Practical Test

General Instructions

December 9, 2024

You may only read the pages in the white envelope.
"EXAMINATION RULES"
"EXAM INSTRUCTIONS"
"SPECIFIC INSTRUCTIONS"
and "SAFETY REGULATIONS"

EXAMINATION RULES

1. **You are NOT allowed to bring any personal items into the examination room, except for personal medicine or approved personal medical equipment.**
   Each team must sit at their designated table.

2. **Each team will have 30 minutes for checking all apparatus and chemicals, and reading instructions and experimental details contained in the brown envelope. Do NOT start checking or reading the papers in the brown envelope before the "CHECK" signal.**

3. **Signature of each competitor will be collected during this period.**
4. **You are NOT allowed to work on the experiments in this 30-minute checking and reading period.**

5. **You may only begin working on the experiments after the "START" signal.**

1. Eating and drinking in the lab are not allowed. If necessary, and for only medical reasons, you may ask an exam supervisor for permission to take a snack break in the provided area.
2. Do NOT disturb or communicate with competitors from the other teams. If you need any assistance, raise your hand and wait for an exam supervisor to come.

3. The team must stay at their table until the time allocated for the examination is over even if they have finished the examination earlier or do not want to continue working.

4. At the end of the examination time you will hear the "STOP" signal. You are NOT allowed to write anything after the signal is given. Arrange the exam sheets and answer sheets neatly on your desk. Do NOT leave the room before all the exam sheets have been collected, and you are given the signal to leave.

5. The exam supervisors will not help with the experiments and will minimize communication with competitors. If your problem is not stated in the examination rules or instructions, use your own judgement.

6. If any injuries occur, you must inform the exam supervisors immediately. There will be no point deduction from the injury, but it must be handled properly and the injured person can only resume to work on the experiments upon the agreement from exam supervisor.

7. There will be only one warning if a team does not comply with the examination rules. Any failure to comply with the rules or instructions of supervisors after the warning results in team disqualification, receiving total of zero points for the team in the practical test.

You may turn to the exam instructions on the next page.

EXAMINATION INSTRUCTIONS

1. Always follow the experiment instructions, but have the right to work on the exam questions in any order.
2. All competitors are expected to work safely, behave responsibly, and keep the work environment clean. When carrying out discussions with your teammates, please keep your voice low so as not to disturb others.
3. Safety goggles and lab coats must be worn all the time. You are allowed to remove the safety goggles only when using the microscope or for a brief goggles adjustment. Prolonged removal of goggles or lab coats results in warning or disqualification.
4. In the case of broken glassware, please raise your hand and seek assistance from exam supervisors.
5. You have **3 hours** to:
   Complete the assigned experimental tasks,
   Carry out calculations,
   Draw graphs,
   Record your results and answers on the answer sheets provided. You must stop working and writing immediately after the "STOP" command is given.
6. Each team has three copies of the complete question sheets printed in white and one copy of answer sheets for each subject: physics, chemistry, and biology. Only the answer sheets will be evaluated.
7. Check the stationery items (pen, pencil, and calculator) provided by the organizers. ONLY use the pen and pencil provided by the organizers.
8. Use only the pen to write your all answers in the sheets, except for drawing where you may use the pencil. All results and answers must be written in the spaces provided within the answer sheets. Data written elsewhere will not be graded. You may use the question sheets and their backside as scratch paper.
9. If you want to change your answer, completely erase or clearly cross out your first answer and write in your new answer. Any ambiguous answers are marked as wrong.
10. Team code is written on every page of the answer sheets. Raise your hand if the information is not correct.
11. If space is provided for calculation, you must show your calculation. Otherwise, no point is awarded for the question.
12. You should write your data and final answers down in the appropriate number of digits.

13. After the “CHECK” signal is given, check that you have a complete set of the exam question sheets. Raise your hand, if you find any missing sheets.

You may turn to the specific instructions on the next page.

SPECIFIC INSTRUCTIONS

1. Checking and reading: You should check all apparatus and chemicals according to the lists given on the first page of each section. No equipment will be handed out after this checking period.
2. Refills/Replacements:
   In the biology section, samples, chemicals, and labware are not refilled or replaced.
   In the chemistry section, chemicals and labware may be refilled or replaced once with the deduction of 1 point per item refilled/replaced. Distilled water and gloves can be replaced without penalty.
   In the physics section, students may ask for replacement equipment.
3. Every piece of the equipment is validated before the examination. If it does not work as expected, it is likely that you do not use it correctly. There will be no inspection of equipment from exam supervisors during the examination.
4. Disposal: Chemical waste must be disposed in the 500 mL beaker.
5. Useful information is provided on the following page.

Safety regulation for chemistry

Read the following instructions carefully

- Wear the lab coat, safety goggles, and protective gloves when you are in the lab.
- Handle each substance with care.
- Do not taste or smell any chemical compound.
- Chemicals must be disposed properly in the beaker of 500 mL.
- Ensure that you keep the answers sheet and question paper away from liquids.
- Immediately report all accidents, injuries to the supervisors present, however minor they may be.
- Eating any kind of food is strictly forbidden during the experimental task.
- Keep the work environment clean.

DO NOT turn to the next envelope before the "CHECK SIGNAL"

Time: 3.0 Hours

Points: 40

IJSO 2024 INTERNATIONAL JUNIOR SCIENCE OLYMPIAD

PHYSICS PRACTICAL EXAMINATION

GENERAL INFORMATION AND OPERATION MANUALS

This practical exam deals with physical measurement methods and instruments:

- Refractometer (first picture below)
- Conductometer and pH-meter, both in one physical device (R-type in the second and S-type in third picture below)
- Digital scale (the last picture below).

Box Content

1. Refractometer
2. pH/conductometer
3. Tray
4. Water bottle
5. Deionized water bottle
6. Digital scale
7. Liquid waste recipient
8. bag A with:
   - A1
     - 72.5%Brix
   - A2
     - CCS1
     - CCS2
     - 1413 µS/cm
   - A3
     - pH 4.00
     - pH 6.86
     - pH 9.18
   - A4
     - calibration weight
9. bag B
   - H1 sample
   - H2 sample
   - H3 sample
   - H4 sample
   - H5 sample
   - wooden pallets
10. bag C
    - EC1
    - EC2
    - EC3
11. bag D
    - ACH2
12. bag E
    - F sample
    - ball
    - syringe
    - wire
    - 100 ml recipient
13. eraser
14. magnifying glass
15. pencil

16. paper towels

P_Experiment A. Science and Measurements. Getting familiar with instruments and their calibration

Science operates with reliable information. The data from measurements must be accurate and precise. The instruments must be checked and, if necessary, recalibrated using certified standard materials.

Following is a description of the operation of the measurement devices used in this physics exam.

P_A1. Working with a Refractometer

General: A refractometer is an instrument measuring the refractive index and/or related parameters. Identify the instrument, it should be in the blue box. It has a prism at one end and an eyepiece (like a microscope) at the other.

You will use an instrument designed for honey quality control: the scale on the right side, **"WATER %"**, is for the honey water content, the central scale, **"BRIX %"**, is for the content of sweet substances (sucrose, sugars in general). You are not going to use the scale on the left side.

Total sugar concentration is expressed in **Brix** (also Bx) percents, showing the mass of sugar in grams present in 100 grams solution (g/100 g). A honey with 65 Bx% contains 65 g sugar in 100 g honey. T**he sugar Brix scale is a conventional scale for sugar content.**

How to measure:

Direct the prism to a light source and look through the eyepiece (don’t use eyeglasses). Check the image clarity. If necessary, rotate the eyepiece until the image of the three scales becomes clear.

Open the prism lid and use a stick to take 2-3 drops of the sample and apply it onto the prism surface (see figure below). Gently press the lid to spread the fluid in a thin layer without air bubbles. Point the prism at an ambient light source and read on the central **"BRIX %"** scale a value at the edge between the blue and the white zones. Similar method to read on the **"WATER %"** scale.

After each measurement clean the prism surface with wet wipes and finish with a soft cloth.

P_A2. Working with a conductometer

Your instrument is a combined „pH-meter /Conductometer".

Identify the instrument; it is labelled „pH-meter /Conductometer".

It has a protective cap at the bottom end. Remove the cap by pulling out: you will see exposed the cell/pH sensor (glass), the cell for conductometric measurements (a pair of metal electrodes), and a temperature sensor (black).

Press the **on/off button**, marked with ⏻ ; it works only as **ON/OFF button**. You will see on display a symbol for battery capacity state, a label like **„pH"** or **"µS/cm"** (press **MODE** key to choose the convenient one), a numerical value, a temperature reading by default in ºC.

Keep the label **"µS/cm"** and the instrument works as conductometer.

Conductivity is 1/resistivity, and conductance is 1/resistance. The conductance is measured in Siemens – symbol S (i.e., Ω^{-1}) – by applying an AC signal (low voltage, 25 – 1000 Hz) to avoid electrolysis. The specific conductivity of solutions is well related to the concentration of dissolved electrolytes.

The instrument switches off automatically after 3 minutes of inactivity to protect the battery. Simply press **ON** to reactivate it.

If you press the **HOLD** button, a small **H** letter appears in the left top corner and the instrument holds the last measured. Reactivate measuring by pressing again **HOLD.**

After each measurement remove the instrument from the solution, wash the sensors area using the wash bottle marked **"Deionized water"** and the **"Liquid waste"** recipient. Use a dry paper towel to remove excess water from sensor area.

How to measure:

Short press (and repeat if it is necessary) ***MODE/CAL*** *button to see the "****µS/cm****" label, the measuring unit for the specific conductivity. You are now in "****Conductivity****" mode of measurement.*

Fix the instrument in the clamp of the laboratory stand (with sensors exposed). Place the bottle with a sample under the instrument and adjust the height in the stand so that sensors are completely immersed in solution (approximately 1 cm depth).

After each measurement clean the electrode area with deionized water using the wash bottle. Gently remove the excess water from electrode area using a dry paper towel. Turn off the instrument when not in use.

Calibration procedure: is attached to the instrument.

For calibration use the solution labeled "*1413 µS/cm".*

Type R conductometer (with the rounded lower edge of the front panel): Press ON. Short press MODE to select "µS/cm" on display (conductometric mode).

Press MODE/CAL for 6 seconds and release it. You will see "CAL", then a blinking value. Long press MODE/CAL to start decrease this value as close as possible to 1413 µS/cm (+/- 20 µS/cm allowed). Avoid a short press of MODE/CAL (you will leave the "µS/cm" mode). If necessary long press HOLD/TEMP to increase the value. When you have the aimed value on display wait until the value is no more blinking. End of calibration.

Type S (straight lower edge of the front panel).

Press ON. Short press MODE to select "µS/cm" on display (conductometric mode).

Press MODE/CAL for 6 seconds and release it. You will see 2 values, an actual reading and (in the second line) the value of the recognised standard (1413 µS/cm).

Short press MODE. The actual reading is blinking. Wait until the value is no more blinking. Short press MODE/CAL once and after a second press it again. You will see four short lines - - - - on display. When a value is displayed again, the instrument is calibrated.

In both cases you can turn off and then turn on again to verify the reading with calibration solution 1413 µS/cm.

P_A3. Working with a pH-meter

General: a pH-meter is used to measure significant variations in the concentration of the H^+ ion (usually identified as hydronium ion $\mathrm{H_3O^+}$).

$\mathrm{pH} = -\log_{10}\ [H^+]$, i.e., $[H^+] = 10^{-\mathrm{pH}}$, where [....] is concentration measured in $\mathrm{mol} / \mathrm{dm}^3$.

The pH-meter measuring cell of the pH-meter generates a voltage that is almost zero at pH = 7 and then varies by about 59 mV/pH unit (see Figure R1).

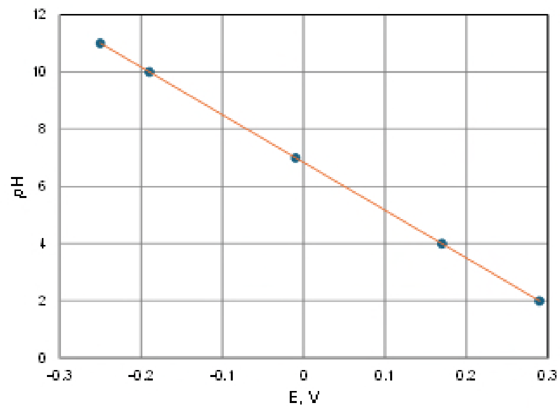


Figure R1. Variation of the electrochemical potential with pH

A pH-meter needs frequent checks and calibrations with calibration solutions having stable pH values (usually known as standard pH buffer solution). You have 3 standard pH buffer solutions for calibration and/or verification.

Your instrument is a combined „pH-meter /Conductometer".

How to measure:

*Press **MODE** key to choose the label „**pH**" and the instrument works as pH-meter.*

REMEMBER: after each change of measured solution, clean the sensor area with deionized water, and absorb the excess water with dry tissue.

Calibration procedure: place the instrument in pH 4.01 standard buffer solution. Verify that the instrument is in the **pH** mode. Long press **MODE/CAL** button for 6 s and wait until the value 4.01 is not blinking anymore. The instrument recognizes automatically the buffer nominal value.

Repeat the same sequence with the other two standard pH buffers (pH 6.86 and pH 9.18). Calibration is finished. Cap the standard pH buffers bottles with the corresponding lid (do not interchange) when not in use.

P_A4. Working with a Digital Weighing Scale

Place the scale on a stable, horizontal surface. Press **ON/OFF** button ⏻ to turn on the scale. Wait 30 seconds before weighing, to allow equilibration.

If you don't see symbol **g** (for gram) in the right up corner, press repeatedly **MODE** button, to change accordingly.

Press **T** (Tare) button for a 0.00 reading (as initial value) or to subtract the mass of a weighing container.

Measuring procedure. Place the object on the scale (*in central position*) and read the value in grams.

Calibration procedure *is attached to the instrument.*

Check the label on the back side of the scale.

a. If the label is WHITE follow this procedure: Press ON . Verify that the unit is g (gram). Wait 20-30 sec.

Long press M (2-3 s) until "100" is on display. If it is not "100" press T to change it.

Press M, a "CAL" message should appear, then '100" is blinking. Put the calibration weight 100.00 g on the scale. Observe the "PASS" message on display.

After that the scale is calibrated and is working in normal way mode. Verify that by removing the weight, you will see 0.00 g on display.

Setting the working time before automatic turn off: Press ON. Long press T. By pressing M, change the value to 180 (seconds). Press ON to confirm. You will see PASS message.

b. If the label is BLACK follow this procedure:

Press ON.

Verify that the unit is g (gram). Wait 20-30 sec.

Long press M (2-3 s) until "100" is on display. If you see "50" or any other value, press M to change to "100".

Press PCS, after one second press again PCS. The '100' message on display will be blinking.

Put the calibration weight on the scale. Observe the display to see "PASS" message.

After that the scale is calibrated and is working in normal way mode. Verify that by removing the weight, you will see 0.00 g on display.

Setting the working time before automatic turn off: Press ON. Long press T. By pressing M, change the value to 180 (seconds) or a higher value (up to 600). Press PCS to confirm. You will see PASS message.

Close the instrument to protect the battery (you have disabled automatic turn off), when not in use.

P_Experiment B. Determination of sugar and water content of honey

Honey, syrups, juices, and sweet drinks have variable concentrations of dissolved solids, especially sucrose, and organic acids or salts. The water content of honey, % [g/100 g or w/w], is that quality criterion that determines the ability of honey to keep its composition and resist alteration by yeast fermentation. The higher the water content, the more likely it is to ferment during long storage periods.

The refractive index allows not only the evaluation of the water content, but also of the total level of sugars, if the available instrument presents multiple evaluation scales. Given the complex composition of many of these foods, their sucrose („sugar") content can be measured with an accuracy of about ± 0.5%. Total sucrose concentration is expressed in Brix % [w/w].

The main physico-chemical characteristics of Romanian honey are shown in **Table R1**.

Table R1. Main physico-chemical characteristics of Romanian honey (EC is the electroconductivity)

Honey type	Water content/ (% w/w)	Sugar content/ (% w/w)	pH	EC/ (mS/cm)
Acacia	13.90 – 19.57	79.95 – 83.00	3.49 – 5.85	0.10 – 0.68
Linden	5.4 – 18.8	79.00 – 81.99	3.60 – 4.70	0.20 – 0.73
Polyfloral	4.8 – 19.6	79.96 – 82.38	3.20 – 4.60	0.23 – 0.83

P_Experiment C. Measuring of honey (electro)conductivity in a 20 % w/w aqueous solution

The electrical conductivity of honey is directly related to the concentration of mineral salts, organic acids, and proteins, varying significantly with the origin. Conductivity determination requires solutions of honey containing 20% (w/w) dry substance in double distilled water.

P_Experiment D. Measuring the pH of a honey solution (10% w/v in water)

Honey contains organic acids (0.6%) and amino acids (0.05%), so its pH can vary between 3.5 and 6. Gluconic acid is the most common, followed by acetic acid, butyric acid, citric, formic, lactin, malic, succonic and pyroglutamic. The most common of the 18 amino acids is proline. **The low pH** value makes honey compatible with foods with low acidity, inhibiting the development of microorganisms.

P_Experiment E. Archimedes' Principle and Application

Theory information

A body submerged (partially or completely) in a fluid is acted upon by a force equal in magnitude to the weight of the displaced fluid (Archimedes' principle).

To every action, there is always opposed an equal reaction (third law in Newtonian mechanics), i.e. in this case the same body exerts a reaction force on the fluid (same magnitude, but opposite direction).

The scheme for the experimental setup is presented in Figure R2 and can be used for measuring the reaction (force) to the buoyant force.

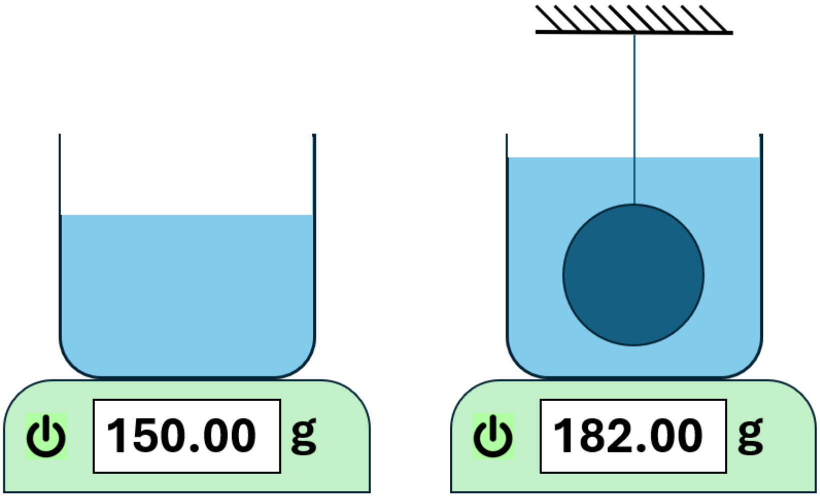


Figure R2. Experimental set-up for measuring the reaction (force) to buoyant force

Note: For your calculations use the following values:

Water density at room temperature is $\rho = 1.000\ \mathrm{g/cm^3}$.

Gravitational acceleration, $g = 9.80\ \mathrm{m/s^2}$.

EXPERIMENTAL PART

Fill-in data only in the Answer Sheets, observing the task code.

***

P_Experiment A1: Verify the BRIX % scale (0.6 pt)

Use the fluid contained in the test tube labelled **“72.5% Brix"** as sample. Report the result in the **Answer Sheets.**

If the reading error is higher than ± 0.5 Brix % ask for assistance. The instrument can be then accurately calibrated. If the error is smaller, accept it, but correct subsequent readings with it.

Go to experiment B, P_Task B1.

P_Experiment A2. Working with a conductometer

P_Task A2.1: Calibrate the conductometer (1.0 pt)

Use the calibration procedure described in "General Information and Operation Manuals", part A2.

After calibration measure:

- available **1413 µS/cm** standard conductivity solution.

and fill-in the first row of the **Observation Table.**

If the reading error is higher than ± 2.0 % ask for assistance.

P_Task A2.2: Measuring with a conductometer (0.3 pt)

After calibration measure:

- conductivity control samples CCS1 and CCS2.

and fill-in the **Observation Table.**

P_Experiment A.3

P_Task A3.1: Verify the pH-meter (0.5 pt)

Fix the instrument in the clamp of the laboratory stand (with sensors exposed). Place the bottle with standard buffer of pH 4.01 under the instrument and adjust the height in the stand so that sensors are completely immersed in solution (approximately 1 cm depth). When the displayed value is stable (after 10 – 20 s), read the pH value and record it in the **Observation Table.**

Repeat the procedure with the other 2 solutions (pH 6.86 and pH 9.18).

P_Task A3.2: Calibrate the pH-meter (1.4 pt)

The instrument has new batteries. The specific calibration is lost when the batteries are replaced.

Calibrate the pH-meter.

Repeat the measurements for all 3 pH buffer solutions with the **calibrated instrument**. Fill-in the obtained values in the **Observation Table.**

If all errors are less than ± 0.1 pH, the pH-meter is in a very good working state.

P_Experiment A4. Working with a Digital Scale

P_Task A4.1: Initial check of the scale (0.10 pt)

Your digital scale is not calibrated. Use the calibration weight of 100 g to check it. Write the result on the answer sheet.

P_Task A4.2: Calibrate the scale and verify the mass of the calibration weight (0.10 pt)

Calibrate the scale. After calibration verify the mass of the calibration weight in normal measuring mode and write the result on the answer sheet.

See the calibration procedure in the GENERAL information.

P_Experiment B

P_Task B1: Measure sugar content and water content (1.8 pt)

There are 5 samples (labelled as H1 to H5): three of them are honey from trusted sources, the other two are adulterated by addition of foreign substance or inferior honey. Measure the sugar content and the water content of each sample using the refractometer.

For each sample propose a diagnostic: **AUTHENTIC or ADULTERATED.**

P_Experiment C.

Please ensure that P_A.2 is completed before continuing with the next task.

Measuring of honey (electro)conductivity in a 20 % w/w aqueous solution

P_Task C1 (0.3 pt)

What mass of honey m_h (having 20 % water content) is required to prepare 100 g of 20% aqueous solution for the electroconductivity determination?

How much water (m_w) would be required to prepare the solution with the above concentration?

P_Task C2 - Measuring of honey (electro)conductivity (1.0 pt)

There are 3 samples (labelled as EC 1 to EC 3) of different honey types, containing 20% dry honey substance. Measure their electroconductivities, in µS/cm, using the instrument in the "**Conductivity**" mode.

Record the conductivity and temperature values in the ***Observation Table*** when the reading is stable (after 10 – 20 s). Repeat the operations with the other samples.

Observe the main characteristics of honey of different origin in **Table R1** and propose a type (acacia, linden, polyfloral), if possible.

P_Experiment D. Measuring the pH of a honey solution (10% w/v in water)

Please ensure that P_A3 is completed before continuing to the next task.

P_Task D1: pH of honey samples (0.5 pt)

There is a 10% aqueous honey solution, labelled ACH2. A sample is **50 mL.**

Place the sample container on the working bench and adjust the pH-meter position (using the stand) so that the sensor area is immersed about 1 cm into the solution.

Read the solution **<u>initial pH</u>** and fill-in the **Observation Table.**

P_Experiment E. Archimedes' Principle and Application

Please ensure that P_A4 is completed before continuing to the next task.

Use the appropiate experimental setup to accomplish the following tasks.

P_Task E.1 (0.5 pt)

Use the weighing scale, the laboratory stand, a beaker, and the small ball to mount the experimental setup in Figure R2.

Turn on the scale and use it after approximately 30 s.

Fill a transparent 150 mL beaker with 100 mL water, place it on the scale and press T button to read 0.00 g. Then completely submerge the ball in water (using the laboratory stand).

Write the value on the display.

P_Task E.2 (0.5 pt)

Draw the free body diagram labeling all the forces acting on the ball in task E.1.

P_Task E.3 (0.3 pt)

Find the mass of the ball.

P_Task E.4 (0.7 pt)

Using the experimental results, calculate the volume of the ball.

P_Task E.5 (0.5 pt)

Calculate the density of the ball.

P_Task E.6 (0.3 pt)

Calculate the buoyant force when the ball is completely submerged.

P_Task E.7 (1.8 pt)

A viscous fluid is provided as sample **F** for measuring its density.

Choose a convenient experimental setup.

Use the known parameters of the ball (from the previous tasks) to calculate the density of the viscous liquid.

P_Task E.8 (1.1 pt)

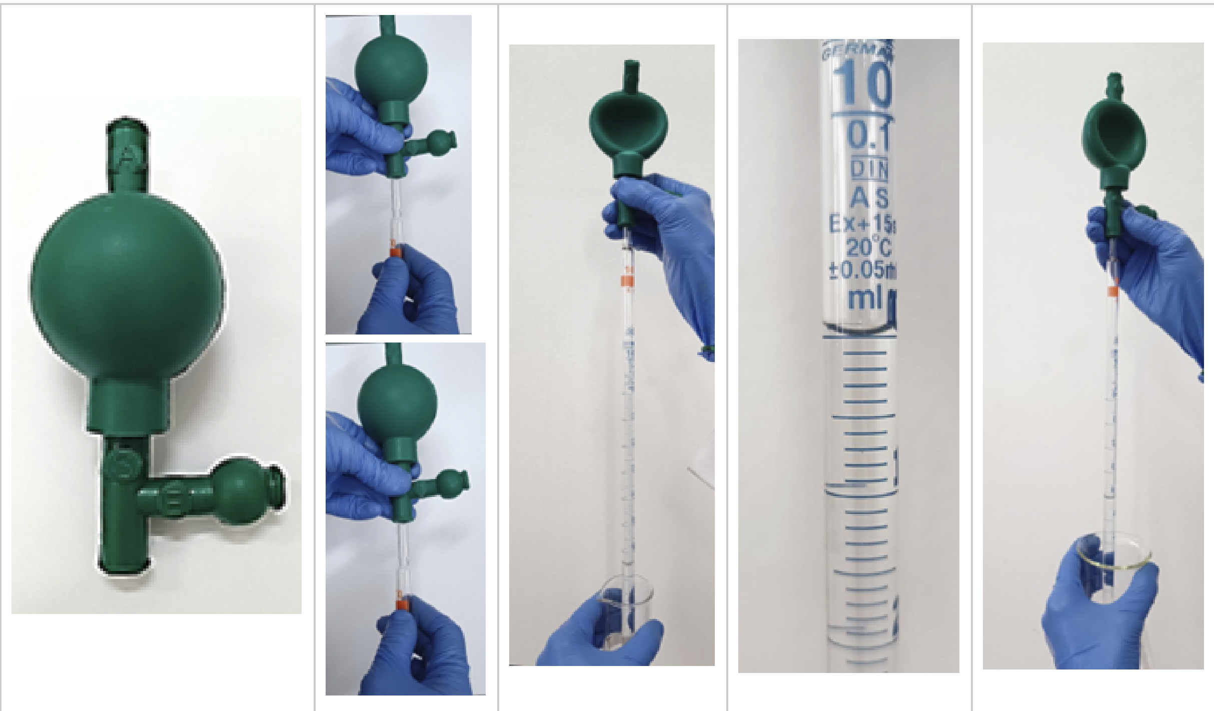
Determine the density of the viscuous liquid using the syringe.

CHEMISTRY

Pipette operation

There are three letters on the pipette filler: “A (air), S (suction) and E (empty).

- Insert the top of the pipette in the bottom of the pipette filler (if is not already assembled).
- Release air from the pipette filler by pressing “A” valve on the top of the pipette filler while simultaneously squeezing the bulb.
- Insert the tip of the pipette into the solution P1 for liquid aspiration by pressing the “S” valve. Adjust the liquid level to the mark using “S” and “E” valves.
- For liquid aspiration, be careful to keep the tip of the pipette in the liquid and not to let the liquid go up into the pipette filler.
- Using “E” valve, release the liquid from the pipette up to the desired volume into the conical flask.
- Recover the solution left in the pipette in the small beaker, in the same way.
- Fill the pipette up to 0 mark and using the "E" valve release the solution down to 8 mark.



C_I. Identification of the compounds in provided solutions (6.9 points)

Materials provided:

- Bottles labelled from 1 to 6 containing the aqueous solution of the inorganic compounds that must be identified
- 16 test tubes and test tubes rack
- 7 plastic Pasteur pipettes
- Tray
- Permanent marker and paper towel
- Wash bottle with distilled water
- Large beaker for residual solutions and washing waters

In the 6 bottles, labelled from 1 to 6, there are aqueous solutions of the following reagents: **barium nitrate, potassium iodide, sodium carbonate, lead nitrate, zinc chloride and sodium sulphite** (not in this order).

Experimental procedure

1. The chemical reactions will be performed in test tubes using about 1 mL of each solutions sampling with plastic Pasteur pipettes. Observe the resulting precipitates after several minutes.
2. Check for each solution possible chemical reactions (forming a precipitate) with the other solutions and write the observations in **C_Table 1** (from the Answer sheet)

C_I.1 In the appropriate cell in C_Table 1, if a precipitate forms, write the colour symbol and "↓", otherwise mark with "-". Use the following colour code:

"W" for white

"R" for red

"Y" for yellow

"G" for green

"B" for black

Based on the experimental observations, fill the **C_Table 2** from Answer sheet:

C_I.2 Identify the chemical compound from each bottle and write the chemical formula of the compounds in the last row of C_Table 2.

C_I.3 In the appropriate cell of C_Table 2, write the chemical formula of the resulting precipitate.

Hint

	Na^{+}	K^{+}	Ba^{2+}	Zn^{2+}	Pb^{2+}
Cl^{-}					↓
I^{-}					↓
CO_3^{2-}			↓	↓	↓
NO_3^{-}					
SO_3^{2-}			↓		↓

↓ - precipitate

Consider all substances used in the experiments as toxic, harmful if swallowed, irritant for eye and skin

C_II. Titrimetic determination of KH_2PO_4(6.5 points)

Materials provided

- Burette
- Stand and burette clamp
- Pipette and pipette filler
- Thymolphthalein indicator solution in a bottle with dropper. Its transition range is: **pH 8.8 (colorless) - pH 10.5 (blue).**
- 2 conical flasks (Erlenmeyer) of 250 mL
- Volumetric flask with cap of 50 mL containing the sample
- 2 small beakers (one for filling the burette and one for sample solution)
- Bottle of 250 mL with the KOH solution
- Permanent marker
- Paper towel
- Wash bottle with distilled water
- 1 large beaker for residual solutions and washing waters.

Potassium dihydrogen phosphate monohydrate $(\mathrm{KH_2PO_4 \cdot H_2O})$ is commonly used as a fertilizer, food additive, and buffering agent in various industrial applications. It is also known as MKP. In agriculture, MKP is used as a source of phosphorus and potassium, which are essential nutrients for plant growth.

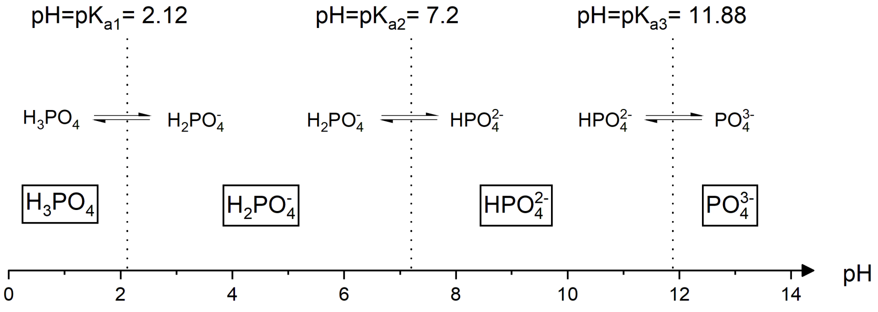
For quantitative determination of $(\mathrm{KH_2PO_4})$ from the volumetric flask carry out the following steps:

1. Carefully fill the clean burette with 0.0973 mol/L of KOH aqueous solution using one of the small beaker (label it “KOH”).
2. Bring the sample solution from volumetric flask to the final marked volume by topping up with distilled water, resulting the **solution P1**. Carefully pour about 30 mL of the solution P1 from the volumetric flask in the other small beaker (label it “P1”).
3. Using the pipette, place 8.0 mL of the solution P1 into the 250 mL conical flask (Erlenmeyer). Add about 25 mL distilled water into the same conical flask and 10 drops of thymolphthalein indicator.
4. Titrate the resulting solution (from 3) to the endpoint with KOH solution (blue colour).
5. Repeat the titration (operations from 3 and 4) and record three values in the answer sheet.

    1. **C_II.1** Record the used volume of KOH for three titrations in **C_Table 3** (from the answer sheet) and calculate the average volume of KOH solution.
    2. **C_II.2** Write and balance the chemical reaction of titration.

3. **C_II.3** Calculate the mass in grams (3 significant digits) of KH_2PO_4 (as anhydrous salt) in 8.00 mL of P1 solution. Show your calculation.
4. **C_II.4** Establish the KH_2PO_4 concentration (mol/L; 3 significant digits) of the P1 solution. Show your calculation.

Molecular weigh of $(\mathrm{KH_2PO_4})$: 136 g/mol



C_Scheme 1. Simplified diagram for the dominant H3PO4 species versus pH in aqueous solution.

DO NOT PROVIDE YOUR FINAL ANSWERS HERE, USE THE ANSWER SHEET.

BIOLOGY

ORPHAN BEARS GET ANOTHER CHANCE

Near the Ursus Valley, Romania, there is a large brown bear population consisting of several families, with a total of 10 cubs.

A physical-spatial location analysis of the bear families demonstrated that three of the families, which together have 5 cubs (marked as ”cub 1”, ”cub 2”, ”cub 5”, ”cub 7” and ”cub 10”) have their dens in the North West (NW) part of the village , two families are located in the South East (SE) part and 3 cubs were identified (”cub 3”, “cub 4” and “cub 9”), and the rest of the bears are located in the Far East part area of the village, where 2 cubs were also identified (“cub 6” and “cub 8”).

Most of the time the bears feed on what they find in the forest, especially different types of berries, but sometimes they can also descend into human settlements to look for food. Residents of Ursus Valley reported to the authorities that a bear cub often visits their village and scares domestic animals. That's why the authorities decided to move this cub, together with its family.

Therefore, the cub needs to be identified, so they enlisted the help of forensic researchers. They collected different types of samples, both from the locality and from the surrounding areas where the different families of bears are located. These samples were represented by faeces, hairs, leaves of most prevalent coniferous tree species. Different coniferous species were present in the NW area, SE area and Far East area.

To ensure that only the bear that visits Ursus Valley will be relocated, the authorities requested the help of forensic geneticists and biologists. They collected biological samples (hair, faeces, etc.) from the location visited by the animal and hair samples from all the 10 bear individuals that make up the population from the forests near settlement Ursus Valley.

Your role, as investigators, is to identify, based on the data obtained from the analysis of the samples, which bear took the habit of visiting the village and therefore could potentially represent a danger for the local inhabitants. You have to undertake different approaches to accomplish this task.

EXPERIMENT 1 - FORENSIC GENETICS

WHICH BEAR?

One important method is based on forensic analyses. Forensic analyses utilize various scientific methods such as DNA profiling, fingerprinting, and dental records comparison to establish distinct individual characteristics within a population, aiding in identification processes.

Therefore, investigators extracted DNA from biological hair samples and performed molecular fingerprinting analysis, using a set of 10 STR type markers, polymorphic DNA regions with 2-6 bp long repeat units. The number of repeats in STR markers is highly variable among individuals, making these markers effective for use in forensic applications. Amplicons for each marker and each individual were separated using a genetic analyser equipment and analysed with the appropriate software, using standard procedures.

You must keep in mind that for a precise identification of the individual, there should be 100% match between the DNA fingerprint of the unknown sample and the DNA fingerprint of the individual.

Glossary of terms used in the practical test of the Biology exam.

STR = Short Tandem Repeats = microsatellite = a set of short repeated DNA sequences at a particular locus on a chromosome, which vary in number in different individuals and so can be used for genetic fingerprinting.

Amplicon = DNA products of a polymerase chain reaction (PCR). They can be visualised and separated according to their size by gel electrophoresis.

DNA profiling (also called **DNA fingerprinting** and **genetic fingerprinting**) = the process of determining an individual's deoxyribonucleic acid (DNA) characteristics.

Polymorphism = the presence of two or more variant forms of a specific DNA sequence that can occure among different individuals.

bp = base pairs.

Using the genotype information presented in **Table I-1**, answer the following questions. Note that the numbers represents the number of repeats for each allele for each STR locus.

Table I-1. Individual wild bear genotypes and the genotype of the unknown sample for each of the 10 STR markers. UA are the name of STR markers. ID1, ID2....ID10 are the identification number of bear individuals from the wild population

STR loci	Unknown sample	Wild bear individual ID									
		ID1	ID2	ID3	ID4	ID5	ID6	ID7	ID8	ID9	ID10
UA03	15, 16	16, 15	16, 16	17, 15	16, 15	16, 15	16, 18	20, 15	15, 15	18, 15	16, 15
UA06	10, 11	11, 10	11, 10	11, 10	11, 10	11, 10	11, 10	11, 10	11, 10	11, 10	11, 11
UA14	8, 10	11, 10	7, 10	10, 10	11, 10	8, 10	8, 12	8, 8	8, 10	8, 10	8, 10
UA17	27, 24	24, 27	24, 27	24, 27	24, 27	24, 27	24, 27	24, 27	24, 27	27, 27	24, 27
UA25	13, 14	13, 14	13, 14	12, 14	13, 14	13, 14	15, 14	13, 14	14, 14	13, 14	13, 14
UA51	12, 14	14, 12	14, 12	14, 12	14, 12	14, 12	14, 12	14, 12	14, 12	14, 12	14, 12
UA57	17, 17	17, 17	17, 17	17, 17	17, 17	17, 17	17, 17	17, 17	17, 17	17, 17	17, 17

UA63	**8, 12**	**12, 9**	**12, 7**	**10, 8**	**12, 8**	**12, 8**	**11, 8**	**12, 8**	**12, 8**	**14, 8**	**13, 8**
UA68	**14, 19**	**19, 14**	**19, 20**	**19, 14**	**17, 14**	**19, 14**	**15, 14**	**19, 14**	**19, 14**	**19, 13**	**19, 14**
UA16	**22, 28**	**22, 28**	**22, 28**	**22, 28**	**22, 28**	**28, 22**	**22, 28**	**22, 28**	**22, 28**	**23, 28**	**22, 28**

UA03, UA06....UA16 – name of STR markers

ID1, ID2....ID10 – identification number of bear individual from the wild population

QUESTIONS:

DO NOT PROVIDE YOUR FINAL ANSWERS HERE FOR ANY OF THE QUESTIONS.

USE THE ANSWER SHEET!

B_I.1 [1.5 points, 0.15 per statement]

Using the information provided in Table I-1, write in the table in the ANSWER SHEET the ID number of each individual bear that has the same genetic profile as the unknown sample, for each marker (found in the STR loci column).

STR loci	Bear individuals with the same genotype as the unknown sample
UA03	
UA06	
UA14	
UA17	
UA25	
UA51	
UA57	
UA63	
UA68	
UA16	

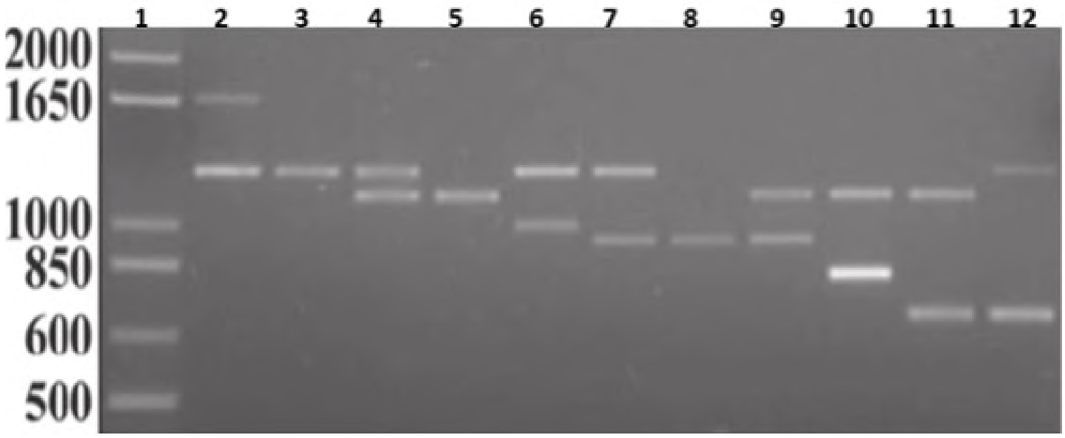
B_I.2 [0.3 points]

Based on your responses in the table, in the ANSWER SHEET, identify the ID number of the bear cub that visited the village. Write your answer in the answer sheet.

The conclusion of your investigation
The ID number of the bear cub that has the habit of visiting the village is:

B_I.3 [1.5 points, 0.3 per statement]

The results of a similar experiment are presented in the figure below. This shows an image of a DNA fingerprint for one STR marker across 11 different individuals (lanes 2 to 12). A DNA ladder was loaded in lane 1, with the length of each fragment labelled beside the image. In lane 1, the numbers 2000, 1650, 1000, 850, 600, 500 represents the length in base pairs = bp of each DNA fragment. Using your knowledge of the existance of different alleles at the specific locus, indicate whether each of the following statements is true or false by marking an "X" on your ANSWER SHEET.



Statement	**TRUE**	**FALSE**
The individual from Lane 10 has a heterozygous genotype for this locus and has the same alleles as the individual in Lane 7.		
Individual from Lane 8 is homozygous for an allele that is between 850 and 1000 bp.		
The allele that individuals from Lane 6 and 11 have in common is approximately 850 bp.		
All 11 individuals from this analysis are heterozygous for this locus.		
Individual from Lane 12 is heterozygous and one of the alleles is slightly above 600 bp.		

EXPERIMENT II

PLANT LEAVES SPECIMENS

In forensic investigations, plant anatomy can play a crucial role in identifying the origin and movement of animals, like bear cubs, by analyzing plant fragments present in collected samples. Coniferous tree species in particular have distinctive anatomical features — such as leaf structure, resin ducts, and stomatal patterns — that can vary significantly between species and even across geographic regions. When these plant parts are found, they provide indirect evidence of an animal's location or habitat.

In this case, the anatomical differences in the leaves of coniferous species from the NW, SE, and Far East regions could serve as geographic markers. By comparing the coniferous leaf anatomy found in the samples to reference samples from these areas, researchers can narrow down where the cub might have been, as the conifer species' specific traits could indicate its environment. This use of plant anatomical analysis helps forensic researchers trace the cub's potential movements and locate its origins.

Materials:

1. 3 envelopes with leaves of three coniferous species (labelled ”A”, ”B” and ”C”)
2. 1 dark bottle with colorant – labelled ”DYE”
3. 1 transparent bottle with DI H2O (distilled water) – labelled ”H2O”
4. 12 pieces of microscope slides placed in one Petri dish
5. 1 box of coverslips
6. 1 scalpel
7. 1 plastic needle
8. 1 tweezer
9. 2 pencils
10. 2 red-capped plastic recipients labelled ”WASTE” for waste
11. 1 pack of tissue paper (for use in all experiments)
12. 3 pairs of gloves - 3 each of sizes S, M, and L (for use in all experiments)
13. 1 compound light microscope

The DYE is specific to cell walls. It stains cellulose walls red and lignified cell walls yellow.

Hypodermis: A layer or layers of cells located just below the epidermis, often serving as a protective or supportive tissue.

Resin duct: Tube-like structures in some plants (like pines and spruces) that transport and store resin, a sticky substance produced to protect the plant from injury or infection.

Plicate chlorenchyma: Refers to the presence of cells with folds that protrude like ridges into the cell.

Palisade chlorenchyma: A type of chlorophyll-rich tissue (chlorenchyma) consisting of elongated, tightly packed cells, usually found just below the upper epidermis of leaves and involved in photosynthesis.

Spongy chlorenchyma: Loosely arranged, irregularly shaped cells with air spaces between them, aiding in gas exchange and photosynthesis.

Vascular bundle sheath: A layer of cells surrounding a vascular bundle (xylem and phloem) in plants, providing structural support and regulating the flow of substances.

Sclerenchyma: A type of plant tissue composed of thick-walled, dead cells that provide mechanical support and strength to the plant.

Collenchyma: A type of flexible plant tissue made up of living cells with unevenly thickened walls, providing structural support while allowing growth.

Vascular bundle: A strand of tissue in plants containing xylem (which transports water) and phloem (which transports nutrients), responsible for internal transport.

Trichome: Hair-like structures on the surface of plant epidermis.

Cuticle: A waxy, protective layer covering the epidermis of leaves, stems, and other plant parts, helping to reduce water loss.

Epidermis: The outermost layer of cells in a plant, serving as a protective barrier against environmental factors like pathogens and water loss.

Aquifer tissue: refers to specialized plant tissue involved in the storage or movement of water.

Aerenchyma tissue: contains large air-filled spaces or cavities between the cells.

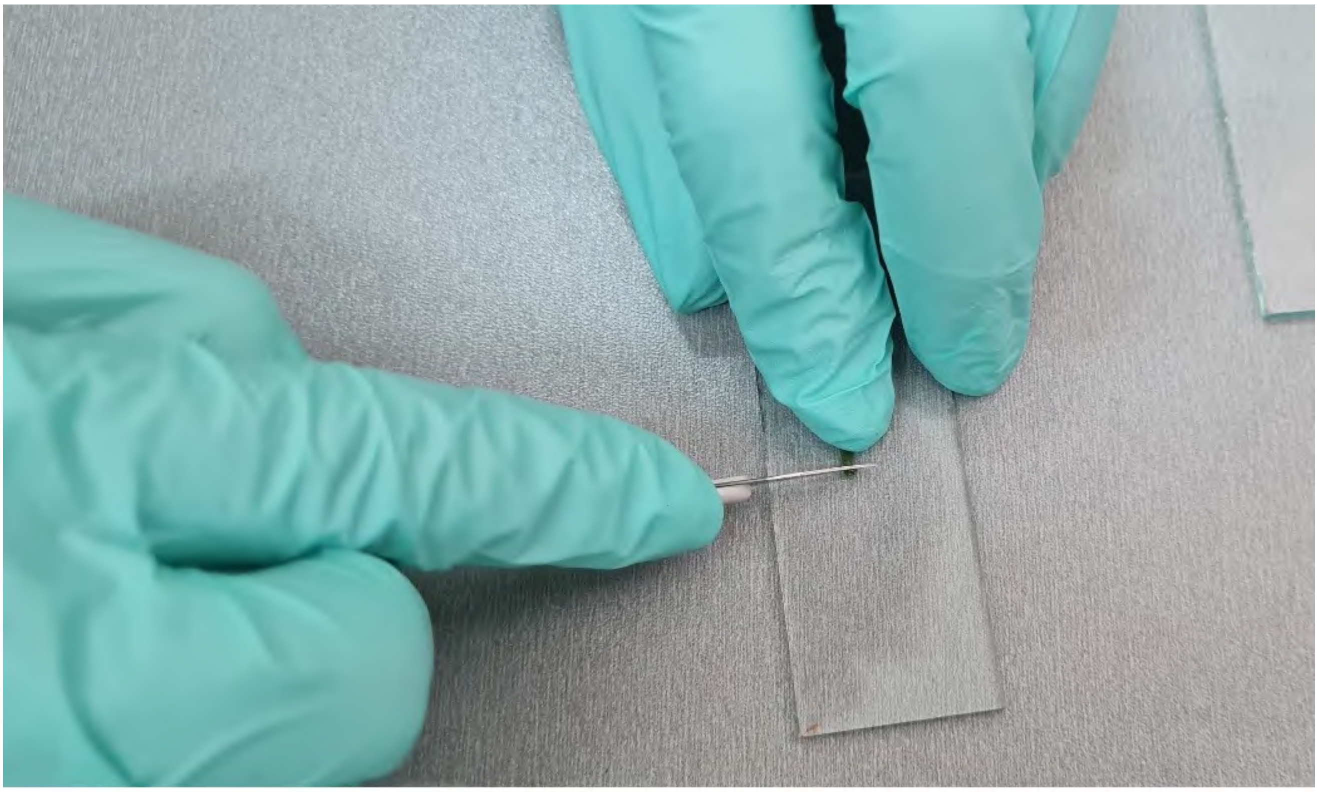
Instructions

Prepare microscope slides from each of the 3 plant leaf specimens, by transverse section and study their anatomies under a microscope. Instructions are as follows.

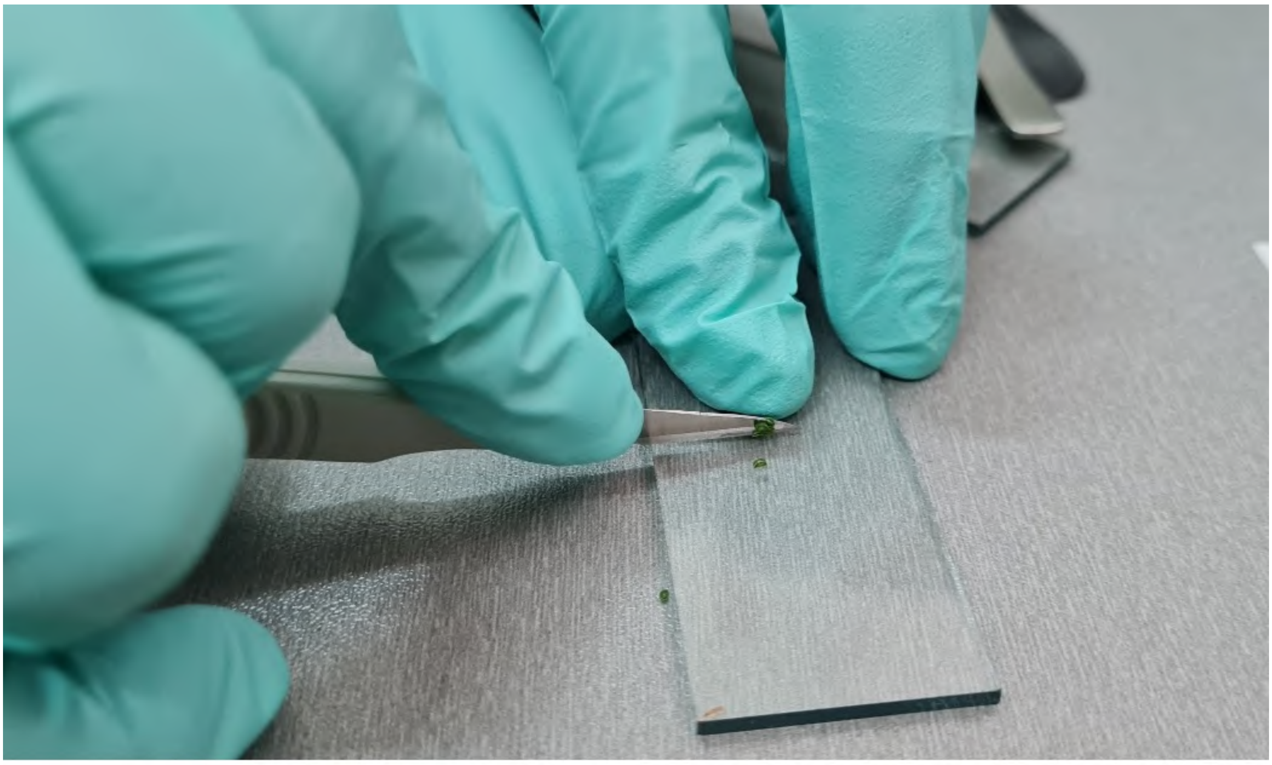
1. Transverse section of specimens: Use the provided scalpel to cut thin cross-sections of each of the plant leaf specimens as follows:

Note: Repeat the following steps for each of the plant leaf specimens provided, independently, so that you do not mix the species specimens.

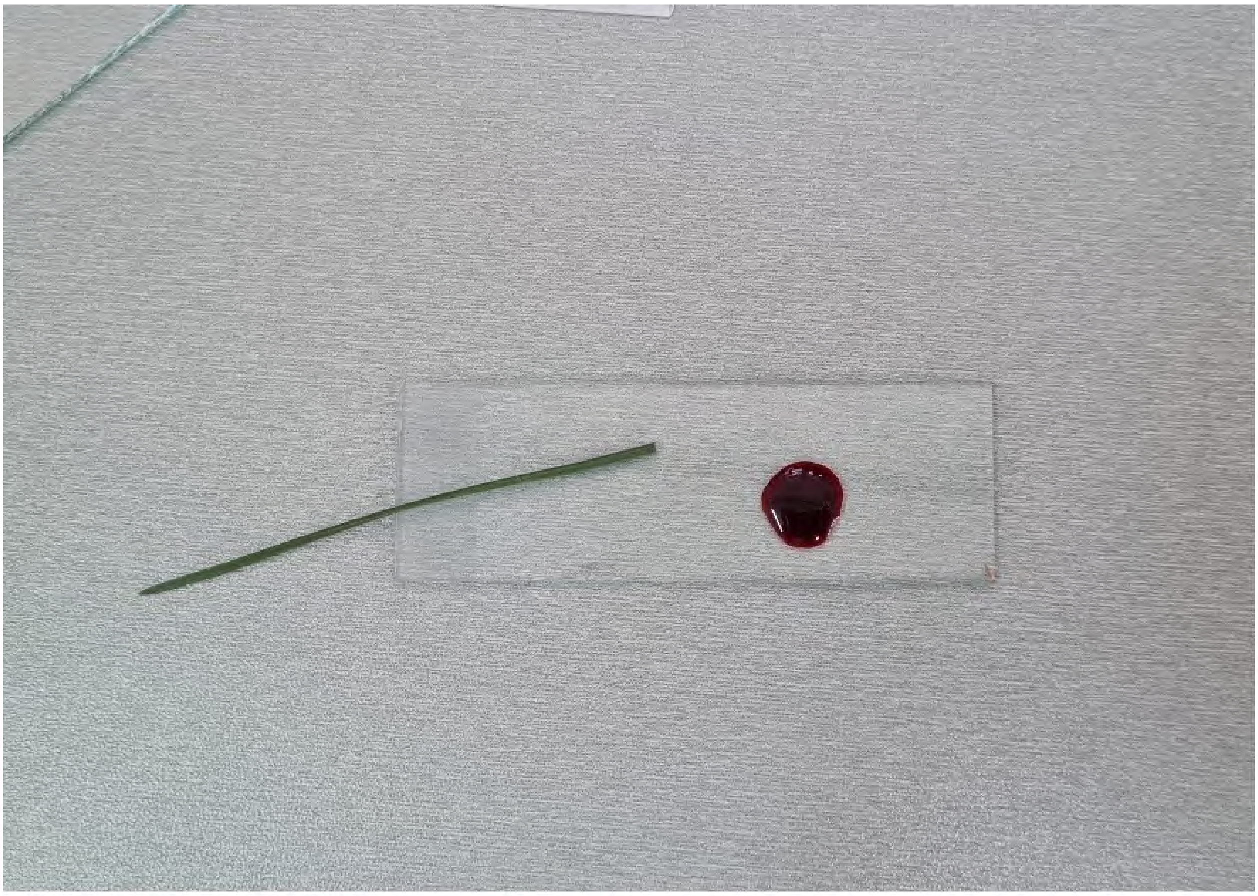
1.1 Hold the specimen down directly on a microscope slide with one hand, as shown in the figure below:



1.2 With the other hand, use the scalpel to cut the specimen vertically to obtain a thin cross-section slice, as shown in the figure below. Repeat this step several times to obtain multiple slices of each of the plant leaf specimens.

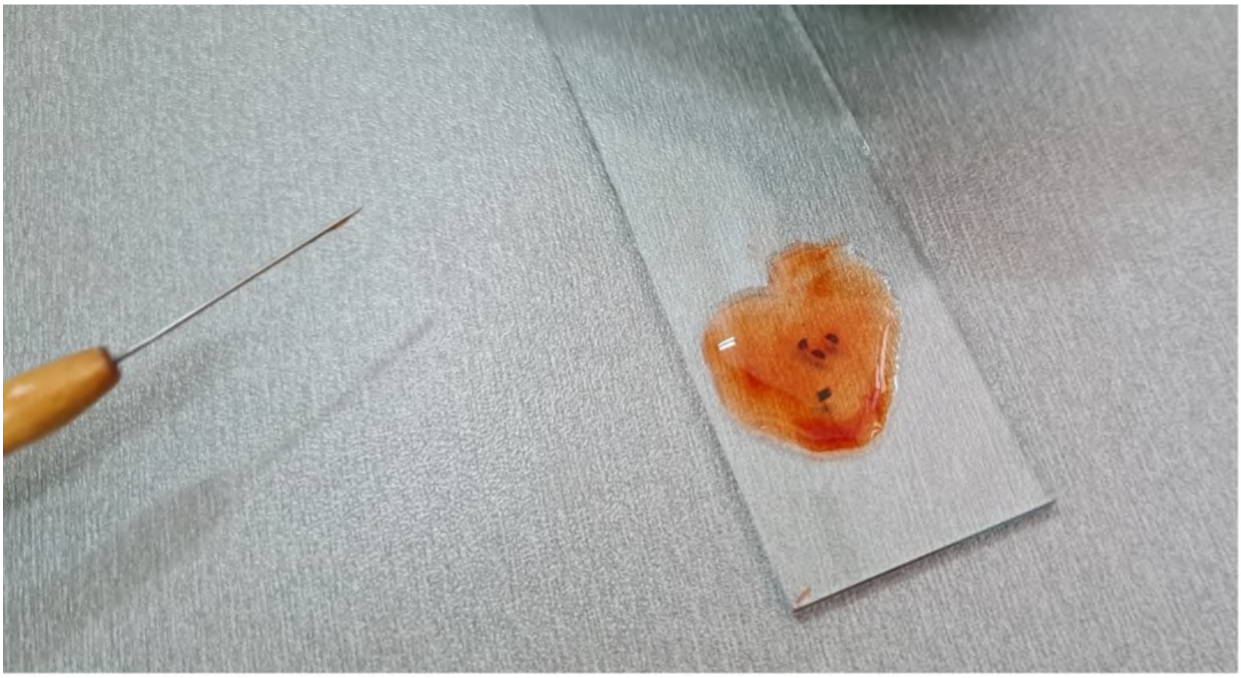


2. Choose as many slices as you like (which you consider the thinnest and whole) of each of the plant leaf specimens and transfer them on a microscope slide. Add a drop of colorant from the bottle labelled ”DYE” on top of the slices from the microscope slide and leave the colorant for approximately 1 minute.

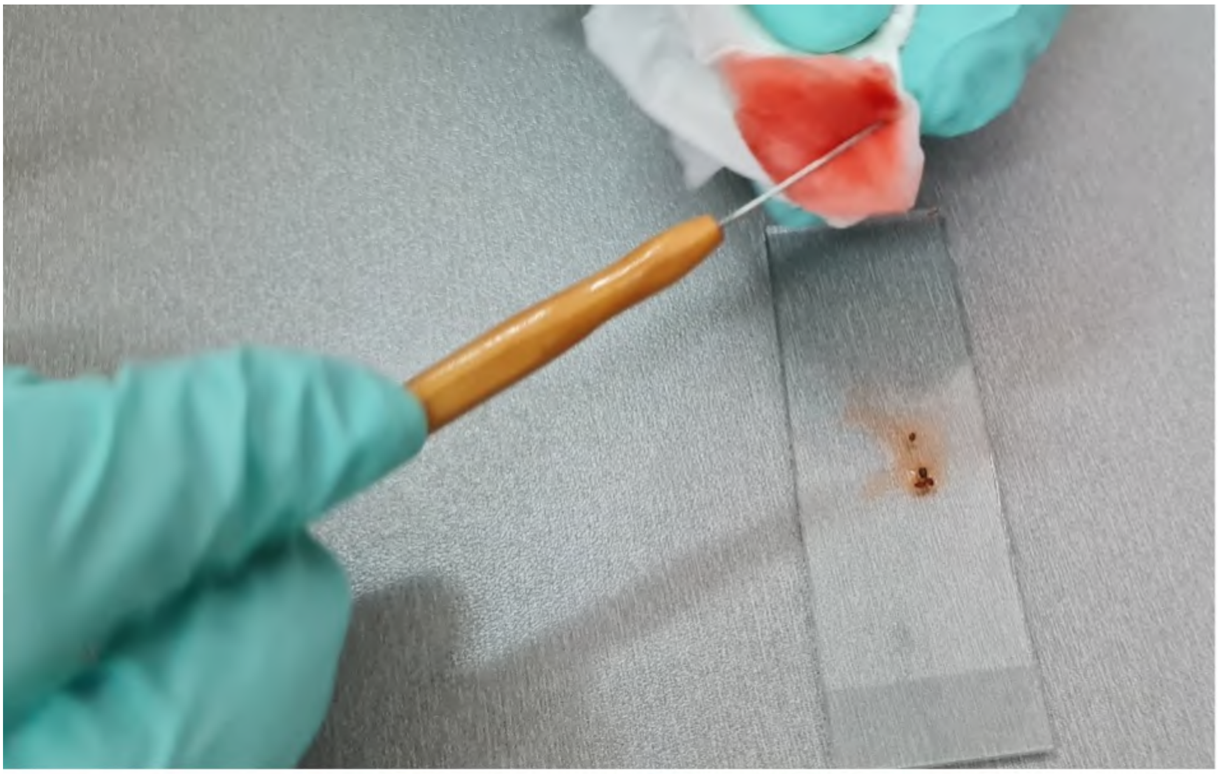


3. After about 1 minute, carefully remove the colorant by draining it with a thin tissue paper. You should be careful not to remove the specimen slices. Use the plastic needle to carefully move the specimen slices on a side, while removing the colorant.

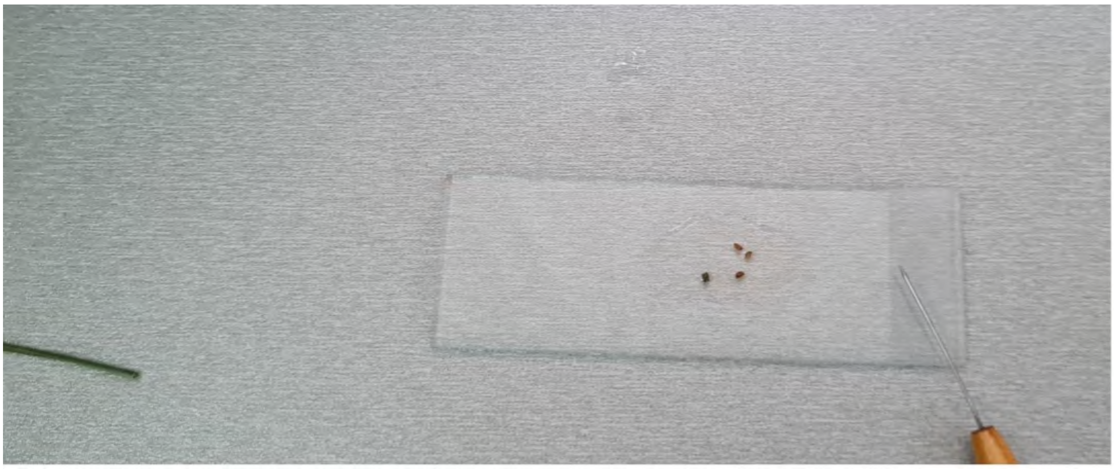
4. Add 1-2 drops of distilled water from the bottle labelled ”H2O” and using the microscope needle, gently move the specimen slices around so that the excess of the colorant is washed out.



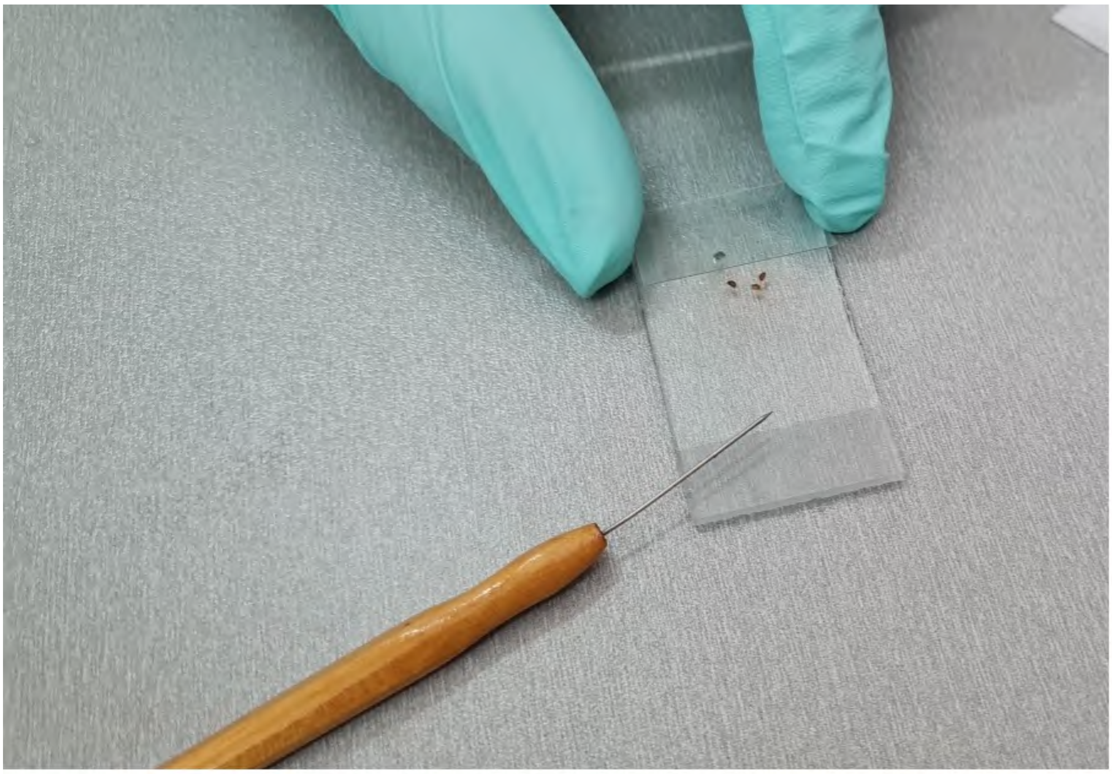
5. Carefully remove the water by draining it with a tissue paper, but take care not to remove also your specimen slices.



6. Add another 1-2 drops of water from the bottle labelled "H2O". If the water becomes red, drain it again, and repeat the procedure until the added water remains colourless. Then proceed to the next step.



7. Cover the specimen slices with a coverslip.



8. Repeat steps 1-7 for each plant specimen.

9. Observe the prepared samples for each of the plant specimens under the provided microscope.

10. Continue to the questions.

QUESTIONS:

DO NOT PROVIDE YOUR FINAL ANSWERS HERE. USE THE ANSWER SHEET!

B_II.1 [3.4 points]

Identify the anatomical characteristics of each leaf specimen. In Table II-1, mark an "X" in the corresponding box to indicate the presence of each identified characteristic. Please note that some tissues are not necessarily present in the cross-section of the specimens. Provide your answers in the ANSWER SHEET.

Table II-1

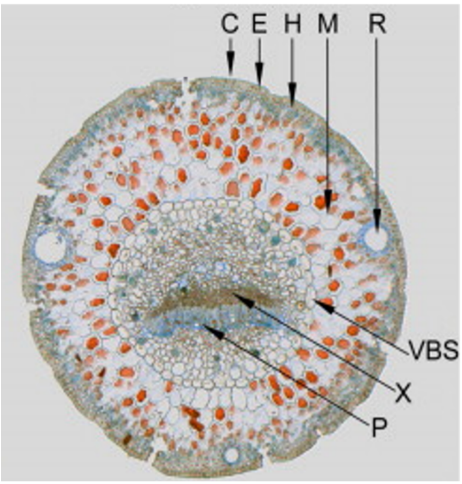
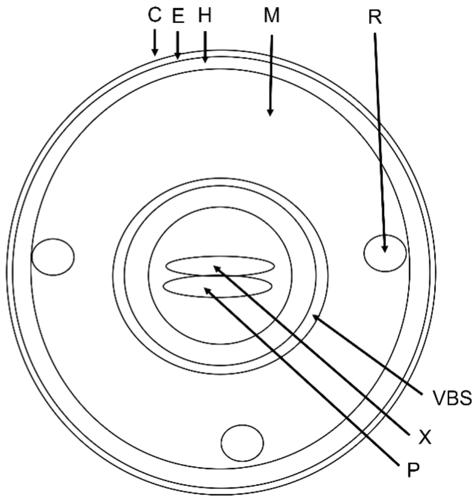
	Specimen A	Specimen B	Specimen C	Tissue is not present in any of the specimens
Cuticle				
Trichomes				
Spongy chlorenchyma				
Palisade chlorenchyma				
Plicate chlorenchyma				
1-2 vascular bundles				
More than 2 vascular bundles				
1-2 resin ducts				
More than 2 resin ducts				
Hypodermis - 1-2 layers				
Hypodermis - more than 2 layers				
Hypodermis – as Sclerenchyma				
Hypodermis – as Collenchyma				
Hypodermis – as Aquifer tissue				
Aerenchyma				

B_II.2 [4.5 points, 1.5 points for each leaf specimen]

Draw in your ANSWER SHEET a schematic representation of each specimen's leaf in the rectangular space provided. Orient the leaf drawing with the upper epidermis facing upward on the page. Use circles to represent resin ducts (if present) and ovals to represent vascular bundles.

Label with:
C – cuticle, E – epidermis, H – hypodermis, M – mesophyll, R – resin duct, VBS – vascular bundle sheath, X – xylem, P – phloem.

Note: an example for schematic representation is given bellow:

Example of a schematic representation – real cross section left, schematic representation right
 

Specimen A – schematic representation

Specimen B – schematic representation

Specimen C – schematic representation

B_II.3 [1.5 points, 0.5 points for each species]

Using the dichotomous identification key provided below, identify the three specimens. Write down the path and the genus of each specimen, in the corresponding table provided in your ANSWER SHEET. An example of an hypothetical species X pathway would be: 1B, 2B, etc.

Dichotomous identification key / decision tree:

Conifer

1.A – Leaves with thin cuticula ---> not in this key

1.B – Leaves with thick cuticula ---> 2

2.A - Leaves scale-like ---> 3

2.B - Leaves needle-like ---> 4

3.A - All leaves short & sharp ---> Sequoia (Sequoioideae)

3.B - Leaves rounded / not sharp ---> Arborvitaes (*Thuja*)

4.A - Leaves in clusters / bundles ---> 5

4.B – Leaves not in clusters / bundles ---> 7

5.A – Leaves in clusters of 2-5 ---> Pine (*Pinus*)

5.B – Leaves in clusters greater than 10 ---> 6

6.A – Needles soft ---> Larch (*Larix*)

6.B – Needles stiff ---> True Cedar (*Cedrus*)

7.A – Needles almost rectangular, stiff ---> Spruce (*Picea*)

7.B – Needles almost flat, flexible & blunt ---> Fir (*Abies*)

Specimen	Path	Genus name of the specimen
A	1B,	
B	1B,	
C	1B,	

B_II.4 [0.6 points]

Identify which plant specimen (A, B, or C) corresponds to the leaf of the plant species dominant in the North West (NW) area of the village. Note that this species has a leaf with only one vascular bundle. Write ”X” in the corresponding box in the table.

	Specimen A	Specimen B	Specimen C
The plant specimen in the NW area of the village			