF	
Wed 15 Nov	8:30-13:00	Practical session	Electrophoresis and MALDI-TOF	C4.556 UNS40
	12:30-13:00	New topic	Carbohydrates - sugars	
Fri 17 Nov	17.00	Deadline protocol	Carbohydrates - sugars	
Mon 20 Nov	12.00	Feedback protocol	Carbohydrates - sugars	
Tue 21 Nov	12.00	Deadline protocol 2nd chance	Carbohydrates - sugars	
Tue 21 Nov	22.00	Deadline lab report trial	Lab report Electrophoresis and MALDI-TOF	
Wed 22 Nov	8:30-13:00	Practical session	Carbohydrates - sugars	C4.556 UNS40
	12:30-13:00	New topic	Enzymes	
Fri 24 Nov	17:00	Deadline protocol	Enzymes	
Mon 27 Nov	12:00	Feedback protocol	Enzymes	
Tue 28 Nov	12:00	Deadline protocol 2nd chance	Enzymes	
Wed 29 Nov	8:30-13:00	Practical session	Enzymes	C4.556 UNS40
Wed 6 Dec	10:00-12:30	Tutorial Meeting	Feedback 1st lab report + discussion results Practical on Enzymes	G6.204 UNS50
	12:30-13:00	New topic	DNA isolation	
Fri 8 Dec	17:00	Deadline protocol (graded; individual)	DNA isolation (graded; no feedback)	
Wed 13 Dec	8:30-13:00	Graded Exp. Practical session Question list (individual)	DNA Isolation	C4.556 UNS40
Wed 21 Dec	23:59	Deadline 2nd practical report	Enzymes	

Note that this is a preliminary schedule. Please check the announcements on EleUM on a regular basis.

Assignments

Available chemicals/solutions/materials:

- Bovine serum Albumin (10 mg/ml in PBS)
- γ-globulins (10 mg/ml in PBS)
- Quickstart Bradford assay reagent
- 1 x PBS
- 1 M Hepes-buffer pH 6.5
- 1 M Tris-buffer pH 8.0
- 1 M Tris- buffer pH 9.2
- 0.5 M Hepes- buffer pH 7.4
- 5 N NaOH
- Saturated NaCl
- Anhydrous sodium carbonate
- Sodium citrate
- Sinapic acid in '50% acetonitrile in water/0.1% TFA' (matrix for MALDI spectral analysis)
- Copper(II) sulfate pentahydrate
- 10% HCl in water
- 10% NaOH in water
- pH indicator strips
- Accutrend plus system + sterile sticks + test strips for glucose measurement
- Finger stick lancing device (sterile)
- Glucose solution: 75 g in 200 ml water
- 1 M $MgCl_2$
- 0.02 % $CaCl_2$
- 0.5 M EDTA
- Amylose, glycogen, glucose, sucrose, lactose, fructose: 2% solutions
- Na_2SO_4 (anhydrous)
- Sulfuric Acid
- Acetic Anhydride
- Para-nitrophenylphosphate (pNPP)
- Iodine reagent
- 10 % SDS (sodium dodecyl sulfate)
- 2% carbohydrate solutions: glucose, fructose, sucrose, lactose, starch
- Glacial acetic acid
- SYBR gold nucleic acid gel stain
- Resorcinol (for Seliwanof's test)
- 5 x sample buffer for SDS-PAGE
- 1 M Benzamidine
- 40 mg/ml PMSF (phenyl methyl sulfonyl fluoride)
- 4-12 % polyacrylamide gels (or 'any kD' - gels)
- 10 x running buffer for SDS-PAGE
- Molecular weight marker for SDS-PAGE: BioRad dual color prestained broad range
- Chloroform
- UV light source
- Isopropanol
- Ethanol
- Methanol
- DNA molecular weight marker
- Coomassie brilliant blue staining solution
- Coomassie destaining solution
- 96-well microtitre plates
- Hydrochloric acid (HCl)

1) Introductory lab: Proteins and protein quantification

Proteins are vital building blocks for the cell. They consist of amino acids and form higher-order structures. The structure of proteins is essential for a correct function and determines the survival of the cell. In each cell, a different array of proteins is produced that determines the function of the cell within the body.
Many scientists work on proteins and their functional aspects and isolate proteins directly from cells and tissues. One of the first things these scientists determine is the concentration of the proteins solution they obtain from their isolation procedures. The question is: "how much protein do I actually have?"
In this part of the lab skills you will have to isolate protein-fractions from blood and blood cells and determine the concentration of the obtained protein solutions. Another issue is the accuracy and confidence in the obtained results. How sure are you of the concentration you just determined? Think about how you want to present numerical data in general, so that a non-expert reader can understand how accurate the data are or how confident you are about the numbers you present.

To do:

1) This is your first practical session. As no protocol proposals could be prepared, a working protocol is given to you. **Before you start, read the protocol thoroughly**. It contains both safety aspects of the reagents you will be working with and all the important steps of the procedure to be executed. Calculations are not in this protocol and should be done by the students before starting the experiment.
2) Prepare a calibration curve. The available standard protein solution is 2 mg/ml BSA. Dilutions are made in phosphate buffered saline (PBS). The standard curve should contain following dilutions: 0-125-200-250-500-750-1000 µg/ml BSA. Calculate dilutions for this protein calibration curve, create a pipetting scheme and show it to the tutor prior to getting started.
3) Dilute unknown protein(s) 10-100-1000 times in PBS. Why diluting the unknown samples?
4) Add Coomassie Brilliant Blue staining solution and incubate for at least 5 min for color stabilization.
5) Measure absorbance at 595 nm.
6) Plot protein concentration of the standards against the corresponding absorbance, use linear regression to calculate the concentration of the unknown protein samples. Optionally, during the laboratory session you can practice your drawing skills on millimeter paper, old school method but still very efficient for understanding curves and linear regression. Ask the lab instructor to help you out on this.

Literature:

- M.M. Bradford. (1976) Rapid and sensitive method for the quantification of microgram quantities of protein utilizing the principle of protein-dye binding. Anal Bioch 72; 248-254.
- Biochemistry, Garrett & Grisham, 4th edition: chapter 4, 5.
- EleUM

2) Cholesterol

Cholesterol has been in the spotlight over the past decades because of its implications on human health. It has been widely recognized that high blood cholesterol levels are a risk factor for cardio-vascular disease. Therefore there has been a trend to reduce fat and especially cholesterol content in the diet. But one should not forget that cholesterol is actually a vital component of the human body. Cholesterol is a precursor of many important molecules like hormones and also plays an important role in cell membranes. In this part of the lab skills biochemistry we will attempt to measure cholesterol in a range of samples, to get an idea about how much cholesterol is in different samples. There are many different methods to measure cholesterol, and some are even highly automated. These methods are used in the hospital to monitor cholesterol levels in patients. We will use a chemical method that is described in the publications that you can find on EleUM.

To do:

1) Extract a work protocol from the available information (EleUM and literature).
   - Give the reactions and describe how the method works.
   - Describe how you intend to quantify the levels of cholesterol in the measured samples.
   - Prepare a pipetting scheme to show in detail how you will conduct this experiment
   - Take a thorough look at the chemicals in this practical. How can you deal with them safely in the laboratory?
2) Determine cholesterol levels from
   1. egg yolk,
   2. egg white
   3. piece of bacon
   4. walnut
   5. margarine
   6. olive oil
   7. whatever you would like to test: your daily peanut butter or cheese or….
3) Calculate how much cholesterol is in the different samples. Make sure your lab journal is updated with calculations and results (in a table). We will check this during the next practical session!
4) What are your expectations? What can you conclude from the results with respect to your own diet or in general the diet of people in the Western world?

Literature:

- Biochemistry, Garrett & Grisham 4th edition, chapter 24.4 : How is cholesterol synthesized?
- Biochemistry, Berg, Stryer 8th edition,Biosythesis of cholesterol p776-788
- T.C. Huang, C.P. Chen, V. Wefler, A. Raferty. (1961) A stable reagent for the Lieberman-Burchard method. Anal Chem 33(10); 1405-1407.
- M.C. Barreto. (2005) Lipid extraction and cholesterol quantification: a simple method. J Chem Educ 82(1); 103-104.
- EleUM

3) Protein electrophoresis and MALDI-TOF mass spectrometry

Proteins mediate virtually every process that takes place in a cell, exhibiting an almost endless diversity of functions. To explore the molecular mechanism of a biological process, a biochemist almost inevitably studies one or more proteins.
A well-known globally used biochemical technique to study/analyze proteins is protein electrophoresis. In this practical session we will focus on (a specific kind of protein electrophoresis called) SDS-PAGE, which is a method to separate proteins on basis of their size i.e. molecular weight. The proteins are separated with the use of a polymerized acryl-amide gel and an electric field across this gel. The SDS (sodium dodecyl sulfate) is required to make this method work.
You will receive (partially) purified unknown proteins. Using SDS-PAGE conclusions can be drawn on size and purity of the proteins.
MALDI-TOF is, as SDS-PAGE, a lab procedure to determine the protein's molecular weight. Proteins mixed with a specific matrix can be ionized by laser irradiation. The resulting 'protein-ions' are analyzed with a time-of-flight tube. Mass spectrometry results in various interesting applications and advantages for the research of biochemists.
Why would we have 2 methods for the same outcome? What are advantages/disadvantages for each?

To do:

1) Describe the principles of SDS-PAGE (SDS polyacrylamide gel electrophoresis).
2) Describe a detailed protocol how to run an SDS-gel with the BioRad gel electrophoresis system followed by Coomassie blue staining and destaining.
   How will you analyze the gel and more specifically: how can you calculate/determine the molecular weight of the proteins on the gel?
3) Briefly describe the principles of the MALDI-TOF mass spectrometry technique. What are pro's and con's with respect to SDS-PAGE?
4) Write an elaborate (practice) report on SDS-PAGE.

Literature:

- Biochemistry, Berg, Stryer 8th edition, chapter 3.1
- Biochemistry, Garret & Grisham chapter 5 appendix, chapter 8
- EleUM

4) Carbohydrates - sugars

Carbohydrates (saccharides) are the single most abundant class of organic molecules found in nature. They exhibit many functions: energy source (glucose) and energy storage (glycogen), structural components (cellulose) , recognition (ABO system, glycoproteins, glycolipics),…
A carbohydrate is a biological molecule consisting of carbon (C), hydrogen (H) and oxygen (O). They can be looked upon as hydrates of carbon but from a structural point of view they can also be termed polyhydroxy aldehydes and ketones. The latter chemical groups can be utilized to measure concentration of carbohydrates in solution.
Carbohydrates are generally classified into four groups: monosaccharides, disaccharides, oligosaccharides and polysaccharides. Monosaccharides are the simple sugars, examples are glucose (also known as dextrose)-, fructose and galactose. Together with disaccharides, monosaccharides are commonly referred to as sugars. Well known disaccharides are lactose (milk) and sucrose, also termed saccharose, which is derived from sugarcane or sugar beet. Sucrose consists of a D-glucose attached to a D-fructose by a glycosidic linkage. It is the most abundantly used sugar in our food industry, well appreciated for its sweet taste.
Oligosaccharides consist of 2 to 10 monosaccharides which can be coupled to other molecules such as glycoproteins and glycolipids. As their name suggests, polysaccharides are polymers of more than 10 monosaccharides. They may contain hundreds or even thousands of monosaccharides. Important molecules are starch, cellulose, glycogen and chitin.

The monosaccharide glucose is critical to human life and its level in blood is kept constant within a narrow range. Polypeptide hormones such as insulin and glucagon regulate glucose levels in blood. Disturbances in regulation can result in diseases such as diabetes. A simple test to screen for dysregulation of glucose levels is the oral glucose tolerance test (OGTT). This test is used routinely in hospitals to screen individuals.

To do:

1) Look for 3 methods which you can use to observe physical and chemical properties of some common carbohydrates: glucose, fructose, lactose, sucrose, amylose and glycogen. The reagent list will help you in finding the correct tests.
2) Describe each method briefly showing us that you understand what is going on in your test tube, give the chemical reaction for each test.
3) Describe the OGTT , write the protocol to perform this test in detail. What are the expected results and what do these results tell you?
   Important: incorporate special safety requirements for the finger stick in your protocol.
4) As a small side step:
   In the process of brewing beer and producing bread, yeast is used for fermentation of mono- , disaccharides and polysaccharides. Describe in short the standard fermentation reaction. Also describe and draw an experiment to show this reaction in the laboratory (most materials not in the list, 2 main possibilities).
   Can all of the sugars (from the list of chemicals) be fermented by baker's yeast? If not, which of the sugars cannot be fermented and why?
   Unfortunately due to time restrains we cannot perform a fermentation reaction in the laboratory so this is a theoretical exercise.

Literature:

- Garret & Grisham, 4th edition, chapter 7.
- Biochemistry, Berg, Stryer 8th edition, chapter 11
- EleUM

5) Enzymes

Enzymes, the catalysts of biological systems, are remarkable molecular devices that determine the pattern of chemical transformations. About a quarter of the genes in the human genome encode enzymes, a reflection of their importance in life. The most striking characteristics of enzymes are their *catalytic power* and *specificity*. Enzymes accelerate reaction rates as much as 10^{21} over uncatalyzed levels, which is far greater than any synthetic catalyst. Moreover, enzymes are very selective, both in the substances with which they interact and in the reactions they catalyze. The substances they catalyze are traditionally called substrates.

Enzymes are very dependent on environmental parameters for their activity. The pH, temperature and concentrations of salts are parameters that can strongly influence enzyme activity.
The kinetics of an enzyme can be described by the K_m and V_{max} of that enzyme for a particular substrate. In this part of lab-skills you are going to determine the K_m and V_{max} of a substrate for an enzyme. Therefore you will have to study the subject of enzyme kinetics and find out how in practice Km and Vmax can be determined. Also you will have to determine the effect of the environmental factor pH.

Enzyme of choice in this practical session: alkaline phosphatase. The substrate used is para-nitrophenyl phosphate (pNPP) which will give a nice yellow color when cleaved by alkaline phosphatase. This yellow color has an absorption maximum of 405 nm thus colour formation can be followed by spectrophotometry. For the determination of the enzyme activity you will need to convert the absorption data to actual amounts of produced substrate. TIP: use the Lambert-Beer law. What is the molar extinction coefficient for the yellow-coloured reaction product?

To do:

1) Describe in detail how you can determine the K_m and V_{max} of alkaline phosphatase for pNPP.
2) Write down a detailed protocol (with elaborate pipetting scheme) on how to determine the Km and V_{max}.
3) Describe how you can change the pH of the reaction in order to study the effect of the environmental factor pH. What are your expectations on enzyme activity upon pH-changing? Why? What is happening to the reaction/or reactants?
4) Write a thorough, elaborate lab report on this practical session. In the report give the exact calculation of the K_m and V_{max}. Also present the graphs you used in the calculation process.

Literature:

- Biochemistry, Garrett & Grisham, 4th edition, chapter 13.1-13.3
- Biochemistry, Berg, Stryer, 8th edition, chapter 8
- EleUM

6) DNA Isolation (= graded practical session – individual)

DNA is the molecule that contains the genetic information in all cells. The DNA is built up of nucleotides and is much more stable than the RNA that is produced in the cell. Therefore, scientists prefer to work with DNA. DNA can be located in the nucleus or the mitochondria. The genomic DNA is located in the nucleus. In recent years the isolation of DNA from small amounts of cells has become more and more common place, especially in forensic science. The DNA is used to determine the exact identity of the cell-donor; it acts as a genetic fingerprint. There are several methods to analyze the genomic DNA found at a crime scene.

We do not have the time to pursue forensic techniques, but we can perform isolation of DNA from different samples. We will have isolated cells from cell culture, (chicken or cow) bone fragments and we will use the famous mouth-swab as source for cells from which we will isolate DNA.

To do:

1) Produce a detailed protocol to isolate genomic DNA from different samples in the time available (= 2 hours). How can you determine the concentration and purity of the isolated DNA? When is the DNA considered: 'high purity'?
2) You will have to isolate DNA and describe how you would demonstrate the presence of DNA (see reagent list).
3) Thoroughly study the theory behind this procedure. Why use certain chemicals…for instance why use chloroform and why the ethanol? There will be a questionnaire (= written test; time = 30 min) after the practical session.
4) Make sure you work safely and accurately, this practical session is the summary of all skills learned in the previous sessions. Tutors walk around, (might) ask questions and watch your lab behaviour.
5) In the afternoon the concentration and purity of your samples will be measured by the tutor (with the 'nanodrop' which is not available in the laboratory). Results will be send to you by e-mail.

Literature:

- Biochemistry, 4th edition, Garrett & Grisham chapter 10 and 12
- Biochemistry, Berg, Stryer, 8th edition, chapter 5
- S. M. Ali, S. Mahnaz, T. Mahmood. (2008) Forensic Science International: genetics Supplement Series 1: 63-65. Rapid Genomic DNA extraction.
- EleUM

Appendix

Elementary laboratory rules

- Lab coats are available and should be worn **at all time** while present in the laboratory.
- It is **not** allowed to eat or drink in the laboratory.
- Safety glasses should be worn when working with chemicals and biological material.
- Gloves should be worn when working with chemicals and biological material. It is **not** allowed to wear gloves outside of the practicum room.
- Volatile liquid chemicals should only be handled in a fume-hood.
- Be present on time.
- Coats and bags should be stored in the lockers.
- Only the participating students are allowed access to the laboratory.
- Coffee or tea breaks are only allowed in consultation with the laboratory staff.
- At the end of each practical, laboratory tables need to be cleaned.

Safe Laboratory Practice (SLP)

In laboratories you always find a high concentration of hazardous substances. Materials that are poisonous (toxic) for humans, animals and plants, aggressive (caustic/corrosive) – like strong acids and bases – infectious (pathogenic/contaminated) – meaning that they contain micro-organisms that could cause disease in humans, animals or plants – inflammable and/or explosive, emitting radiation (UV, IR, electromagnetic, radioactive), very hot or very cold, or carcinogenic, teratogenic (causing damage to posterity) or mutagenic can all be present in a laboratory. Therefore it is essential to work safely. In order to be able to work safe, you need to be familiar with safety rules and regulations. Safe Laboratory Practice does not concern the experiment itself, but the person who is conducting it and everybody around. When you work in a safe way, you will be able to prevent many dangerous situations. A few guidelines can help to achieve SLP.

Personal conduct

- Eating, drinking, smoking, mobile chatting or using make up in the laboratory is **strictly forbidden**.
- Materials which are not required for the experiment should be kept in lockers. This includes mp3-players and mobile phones.
- Wearing a white laboratory coat is **obligatory**.
- Always keep you coat closed.
- Make sure the sleeves of you lab coat cover your own clothes.
- **Never** wear your lab coat outside the laboratory.
- Remove rings, watches, bracelets, etc.
- Long hair should be put together.
- **Always wear gloves** when working with chemicals or biological materials – **remember**: gloves do not offer complete protection, so keep working as careful as if you were not wearing them.
- **Always wear safety glasses** when working with chemicals or biological materials.
- Instructions from the laboratory staff should be followed immediately at all times.
- In case of accidents, immediately notify the laboratory staff; this includes accidents that seem harmless.
- In case of accidents involving eyes (e.g. chemicals in the eye), first flush with water, then warn the laboratory staff: "**first water, then bother**".
- **Always wash your hands** before leaving the laboratory; use disinfecting soap and disposable drying paper.

Work area

- **Clean and disinfect the working area** before each experiment.
- Put **only the necessary equipment** in the working area.
- **Always clear away unused materials**, not only at the end of the practical but also during the practical.
- **Immediately remove and clean spilled solutions or chemicals**.
- **Always close** bottles or jars immediately after using it.
- **Never** lift a bottle or a jar by its stopper, lid or neck.
- When using aggressive, volatile or inflammable substances, **always work inside a fume hood**.
- **Always make sure** that people around you know what you are working with.
- **Always** work in a logical, tidy manner, **minimizing risks**.
- **Always label or mark glassware** - write down the content (even if it is merely water) and the concentration.
- **Always remain focused** on your work. Carelessness produces accidents!

Waste handling

Proper segregation of laboratory waste is essential to good chemical hygiene and a safe workplace environment. You should aim to minimize the quantity of waste products and correctly dispose of all waste products. Many researchers often tend to put all of their wastes into the same cabinet or fume hood. Doing so can have disastrous results!
The guidelines for temporary storage of chemical wastes in the laboratory are really no different than those that you use for the storage of your usual lab chemicals. There are 6 main categories in chemical waste:

Category 1 **Acidic and neutral inorganic waste in solution**
Acidic laboratory waste (mixtures) Fixative Bleach fixative Sulfuric acid (diluted) Phosphoric acid (diluted) Other inorganic acids

Category 2 **Alkaline inorganic waste in solution**
Alkaline laboratory waste (mixtures) Inorganic bases Ammonia solution

Category 3 **Halogen-deficient organic waste**
Waste water containing organic substances Waste oil Paint Medicine waste Solvents Liquid alcohol

Category 4 **Halogen-rich organic waste**
Laboratory waste, organic, halogen-rich Methylene chloride Chlorinated aromatics

Category 5 **Special waste**
Waste containing heavy metals and metaloids Laboratory waste containing heavy metals Batteries Mercury-containing objects

Category 6 **Waste with exceptional risks**
Asbestos-containing objects Extremely corrosive waste (e.g. concentrated acids) Explosive substances Gas cylinders Organic peroxides Extremely toxic substances (beryllium/selenium/arsenicum) PCB/PCT-containing objects or substances

The most important rule is to make sure that any chemicals or wastes that stored together are **compatible** with each other. Therefore, proper segregation of wastes involves making sure that wastes within a bottle

are compatible. Only chemically compatible waste can be mixed together and placed in a common container for disposal.

NEVER store the following types of wastes near each other:

- Acids and bases.
- Organics and acids.
- Cyanide, sulfide or arsenic compounds and acids.
- Alkali or alkali earth metals, alkyllithiums etc. and aqueous waste.
- Powdered or reactive metals and combustible materials.
- Mercury or silver and ammonium containing compounds.

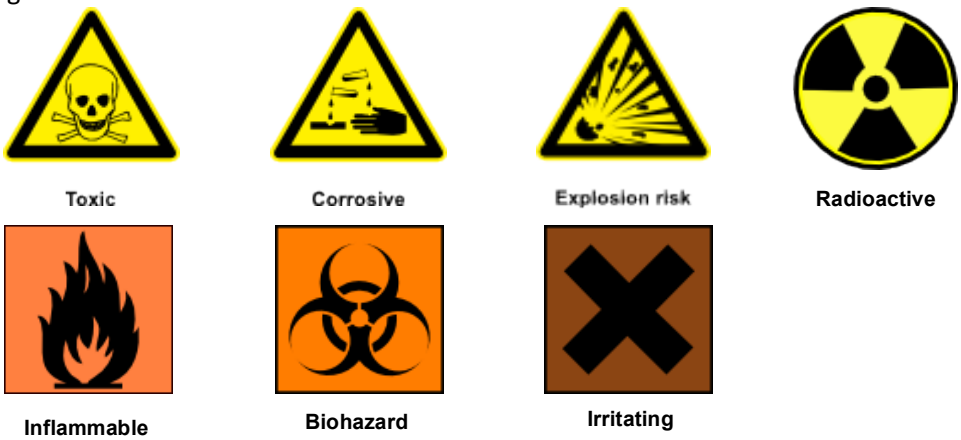
If a bottle broke in a waste storage area where incompatibles were present, the results could be disastrous. Remember: incompatible bottles of wastes should be stored in separate cabinets, preferably as far apart as possible.

The rules and regulations regarding various categories of waste products are presented below.

- All glassware must be rinsed with tab water and labels and marks must be removed before placing it into the white container.
- All reaction tubes must be rinsed with tab water. All contents must be removed – if necessary by using a tube brush – and put upside-down in the baskets.
- All stoppers must be rinsed and deposited into the containers marked with "VUIL".
- All glass pipettes must be rinsed with tab water and put upside-down into the pipette-holders.
- Chemical waste products must be stored in the appropriate containers (ask the laboratory staff).
- Risk waste products and all materials contaminated with those substances are disposed in special yellow bags (ask the laboratory staff).
- "Biohazard" waste products, i.e. biological substances, and all materials contaminated with those substances disposed in stainless steel sterilization jars, equipped with transparent plastic bags.
- All needles and scalpels are discarded in the small yellow/red containers present on the laboratory tables.
- Lab tables must be thoroughly cleaned and dried – when hazardous materials or bio-materials have been used, tables should be disinfected with 70% ethanol.
- All lab tables must be left neat and tidy (electrical instruments can remain plugged-in).

Chemical safety

You need to know what you are doing when working with chemicals. Therefore, it is essential to know where to find chemical information, risk indicators and safety advice. The category or risks of substances can be judged from the pictogram of logo displayed on bottles or jars. A few of the most frequently used logos are shown below:



In addition to these logos, bottles and jars usually contain safety risk codes, referring to Risk and Safety Statements. These statements contain information on risks (R-phrases) and safety (S-phrases). Additional information on chemical characteristics, risks and safety can be found in the "Merck Index".

R-phrases

R1	Explosive when dry
R2	Risk of explosion by shock, friction, fire or other sources of ignition
R3	Extreme risk of explosion by shock, friction, fire or other sources of ignition
R4	Forms very sensitive explosive metallic compounds
R5	Heating may cause an explosion
R6	Explosive with or without contact with air
R7	May cause fire
R8	Contact with combustible material may cause fire
R9	Explosive when mixed with combustible material
R10	Flammable
R11	Highly flammable
R12	Extremely flammable
R14	Reacts violently with water
R15	Contact with water liberates extremely flammable gases
R16	Explosive when mixed with oxidizing substances
R17	Spontaneously flammable in air
R18	In use, may form flammable/explosive vapor-air mixture
R19	May form explosive peroxides
R20	Harmful by inhalation
R21	Harmful in contact with skin
R22	Harmful if swallowed
R23	Toxic by inhalation
R24	Toxic in contact with skin
R25	Toxic if swallowed
R26	Very toxic by inhalation
R27	Very toxic in contact with skin
R28	Very toxic if swallowed
R29	Contact with water liberates toxic gas.
R30	Can become highly flammable in use
R31	Contact with acids liberates toxic gas
R32	Contact with acids liberates very toxic gas
R33	Danger of cumulative effects
R34	Causes burns
R35	Causes severe burns
R36	Irritating to eyes
R37	Irritating to respiratory system
R38	Irritating to skin
R39	Danger of very serious irreversible effects
R40	Limited evidence of a carcinogenic effect
R41	Risk of serious damage to eyes
R42	May cause sensitization by inhalation
R43	May cause sensitization by skin contact
R44	Risk of explosion if heated under confinement
R45	May cause cancer
R46	May cause heritable genetic damage
R48	Danger of serious damage to health by prolonged exposure
R49	May cause cancer by inhalation
R50	Very toxic to aquatic organisms
R51	Toxic to aquatic organisms
R52	Harmful to aquatic organisms
R53	May cause long-term adverse effects in the aquatic environment
R54	Toxic to flora
R55	Toxic to fauna
R56	Toxic to soil organisms
R57	Toxic to bees
R58	May cause long-term adverse effects in the environment
R59	Dangerous for the ozone layer
R60	May impair fertility

R61	May cause harm to the unborn child
R62	Possible risk of impaired fertility
R63	Possible risk of harm to the unborn child
R64	May cause harm to breast-fed babies
R65	Harmful; may cause lung damage if swallowed
R66	Repeated exposure may cause skin dryness or cracking
R67	Vapors may cause drowsiness and dizziness
R68	Possible risk of irreversible effects

Combinations of R-phrases

R14/15	Reacts violently with water, liberating extremely flammable gases
R15/29	Contact with water liberates toxic, extremely flammable gases
R20/21	Harmful by inhalation and in contact with skin
R20/22	Harmful by inhalation and if swallowed
R20/21/22	Harmful by inhalation, in contact with skin and if swallowed
R21/22	Harmful in contact with skin and if swallowed
R23/24	Toxic by inhalation and in contact with skin
R23/25	Toxic by inhalation and if swallowed
R23/24/25	Toxic by inhalation, in contact with skin and if swallowed
R24/25	Toxic in contact with skin and if swallowed
R26/27	Very toxic by inhalation and in contact with skin
R26/28	Very toxic by inhalation and if swallowed
R26/27/28	Very toxic by inhalation, in contact with skin and if swallowed
R27/28	Very toxic in contact with skin and if swallowed
R36/37	Irritating to eyes and respiratory system
R36/38	Irritating to eyes and skin
R36/37/38	Irritating to eyes, respiratory system and skin
R37/38	Irritating to respiratory system and skin
R39/23	Toxic; danger of very serious irreversible effects through inhalation
R39/24	Toxic; danger of very serious irreversible effects in contact with skin
R39/25	Toxic; danger of very serious irreversible effects if swallowed
R39/23/24	Toxic danger of very serious irreversible effects through inhalation and in contact with skin
R39/23/25	Toxic; danger of very serious irreversible effects through inhalation and if swallowed
R39/24/25	Toxic; danger of very serious irreversible effects in contact with skin and if swallowed
R39/23/24/25	Toxic; danger of very serious irreversible effects through inhalation, in contact with skin and if swallowed
R39/26	Very Toxic; danger of very serious irreversible effects through inhalation
R39/27	Very Toxic; danger of very serious irreversible effects in contact with skin
R39/28	Very Toxic; danger of very serious irreversible effects if swallowed
R39/26/27	Very Toxic; danger of very serious irreversible effects through inhalation and in contact with skin
R39/26/28	Very Toxic; danger of very serious irreversible effects through inhalation and if swallowed
R39/27/28	Very Toxic; danger of very serious irreversible effects in contact with skin and if swallowed
R39/26/27/28	Very Toxic; danger of very serious irreversible effects through inhalation, in contact with skin and if swallowed
R42/43	May cause sensitization by inhalation and skin contact
R48/20	Harmful; danger of serious damage to health by prolonged exposure through inhalation
R48/21	Harmful; danger of serious damage to health by prolonged exposure in contact with skin
R48/22	Harmful; danger of serious damage to health by prolonged exposure if swallowed
R48/20/21	Harmful; danger of serious damage to health by prolonged exposure through inhalation and in contact with skin
R48/20/22	Harmful; danger of serious damage to health by prolonged exposure through inhalation and if swallowed
R48/21/22	Harmful; danger of serious damage to health by prolonged exposure in contact with skin and if swallowed
R48/20/21/22	Harmful; danger of serious damage to health by prolonged exposure through inhalation, in contact with skin and if swallowed
R48/23	Toxic; danger of serious damage to health by prolonged exposure through inhalation
R48/24	Toxic; danger of serious damage to health by prolonged exposure in contact with skin
R48/25	Toxic; danger of serious damage to health by prolonged exposure if swallowed
R48/23/24	Toxic; danger of serious damage to health by prolonged exposure through inhalation and in contact with skin
R48/23/25	Toxic; danger of serious damage to health by prolonged exposure through inhalation and if swallowed
R48/24/25	Toxic; danger of serious damage to health by prolonged exposure in contact with skin and if swallowed
R48/23/24/25	Toxic; danger of serious damage to health by prolonged exposure through inhalation, in contact with skin and if swallowed
R50/53	Very toxic to aquatic organisms, may cause long-term adverse effects in the aquatic environment

R51/53	Toxic to aquatic organisms, may cause long-term adverse effects in the aquatic environment
R52/53	Harmful to aquatic organisms, may cause long-term adverse effects in the aquatic environment
R68/20	Harmful; possible risk of irreversible effects through inhalation
R68/21	Harmful; possible risk of irreversible effects in contact with skin
R68/22	Harmful; possible risk of irreversible effects if swallowed
R68/20/21	Harmful; possible risk of irreversible effects through inhalation and in contact with skin
R68/20/22	Harmful; possible risk of irreversible effects through inhalation and if swallowed
R68/21/22	Harmful; possible risk of irreversible effects in contact with skin and if swallowed
R68/20/21/22	Harmful; possible risk of irreversible effects through inhalation, in contact with skin and if swallowed

R-phrases no longer in use

R13	Extremely flammable liquefied gas.
R47	May cause birth defects.

S-phrases

(S1)	Keep locked up
(S2)	Keep out of the reach of children
S3	Keep in a cool place
S4	Keep away from living quarters
S5	Keep contents under ... (*appropriate liquid to be specified by the manufacturer*)
S6	Keep under ... (*inert gas to be specified by the manufacturer*)
S7	Keep container tightly closed
S8	Keep container dry
S9	Keep container in a well-ventilated place
S12	Do not keep the container sealed
S13	Keep away from food, drink and animal feeding stuffs
S14	Keep away from ... (*incompatible materials to be indicated by the manufacturer*)
S15	Keep away from heat
S16	Keep away from sources of ignition - No smoking
S17	Keep away from combustible material
S18	Handle and open container with care
S20	When using do not eat or drink
S21	When using do not smoke
S22	Do not breathe dust
S23	Do not breathe gas/fumes/vapor/spray (*appropriate wording to be specified by the manufacturer*)
S24	Avoid contact with skin
S25	Avoid contact with eyes
S26	In case of contact with eyes, rinse immediately with plenty of water and seek medical advice
S27	Take off immediately all contaminated clothing
S28	After contact with skin, wash immediately with plenty of ... (*to be specified by the manufacturer*)
S29	Do not empty into drains
S30	Never add water to this product
S33	Take precautionary measures against static discharges
S35	This material and its container must be disposed of in a safe way
S36	Wear suitable protective clothing
S37	Wear suitable gloves
S38	In case of insufficient ventilation wear suitable respiratory equipment
S39	Wear eye/face protection
S40	To clean the floor and all objects contaminated by this material use ... (*to be specified by the manufacturer*)
S41	In case of fire and/or explosion do not breathe fumes
S42	During fumigation/spraying wear suitable respiratory equipment (*appropriate wording to be specified by the manufacturer*)
S43	In case of fire use ... (*indicate in the space the precise type of fire-fighting equipment. If water increases the risk add -* ***Never use water***)
S45	In case of accident or if you feel unwell seek medical advice immediately (show the label where possible)
S46	If swallowed, seek medical advice immediately and show this container or label
S47	Keep at temperature not exceeding ... °C (*to be specified by the manufacturer*)
S48	Keep wet with ... (*appropriate material to be specified by the manufacturer*)
S49	Keep only in the original container
S50	Do not mix with ... (*to be specified by the manufacturer*)

S51	Use only in well-ventilated areas
S52	Not recommended for interior use on large surface areas
S53	Avoid exposure - obtain special instructions before use
S56	Dispose of this material and its container at hazardous or special waste collection point
S57	Use appropriate containment to avoid environmental contamination
S59	Refer to manufacturer/supplier for information on recovery/recycling
S60	This material and its container must be disposed of as hazardous waste
S61	Avoid release to the environment. Refer to special instructions/safety data sheet
S62	If swallowed, do not induce vomiting; seek medical advice immediately and show this container or label
S63	In case of accident by inhalation; remove casualty to fresh air and keep at rest
S64	If swallowed, rinse mouth with water (only if the person is conscious)

Combinations of S-phrases

(S1/2)	Keep locked up and out of the reach of children
S3/7	Keep container tightly closed in a cool place
S3/7/9	Keep container tightly closed in a cool, well-ventilated place
S3/9/14	Keep in a cool, well-ventilated place away from … (*incompatible materials to be indicated by the manufacturer*)
S3/9/14/49	Keep only in the original container in a cool, well-ventilated place away from … (*incompatible materials to be indicated by the manufacturer*)
S3/9/49	Keep only in the original container in a cool, well-ventilated place
S3/14	Keep in a cool place away from … (*incompatible materials to be indicated by the manufacturer*)
S7/8	Keep container tightly closed and dry
S7/9	Keep container tightly closed and in a well-ventilated place
S7/47	Keep container tightly closed and at temperature not exceeding … °C (*to be specified by the manufacturer*)
S20/21	When using do not eat, drink or smoke
S24/25	Avoid contact with skin and eyes
S27/28	After contact with skin, take off immediately all contaminated clothing, and wash immediately with plenty of … (*to be specified by the manufacturer*)
S29/35	Do not empty into drains; dispose of this material and its container in a safe way
S29/56	Do not empty into drains, dispose of this material and its container at hazardous or special waste collection point
S36/37	Wear suitable protective clothing and gloves
S36/37/39	Wear suitable protective clothing, gloves and eye/face protection
S36/39	Wear suitable protective clothing and eye/face protection
S37/39	Wear suitable gloves and eye/face protection
S47/49	Keep only in the original container at temperature not exceeding … °C (*to be specified by the manufacturer*)