

Practical test

Practical Test

General remarks about the practical tests

In practical tests you are expected to demonstrate that you know some basic methods, processes and techniques of biology and that you are able to use them for experimental purposes.
This will be tested in a series of tests, organised in four different laboratories.

Laboratory 1: Plant anatomy, morphology and taxonomy

Laboratory 2: Animal anatomy, morphology

Laboratory 3: Plant pigment analysis

Laboratory 4: Ethology

You have approximately 60 minutes in each laboratory. You can score 50 points at a maximum in each laboratory, which means a total amount of 200 points for the practical test. If at some task you mark some incorrect alternative(s) as well as some correct ones, you will get a lower score at that particular task than if you only mark the correct answers.

Laboratory 1: Plant anatomy, morphology and taxonomy

Introductory remarks

In this laboratory you will be given three separated tasks. It is of no importance with which you start. The time to perform all the tasks is 70 minutes. Please be sure that the task paper given to you fits the task you are performing.

The dissection case must be used in two laboratories. It's a gift. Take it with you.

Task 1: making sections of plant organs. Identification of the organs. Taxonomic classification.

Task 2: identification of 3 representatives of a plant taxonomic group by selecting distinct parts of the species.

Task 3: identification of flowering plant families on the basis of flower buds and reference slides of transverse section of the buds.

TASK 1: Making sections of plant organs. Identificarion of the organs. Taxonomic classification. (Total points 11)

Material
Plant organ samples (2 types A and B)
Instruments and equipment:

Razor blade
Stain and glassware +H_2O
Slides and coverslips
Microscope
Reference slides of transverse section of plant organs of 7 different taxonomic groups

1. Make a cross section of sample A. Prepare a wet mount and use the stains if necessary.
You have a set of reference slides (1-7) with cross sections of different plant organs.
Examine your preparation under the microscope and compare with the reference slides. Identify the corresponding reference slide and fill in the correct number of that slide on the answer form in the blank **1A1**.
(1,5 points)
You have a list of organ types. Select the correct number out of the list and fill in the blank **1A2** on your task 1 answer form. **(2 points)**
You have a list of taxonomic group. Select the correct number out of the list and fill in the blank **1 A3** on your task 1answer form. **(2 points)**

2. Make a cross section of sample B. Proceed as in 1 and fill in blanks on your task 1answer form .
1B1 (1,5 points) 1B2 (2 points) 1B3 (2 points)

TASK 1. List of organ types

1. Primary root
2. Primary stem
3. Rhizome

4. Leaf petiole
5. Leaf rachis
6. Secondary root
7. Secondary stem

TASK 1. List of taxonomic groups

1. Flowering plant monocotyl (Liliaceae)
2. Flowering plant dicotyl (Magnoliaceae)
3. Conifer
4. Fern
5. Clubmoss
6. Horsetail
7. Cycad

TASK 2: Identification of 3 representatives of a plant taxonomic group by selection of distinct parts of the specimen.

Material

Mixture of plant parts of 3 representative species of different plant taxonomic groups
Binocular microscope
Dissecting equipment (tweezers)
Glassware (petri dishes)

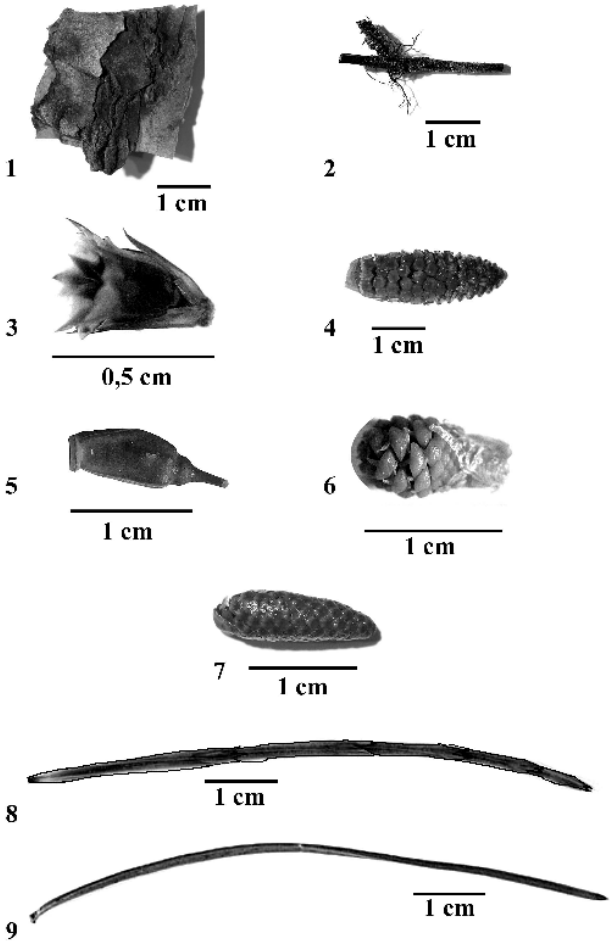
1. Transfer the mixture of plant parts into a large petri dish. Select the parts which belong to the same species (if necessary the binocular microscope can be used) and transfer the items that belong together.
In petri dish A you have to collect **4** items.
In petri dish B you have to collect **3** items.
In petri dish C you have to collect **2** items.
You have a figure with several plant parts of different plant species belonging to several taxonomic groups. Select those figures, that corresponds with the items in the petri dishes. Fill in the blanks in **211**, **212** , **213** and **214 (each 2 points)** for the items belonging to **species 1**, on the **task 2 answer form.**
Do the same for **species 2** and **3**. Fill in for petri dish A and petri dish B **221**, **222** and **223 (each 2 points).** Fill in for petri dish C **231**, **232 (each 2 points)** on the **task 2 answer form** .

2. You have a list of taxonomic groups: select the appropriate group for the species in Petri dishes A, B and C and fill in the corresponding number in the blank code **215, 224**, **233 (each 2 points)** on the **task 2 answer form**.

TASK 2. List of several taxonomic groups.

1. Monocotyl
2. Dicotyl
3. Cycad
4. Conifer
5. Moss
6. Alga
7. Horsetail
8. Clubmoss

Task 2. Figure of different plant parts



TASK3: Identification of flowering plant families on the basis of flower buds and refernce slides of transverse section of buds (Total points 15).

Material
Samples of flower buds of 2 plant species belonging to 2 different families A and B.
Reference slides of cross sections of flower buds (1, 2, 3)
Binocular microscope
Dissection tools
Petri dishes

3.1. Take the flower bud out of petri dish A. Dissect the flower and observe its characteristic diagram.
You have a set of selected reference slides (1-3).
Select, on the basis of these characteristics, the corresponding reference slide and fill in the number in the blank code **311**. **(1,5 points)**

You have a list of families of flowering plants.
Select the correct number corresponding with the family of the sample A flower bud and fill in the number in the blank code **312 (2 points)** on your answer form task 3.

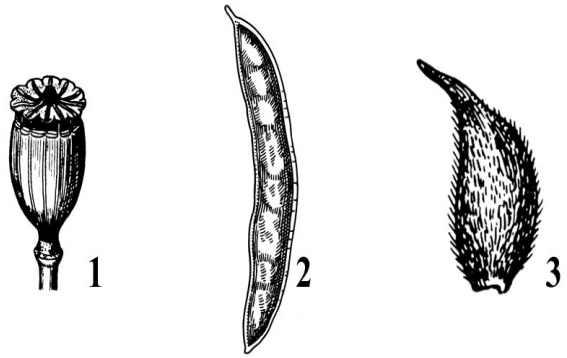
You have pictures of fruit types.
Select the number of the type of fruit belonging to this family and fill in the number on the blank code 313 **(1,5 points)**.

3.2. Do the same for the petri dishes B and C.
Fill in for petri dish **B** the number in the blank code **321 (1,5 points)**, **322 (2 points)**, and **323 (1,5 points).**
Fill in for petri disch **C** the number in the blank code **331 (1,5 points)**, **332 (2 points)**, and **333 (1,5 points).**

TASK 3. List of families of flowering plants

1. Ranunculaceae
2. Lamiaceae
3. Oleaceae
4. Fabaceae
5. Apiaceae
6. Asteraceae
7. Poaceae
8. Liliaceae
9. Fagaceae
10. Brassicaceae

Task 3. Pictures of fruit types



Laboratory 2: Animal anatomy, morphology

Introductory remarks

The insect to be dissected is a cockroach : Insecta, Dictyoptera, Blattidae, from the genus *Periplaneta*. These insects are well known to you. They have invaded nearly all the buildings in some cities. As they are nocturnal, the humans may considerably underestimate their number. They are sometimes seen as mark of dirtiness, however, they can be very abundant even in very clean environment, like hospital. A main factor of their proliferation is the climate : summer, with both high temperature and humidity are extremely favourable to the species.

Dissection of *Periplaneta*

Material
Periplaneta
A dissection tray
6 entomological needles
8 coloured needles (one to eight)
Scalpel

H_2O
tweezers
fine scissors
slides and cover-glasses
binocular microscope + light

Manipulation

1. add tap water in the dissection tray (1,5 cm high).
2. fix the animal properly. The dissection can be made under binocular microscope : start cutting it with fine scissors beginning at the anus ; cut along the two lateral sides as far as the prothorax.
3. lift up the cut off tergite (dorsal plate) and turn them over. (**5 points)**
4. unroll the digestif tract, lift it away from the body without cutting it and pin the different sections with labelled needles in the following order

Use needle	red 1. for the crop / stomodaeum.	**(5 points)**
	blue 2. for the gizzard / proventiculus	**(5 points)**
	orange 3. for the digestive caeca	**(5 points)**
	black 4. for the mesenteron / midgut	**(5 points)**
	yellow 5. for Malpighi tubules	**(5 points)**
	green 6. for proctodaeum	**(5 points)**
	white 7.for nerve cord	**(5 points)**
	pink 8. fat body	**(5 points)**

5. Cut a piece of trachea and make a wet mount of it, bring it under the binocular microscope and ensure you have a thin preparation. Take this between slide and cover-glass and examine it under the binocular microscope **(5 points)**.

The dissection must be completed within 40 minutes.
The dissection must be checked by an assistant.
When you have finished the dissection, raise your hand to call for an assistant.
The assistant and you must sign the evaluation sheet.
Re-evaluation is supervized by a Professor.

Laboratory 3: Plant pigment analysis

Reversed Phase Thin-Layer Chromatography of Photosynthetic Pigment

Introduction remarks

Thin-Layer Chromatography is an important technique for analysis of molecules. How do biologists and biochemists know so much about molecules

such as, for example, proteins, lipids and plant pigments? The answer is of course that they have been isolating, separating and studying these molecules for more than a century. One important technique for the analysis of molecules is thin-layer chromatography (TLC). This technique can separate different kinds of molecules based on their relative affinities for a hydrophilic stationary phase and a hydrophobic mobile phase. The stationary phase is usually a thin layer of silicic acid (silica gel) on a glass or metal plate, while the mobile phase is a mixture of appropriate solvents. Although largely replaced by several new and more efficient and accurate techniques, TLC remains a rather simple but effective technique to make a first qualitative analysis of photosynthetic pigments in samples.
For the analysis of photosynthetic pigments some problems may appear, such as the formation of pheophytins due to the reaction of pigments with H_2SiO_3 molecules, present in the silica gel. The problem can be avoided by using silica gel from which the polar groups are saturated with C_{18}-alkylchains. This results in a non-polar silica gel and the technique is specified as reversed phase thin layer chromatography (RP-TLC) with an appropriate solvent for the mobile phase (e.g. mixtures of ethylacetate, methanol and water).

Equipment and material available for each student

1. Simple chromatography tank (high-200/250ml-beaker filled with small but suitable volume of solvent, covered with petri dish)
2. sheet of parafilm (spare)
3. 2 TLC-strips cut at 40 x 100 mm , dried for at least 30 min. at 60 - 80 ° (1 spare strip)
4. pencil
5. ruler
6. piece of aluminium foil
7. 3 test tubes (glass 20 ml)
8. graduated measuring cylinder (10ml)
9. chemicals : petroleum ether 40 - 60° (bottle A); acetone 100 % (bottle B)
10. distilled water in test tube
11. fine sand in test tube
12. mortar + pestle
13. 2 capillary tubes (20 µl)
14. bean plant
15. task description with experimental protocol
16. Multiple choice questions
17. answer leaflets

TASKS:

1. Separation and identification of plant pigments by reversed phase TLC
2. Solving MC questions related to TLC and Plant Pigments (use answer leaflet no 1)
3. Completing the answer leaflets no 2 for task evaluation

At the end of the session each student presents two answer leaflets and 1 TLC-plate.

Task 1: Separation and identification of pigments by reversed phase TLC

Preparation (takes approx. 15 minutes)

1. Take about 8 cm^2 of plant material.
2. Grind the plant material in a mortar using a bit of sand.
3. Add **100 % acetone** (max. 10 ml) bottle B.
4. Pour in test tube, decant after 5 minutes (meanwhile you can prepare the TLC-plate, see step 7).
5. Add approximately 20 drops petroleum ether (bottle A) and ap proximately 5 ml distilled water.
6. Shake well and allow to separate into two layers (wait 1 min for the separation).
7. Take a TLC plate and draw carefully and faintly a start line (**use pencil**) at 1.5 cm from the bottom (avoid damaging the silica gel layer).

Spotting (takes approx. 5 minutes)

1. Spot the pigment extract on the start line with a small capillary tube in three different locations (do it gently and avoid damaging the silica gel layer).
2. Dry the spots by gently blowing.
3. Repeat this 4 to 5 times.

Running (takes approx. 25 minutes)

1. Put the TLC plate in the tank and allow to develop in the dark (cover the jar with aluminium foil). **IMPORTANT: Use your free time to solve the MC questions (task 2).**
2. After approx. 20 minutes, take the plate out of the tank. In any case take the plate out when the front line reaches 1 cm from the top of the TLC plate.

Interpretation

1. Mark the solvent front line (**use pencil**).
2. Use the answer leaflet to draw a copy of your TLC plate, including the front and start line and the pigments observed.
3. Calculate R_f-values for the photosynthetic pigments you have sepa rated.

Remarks:
Photosynthetic pigments with a R_f-value equal or greater than 0,4 might be only slightly visible. Examples for this RP-TLC:

neoxanthin	R_f =0,6
violaxanthin	R_f =0,5
luteïn	R_f =0,4

Despite the precautions pheophytins may appear. If they show up, you might see them as a grey-blue haze. We do not consider them as photosynthetic pigments because they are derived from photosynthetic pigments through chemical alteration.

4. Mark on your drawing, each of the observed photosynthetic pig ment spots. **Numbering of your spots starts with no 1 for the spot with the lowest R_f-value**. Try to identify four photosynthetic pigments with the lowest R_f-values.
5. Cover your TLC plate with aluminium foil to prevent deterioration of the pigment colors.
6. Continue with **task 3** by completing n° 2 answer leaflet, using all available information including that from the MC questions.

Task 2: Answer the MC questions related to TLC and Plant Pigments
Choose the one best answer for each question.

Scoring for each question:
- Right answer = + 3 points
- Wrong answer = - 1 point (guess correction)
- No answer = no points

Mark your answers on leaflet no 1

1. Which of the following statement is correct?

A. Plants have chloroplasts and therefore can live without mitochon-dria.
B. Both chlorophyll and heme contain an extensive system of double bonds that allow them to absorb visible light.
C. The role of chlorophyll in photosynthesis is equivalent to that of heme in mitochondrial electron transport.
D. All the statements above are wrong.

2. Study the molecular structure of major plant pigments to predict the relative solubility in polar or non-polar solvents (**see figure on page 17**). To determine polarity of these pigments, one can count the number of atoms per molecule. Which count is the most suitable?

A. H-atoms
B. N-atoms
C. C-atoms
D. O-atoms

3. Acetone is very suitable to extract pigments from chloroplasts. Choose a correct answer that supports this fact.

A. Non-polar molecules only dissolve in non-polar solvents.
B. Chloroplast-membranes are polar structure.
C. Non-polar molecules only dissolve in polar solvents.
D. The stroma in chloroplasts contains a huge number of non-polar molecules.

4. Plant material often contains red pigments; their structure reveals that they have no function in photosynthesis and are soluble in water. A perfect location for these pigments in plant cells is:

A. Membranes of chloroplasts.
B. Membranes of mitochondria.
C. Lumen of vacuoles.
D. Cytosol of the cell.

5. Chlorophyll is a complex molecular structure with a magnesium atom held in the centre of a porphyrin ring, which is structurally similar to the porphyrin ring that binds iron in heme. Which of the next statements is correct and completes the functional description of the molecule?

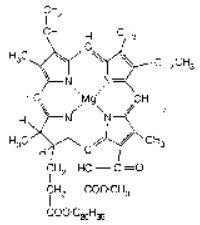
A. Electrons within the bond network of the porphyrin ring absorb light, while the long hydrophilic tail helps to hold the chlorophyll in the thylakoid membrane.
B. Electrons within the bond chain of the long hydrophilic tail absorb light, while the network of the porphyrin ring helps to hold the chlorophyll in the thylakoid membrane.
C. Electrons within the bond network of the porphyrin ring absorb light, while the long hydrophobic tail helps to hold the chlorophyll in the thylakoid membrane.
D. Electrons within the bond chain of the long hydrophobic tail ab sorb light, while the network of the porphyrin ring helps to hold the chlorophyll in the thylakoid membrane.

6. A researcher has just discovered a new bacteria species that has a unique photosynthetic pigment. In a TLC, the pigment appears to be reddish yellow. What colours of the visible light spectrum are not absorbed?

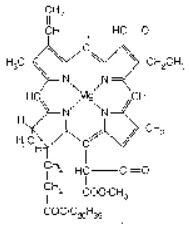
A. Red and yellow.
B. Blue and violet.
C. Green and yellow.
D. Blue, green, and red.

7. If you know the absorption spectrum of a plant pigment, you can be reasonably certain of:

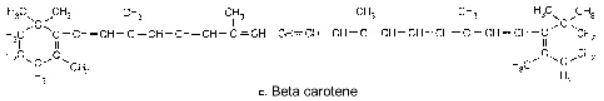
A. The wavelength of visible light it absorbs.
B. The wavelength of visible light it reflects.
C. Tthe energy of the visible light it absorbs and reflects.
D. All of the above.



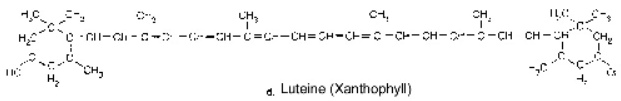
a. Chlorophyll A



b. Chlorophyll B



c. Beta carotene



d. Luteine (Xanthophyll)

ANSWER LEAFLET N° 1: MC QUESTIONS (21 points)
Scoring for each question:

right answer	**+ 3 points**
wrong answer	**- 1 point**
no answer	**no point**

Question 1	A	B	C	D
Question 2	A	B	C	D
Question 3	A	B	C	D
Question 4	A	B	C	D
Question 5	A	B	C	D
Question 6	A	B	C	D
Question 7	A	B	C	D

Code number:

ANSWER LEAFLET N° 2 (29 points)

Fill in for RP-TLC task evaluation

RP-TLC plate n°:.....

Photosynthetic pigment n° 1
name:..... **(3 points)**
R_f -value:..... **(3 points)**

Photosynthetic pigment n° 2
name:..... **(3 points)**
R_f -value:..... **(3 points)**
Photosynthetic pigment n° 3
name:..... **(3 points)**
R_f -value:..... **(3 points)**

Photosynthetic pigment n° 4
name:..... **(3 points)**
R_f -value:..... **(3 points)**

Draw a copy of your TLC-plate in the box **(5 points).**
Mark the most abundant photosynthesis pigment spots.
Present answer leaflet n° 1, n° 2, and your TLC-plate to the panel of examiners.

Laboratory 4: Ethology

Introductory remarks

In this part, you will see 6 film sequences dealing with the behavior of insects, birds and mammals. Each sequence will be shown twice.
We suggest you proceed as follows:
First, watch the sequence.
Second, read the relevant question.
Third, watch the sequence a second time.
Fourth, answer the question on the answer sheet correctly.

Good Luck

Questions about sequences

1. Butterfly chrysalis : the chrysalis of this species is either green or brown. Both morphs are cryptic in their natural environment (trees) **(8 points).**

A. Chrysalis colour is conditioned by the texture of the substrate on which the caterpillar moves before pupate.
B. Chrysalis colour is conditioned by the colour of the twig on which the caterpillar ran before pupate.
C. Both A and B are correct.
D. Neither A nor B is correct

2. Path selection by ants: two video sequences are displayed. During the first sequence, a bridge made of two unequal lengths connects the nest to a food source. During the second sequence, the ants have access to the food only by using the long path of the bridge during the first part of the experiment. The ants, however, can use both the long and the short paths in the second part of the experiment, which started after a heavy traffic was first established on the long path during the first part of the experiment **(8 points).**

A. The ants randomly select one or the other path.
B. The ants are able to evaluate travelled distances and to remem ber the shorter path.
C. Path selection depends on the number of ants which had previ ously travelled along each path.
D. Ants never travel along a path that they have not marked chemi cally.

3. Honeybee dance: in the video sequence, a bee loaded with pollen per-

forms a waggled dance for recruiting nest-mates towards a food source. The bee dance informs nest-mates on the direction they must follow to find the food source: **(8 points)**

A. About 45° right to the sun.
B. About 135° left to the sun.
C. South-west from the hive.
D. North-east from the hive.

4. Social interactions between cows: the video sequences illustrate dominance relationships between feeding cows in five experimental conditions. Out of the following assertions, which one is correct **(8 points)**?

A. Visual stimuli are required for holding dominance position.
B. A dominant cow always expresses its status whatever the experimental condition.
C. Dominance is not expressed without head contact between cows.
D. All the above answers A, B and C are wrong.

5. Maternal behavior in sheep. Three video sequences are shown: the first illustrates the behavior of a mother and her lamb soon after parturition; the second, a lamb is washed immediately after parturition before its mother was able to care for it, after what it is given to its mother who had never cared for lambs before; the third, the lamb was washed soon after parturition and then given to a foreign ewe who had experienced lamb care before **(10 points).**

A. Suckling acceptance requires that the ewe had the opportunity to care for a lamb after parturition.
B. An ewe is more prone to accept a lambkin if she marked it by licking it before.
C. Washing eliminates all odours, preventing suckling acceptance.
D. The three answers A, B, C are correct.

6. Emperor-pinguin :four videos are shown:1)egg-laying and egg-transfer to the male ; 2) incubation of eggs by aggregated males ; 3) females join the males and chickens are tranferred to them; 4) chickens are grouped in a nursery **(8 points).**

A. Only males take care of chickens.
B. Only females take care of chickens.
C. Brood-care by males and females is similar.
D. Both males and females take care of the chickens, but there is a division of tasks between them.