St. Xavier's College, Mapusa-Goa
Department of Botany

Standard Operating Procedure (SOP)

a) Instruments

b) Preservation of Plant Specimens

Staff Members

Dr. Wendy F. Xavier Martins (HOD)
Ms. Patricia A. Fernandes (Laboratory Assistant)
Ms. Siya Kandolkar (Field Plant Collector)
Mr. Manuel Cruz (MTS)
Mr. Regi M. Rodrigues (MTS)

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1. Standard Operating Procedure (SOP): Operation of pH Meter

Purpose:

To provide detailed instructions for the proper operation of the pH meter. To ensure accurate and reliable pH measurements, which are essential for quality control, research, and analytical purposes in various industries.

Scope:

This SOP applies to all personnel involved in operating and maintaining the pH meter in a laboratory or testing environment.

Equipment and Materials:

a) pH meter
b) pH electrode(s)
c) Buffer solutions (pH 4, 7, and 10)
d) Distilled or deionized water
e) Cleaning materials (lint-free cloth, electrode cleaning solution, etc.)

Procedure:

Calibration:

a) Before starting any measurements, ensure that the pH meter is properly calibrated according to the manufacturer's instructions.
b) Prepare fresh buffer solutions of known pH values (pH 4, 7, and 10) using the appropriate stock solutions and following the recommended preparation procedures.
c) Immerse the pH electrode(s) in each buffer solution, allowing sufficient time for stabilization.
d) Adjust the pH meter reading to match the corresponding buffer pH value by following the calibration procedures provided by the manufacturer.
e) Document the calibration process and results as per the laboratory's standard operating procedures.

Measurement Procedure:

a) Rinse the pH electrode(s) with distilled water to remove any residual substances.
b) Gently blot the electrode(s) with a lint-free cloth to remove excess water without damaging the sensitive glass membrane.
c) Immerse the electrode(s) into the sample solution, avoiding contact with the container walls or other solid particles.

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d) Allow the pH reading to stabilize on the meter display.
e) Record the pH reading obtained from the sample solution.

Maintenance and Storage:

a) After each measurement, rinse the pH electrode(s) with distilled or deionized water to remove any residual sample.
b) If necessary, clean the electrode(s) using an appropriate electrode cleaning solution or procedure recommended by the manufacturer.
c) Store the pH electrode(s) in a suitable storage solution or container to maintain their performance and integrity.

2. Standard Operating Procedure (SOP): Operation of Visible Spectrophotometer

Purpose:

To provide detailed instructions for the proper operation of a visible Spectrophotometer.

To ensures accurate and reliable measurements of ultraviolet-visible (UV-Vis) absorbance or transmittance of samples, which is essential for various laboratory applications such as quantitative analysis, purity determination, and method development.

Scope:

This SOP applies to all personnel involved in operating and maintaining the UV Spectrophotometer in a laboratory or testing environment

Equipment and Materials:

a) Visible Spectrophotometer
b) Appropriate UV-Vis cuvettes or cells
c) Samples to be analyzed
d) Blank solution (if applicable)
e) Cleaning materials (lint-free cloth, laboratory-grade solvents, etc.)

Sample Measurement Procedure:

a) Prepare the samples to be analyzed, ensuring they are in a suitable form and concentration for UV-Vis measurements.
b) If required, prepare a blank solution using the appropriate solvent or matrix to account for any background absorbance or interference.
c) Set the desired wavelength or spectral range based on the analytical requirements.
d) Insert the cuvette or cell containing the blank solution into the sample compartment and

set it as the reference.

e) Remove the blank cuvette and insert the cuvette containing the sample into the sample compartment.
f) Record the absorbance or transmittance reading displayed by the Visible Spectrophotometer.
g) If multiple readings are required, rinse the cuvette with the appropriate solvent, dry it, and repeat the measurement process for each sample.
h) If necessary, calculate the concentration or perform further data analysis based on the specific analytical method.

Post-Measurement Procedures:

a) Clean the cuvettes or cells after each measurement by rinsing with suitable solvents, wiping with a lint-free cloth, and ensuring they are free from any residual sample or contaminants.
b) Clean the sample compartment of the Visible Spectrophotometer using appropriate cleaning materials as recommended by the manufacturer or the laboratory's standard operating procedures.
c) Store the Visible Spectrophotometer in a clean and secure location, following the manufacturer's guidelines for proper storage.

3. Standard Operating Procedure (SOP): Operation of Laminar Air Flow

Purpose:

The purpose of a standard operating procedure (SOP) for laminar air flow (LAF) is to ensure the correct operation, cleaning, and monitoring of the LAF system

Scope:

To carry out the procedure Operation of Laminar airflow in the laboratory

Procedure:

a) Connect the main switch to the main supply. Switch "ON" the mains.
There are three switches and a Magnehelic gauge on the side panel of an instrument.
- Air flow
- Light
- UV

b) Clean the working bench of LAF with cotton moistened in filtered 70% Ethanol
c) The airflow and UV should be kept "ON" for 20-30 minutes before carrying out work under LAF. Check the reading of the Magnehelic gauge; it should be between 10 to 15 pascal when airflow is on.

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d) During working switch “OFF” the UV light and switch “ON” the tube light.
e) After working, switch “OFF” the tube light and airflow of LAF.
f) After completion of work, clean the working bench of LAF with cotton moistened in filtered 70% ethanol.

Precaution:

a) Clean the LAF after every operation and record the cleaning detail in the logbook.
b) Switch off the UV light before starting the operation; otherwise, it can harm your body.
c) Maintain the working plate. It should be horizontally planned during operation hours.

4. Standard Operating Procedure (SOP): Operation of Compound Microscopes

Purpose:

This SOP provides guidelines for the proper use and maintenance of the compound microscope to ensure accurate and reliable microscopy observations.

Scope:

Operation and Cleaning of Microscope installed in the laboratory.

Procedure:

a) Connect the plug to main power socket and switch ON the mains to the instrument and switch ON the lamp.
b) Adjust the intensity of the light by turning the knob provided at base of Microscope.
c) Adjust the condenser by raising or lowering it.
d) Fix the slide in position on the stage.
e) Turn the low power (4xs) objective into position. Make sure it has clicked into position.
f) Lower the condenser as low as possible and open the iris diaphragm wide
g) Gradually raise the mechanical stage till the objective is almost touching the slide
h) Then looking through the eyepiece and using the coarse adjustment knob lower the mechanical stage so as to bring the object roughly into focus.
i) By using the fine adjustment knob bring the object sharply into focus.
j) Make any final adjustment with the condenser and iris diaphragm.
k) If the high power objective is to be used, rotate the eyepiece until the high power objective occupies the position of the low power objective. Then use the coarse and fine adjustment knobs to focus the object.
l) Sharpen the focus using fine adjustment knob.

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Use of oil immersion lens:

a) After focusing the object under low power, turn the eyepiece and put the oil immersion objective in place.
b) Place a drop of oil (cedar wood) on the slide.
c) Gradually raise the mechanical stage till the oil drop touches the objective and just flattens it, without touching the object itself.
d) During this procedure the objective should be viewed from the side with the eyes on level with the slide.
e) Now look down the ocular and slowly lower the mechanical stage by means Of the coarse adjustment knob until the object is just in view.
f) Then using the fine adjustment knob bring the object into focus.

Note: 100 x objective is used only as an oil immersion lens.

Cleaning:

a) Before using check that the objectives, eyepieces and all external parts of the microscope are clean.
b) Clean the magnification objectives and clean the surface of small concave front lens with a lint free cloth or tissue paper.
c) Wipe the outer surfaces of the microscope with lint free duster.
d) Wipe the underneath portion of the oil immersion lens with a fast absorbing tissue paper or cloth before and after using the lens
e) Clean the 100X oil emulsion objective after each use.

5. Standard Operating Procedure: Operation of Autoclave

Purpose:

To ensure that all potentially infectious waste materials, and waste that must be made biologically inactive before disposal, are adequately sterilized when subjected to autoclaving.

Scope:

This SOP applies to all autoclaves that are used to decontaminate infectious waste materials and plant (and plant associated) materials that must be made biologically inactive prior to disposal into the normal solid waste stream.

Procedures:

Waste Collection:

a) All potentially infectious waste must be collected into a solid walled container marked with the universal biohazard symbol.

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b) Non-infectious waste (such as plant material that requires autoclaving) may be collected in bags that are not marked with the biohazard symbol.
c) Do not mix potentially infectious waste with non-infectious waste at any time.

Autoclaving:

a) Place bag to be autoclaved into a shallow pan or tub.
b) Affix a small piece of autoclave indicator tape or test strip to the outside of the bag.
c) Place the pan and bag inside the autoclave.
d) Do not overload the autoclave.
e) No other materials should be autoclaved together with waste in the same load.
f) Run the autoclave at a chamber temperature of 121°C for 60 minutes , using a dry cycle run. 121°C is a standard temperature for autoclave operation, and generally achieved when chamber pressure is 15-16 psi.
g) When the cycle has been completed, the chamber may now be opened and the waste bag removed.
h) Wear autoclave gloves when handling hot items.
i) Once the bag has cooled, place the treated waste bag inside a black plastic bag and close the bag either by knotting or with a twist tie.
j) The treated waste may now be discarded as normal solid waste.
k) The above instructions are for solid waste
l) For liquid cultures, always use secondary containment when autoclaving such as a shallow pan or tub.

Precautions:

a) Always wear thermal protective gloves when handling items that have been recently autoclaved.
b) Use caution when opening an autoclave door.
c) Always verify that the chamber pressure has come down to 0 psi before trying to open the door.
d) Still, a little bit of steam may be released when you open the autoclave door.
e) Be cautious about minimizing exposure during placement and retrieval of the testing vial.
f) At a minimum use personal protection equipment (PPE) such as gloves, safety glasses, and a lab coat and mechanical methods such as forceps to avoid exposures.

The following items should not be autoclaved:

a) Polyethylene plastics (LDPE and HDPE)
b) Solutions or waste products that contain chemicals characterized as corrosives (bleach), solvents, flammables, volatiles, or radioactive materials
c) Anything in a sealed container

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6. Standard Operating Procedure: Operation of Plant growth Chamber

Purpose:

To ensure consistent operation and compliance with regulations.

Scope:

To study plant growth controlling environmental factors such as temperature, humidity, light and carbon dioxide level.

Operating Instructions:

a) Place the Plant Growth Chamber on the Level surface.
b) Open the door, fix the side runners & Place the S.S. Rod trays at the required distance with sample inside the chamber. Close the door firmly.
c) Give water connection to Water Inlet of Reservoir Pot. (at back side) DM water.
d) Connect the chamber to 230 v ac., 50 Hz Mains supply. Proper Earthing must be provided to avoid unnecessary shock Hazard.
e) Put the Plug into Mains socket & switch the supply "ON" with the help of "MAINS" ON/OFF switch, now "MAINS" on indicator will Glow.
f) Set the required Dry Temperature, Humidity (R.H), Lux, please refer the PLC Controller and Touch screen colour HMI.
g) Switching "ON" & "OFF" of the Cooling system for lower Temperature thought the Controller menu. (factory setting) automatic operations as follows
    - OFF Mode: Between the Range of 40 °C above to 60 °C.
    - ON Mode: Between temp Range 10 °C upto 40°C.
h) WATER Level: When the water level of water tank will go down to the normal level, the Buzzer will ring and supply connection to boiler Heater will get disconnected.

- **Note: Connect the Over Flow to drain the water.**

Maintenance:

a) A routine maintenance is required for Wet bulb sensor used to indicate Humidity (R.H) reading provided to the controller.
b) Note the Humidity sensor must be clean with fresh air periodically every month to avoid incorrect reading on the controller.
c) The PGC outer surface may be cleaned with a damp cloth. Do not allow water to enter the interior of the controller panel case. Do not clean with organic solvents. Fan models: the motor is sealed for life; no lubrication is required.
d) Check the door seal regularly for cuts or tears.

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Calibration

a) After prolonged use the controller and/or thermocouple could require recalibration. This would be important for processes that require accurate temperature readings.
b) A quick check using an independent thermocouple and temperature indicator should be made from time to time to determine whether full calibration is required.
c) Depending on the controller, the controller manual may contain calibration instruction.

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Preservation of Plant Specimens

1. Wet preservation of plant specimens

Wet preservation of plant specimens is a technique that involves preserving plant material in air-tight glass or plastic jar with a liquid fixative and storing it in a permanent solution. This method is also known as fluid-preserved specimens or embalmed specimens.
The type of preservative used should be clearly labelled on the jar. A common preservative is a mixture of formaldehyde with acetic acid and ethyl alcohol.

a) Preservation

To preserve the plant specimens in the Botany dept. a mixture of formaldehyde: acetic acid: alcohol (9: 0.5: 0.5) is used. The specimen is placed in a jar containing the preservative solution. Care is taken that the specimen is submerged in it.

b) **Sealing**

Using wax the jars are sealed to reduce evaporation.

c) **Labelling**

Each preserved specimen is labelled with its Botanical name, common name and family.

d) **Maintenance**

The fluid level of the preservative in the specimen jars is maintained.

2. Dry preservation of plant specimens - Herbarium

A Herbarium is defined as a collection of plants that have been dried, pressed, preserved on sheets, labeled and arranged according to **Bentham and Hooker's system** of classification.

Methods of preparation of herbarium specimens:

The preparation of a herbarium involves:

a) Collection of plant specimens

Herbs, very small shrubs should be collcyed complete in glowering stage, along with leaves and roots. Trees and shrubs should be collected with both, vegetative and glowering shoots.. All information concerning planst should be recorded in the field note

book and a tag from the sheet attached to the concerned specimen.

b) Poisoning

The specimen is dipped in **2%** solution of **mercuric chloride with 70% ethyl alcohol**

c) Pressing and Drying

Specimens shorter than 15 inches, should be kept directly in the folded newspaper after loosely spreading the leaves and branches. If longer than 15 inches, can be folded in the form of **N**' or' **W**' or '**V**' shapes ensuring the terminal part of the plant with leaves, flowers is erect.

d) Mounting

The dried specimens are mounted on herbarium sheets of standard size (**41 x 29 cm**). Mountingis done with the help of adhesive. The bulky plant parts like dry fruits seeds, cones etc. are dried without pressing and are put in small envelops called fragment packets.

e) Labelling

A label is pasted on the lower right hand corner. The label indicates the information about the collector's name, collector's number, Botanical name, common name, habit, date and time of collection and locality.

f) Filing and deposition in herbarium cabinets

Properly dried, pressed and identified plant specimens are placed in thin paper folds (specimen covers) which are kept together in thicker paper folders genus overs), and finally they are incorporated into the herbarium cupboards in their proper position according to **Bentham and Hooker's system** of classification

g) Maintenance of herbarium specimens.

Use of repellents like naphtalenene is being put in the small muslin bags and kept in pigeonholes.

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