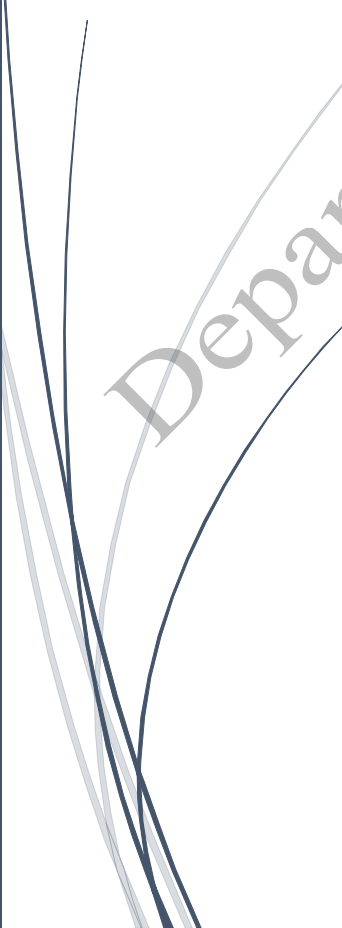


Standard Operating Procedures (SOP)

Department of Biotechnology

St. Xavier's College, Mapusa Goa

Department of Biotechnology



STANDARD OPERATING PROCEDURES AUTOCLAVE

1. Pre-Operation Checks

- Inspect the autoclave for visible signs of damage or leaks.
- Check water levels according to the manufacturer's instructions.

2. Preparation of Materials

- Place materials in autoclave-safe containers. Do not overfill, as steam circulation is essential.
- Use autoclavable bags for waste; ensure they are loosely closed to allow steam penetration.

3. Loading the Autoclave

- Arrange items to allow for steam circulation.
- Place containers on trays, and avoid direct contact with the walls of the autoclave.
- Avoid overloading the autoclave, as this can impact sterilization efficiency.

4. Selecting the Cycle

- Choose the appropriate cycle based on the type of material:
- Liquids: Usually 121°C for 30 minutes.
- Solid Waste: Usually 121°C for 20–30 minutes, depending on volume.
- Glassware and Equipment: Follow specific lab recommendations.

5. Running the Autoclave

- Close and lock the autoclave door securely before starting.
- Start the autoclave cycle and monitor for any unusual noises or alarms.
- Stay nearby in case of emergency, especially during the startup phase.

6. Unloading the Autoclave

- Wait for the cycle to complete and the pressure to normalize before opening the door.
- Wear heat-resistant gloves and a face shield when opening the autoclave.
- Crack the door open slightly to let steam escape and allow cooling. (5–10 minutes).

- Remove items carefully, keeping in mind that surfaces and containers may be hot.

7. Post-Operation and Cleanup

- Turn off the autoclave and allow it to cool if not in continuous use.
- Dispose of waste materials according to laboratory waste disposal guidelines.

Maintenance and Troubleshooting

- Routine maintenance should be scheduled according to the manufacturer's guidelines.
- Report any malfunctions immediately and tag the autoclave as "out of service" if necessary.
- Do not attempt repairs unless you are trained and authorized.

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HOT AIR OVEN

1. Pre-Operation Checks

- Inspect the oven for any signs of damage, wear, or contamination from previous use.
- Verify that the oven is clean and free from flammable residues.
- Ensure that exhaust ventilation is working properly, particularly if heating materials that may release fumes.
- Confirm power connections and check for any electrical hazards.

2. Preparation of Materials

- Select appropriate containers for the materials, ensuring they are compatible with oven use and will not melt or degrade.
- Arrange materials in a single layer within trays or containers, avoiding overcrowding to allow proper heat circulation.
- Label all items with sample identifiers, dates, and other relevant information if required by lab protocol.

3. Setting the Temperature and Time

- Determine the appropriate temperature and duration for the material being heated (refer to lab protocols or Material Safety Data Sheets, if applicable). Typical temperatures vary by use, e.g., drying samples at 60°C–110°C or heat treatment at higher temperatures.
- Set the temperature and timer on the oven. Allow the oven to preheat to the desired temperature if necessary.

4. Loading the Oven

- Wear appropriate PPE (e.g., heat-resistant gloves) to protect from burns.
- Open the oven door carefully, as hot air may escape.

- Place trays and containers securely in the oven, ensuring they do not touch the sides.
- Close the oven door firmly to prevent heat loss.

5. Running the Oven

- Monitor the oven temperature periodically to ensure it remains at the set level.
- Avoid opening the oven door frequently, as this will cause temperature fluctuations.
- Remain in the vicinity of the oven while it is in use, particularly at high temperatures.

6. Unloading the Oven

- Wait for the heating cycle to complete and allow the oven to cool if necessary.
- Wear heat-resistant gloves and safety goggles.
- Open the door slowly to allow any remaining hot air to escape safely.
- Remove items carefully, placing them on a heat-resistant surface or cooling rack.

7. Post-Operation and Cleanup

- Turn off the oven and allow it to cool completely if it will not be used for another cycle.
- Clean the interior if required, removing any residue or spills after cooling.
- Record details of the operation in a logbook, including date, operator name, temperature, duration, and any issues.

Maintenance and Troubleshooting

- Schedule regular maintenance according to the manufacturer's instructions.
- Report malfunctions immediately and take the oven out of service if necessary.
- Do not attempt repairs unless trained and authorized.

LAMINAR AIR FLOW HOOD

1. Pre-Operation Checks

- Inspect the LAF hood for visible damage or contamination.
- Verify that the hood is functioning properly by checking the airflow indicator (if present).
- Check the filter status to ensure that HEPA filters are intact and functioning. If the hood has not been used recently, allow it to run for at least 10-15 minutes before starting work.
- Put on a lab coat, gloves, and any other required PPE.

2. Preparation of the Work Area

- Disinfect the interior surfaces by wiping them down with an approved disinfectant (e.g., 70% ethanol). Start with the back wall, then the sides, and finally the work surface.
- Place all necessary materials and equipment inside the hood before beginning work to minimize movement in and out of the LAF area.
- Arrange items systematically to allow free airflow across the work surface. Avoid blocking airflow by positioning larger objects near the sides or back of the hood.
- Only bring essential items into the LAF hood to maintain cleanliness and reduce clutter.

3. Operation in the LAF Hood

- Perform work at least 6 inches inside the hood, away from the edge, to ensure materials remain within the sterile airflow.
- Avoid blocking the airflow from the HEPA filter by keeping hands and materials below the airflow path.
- Use aseptic techniques, such as flame sterilization of equipment if necessary, and avoid touching any surfaces with gloved hands after they contact nonsterile surfaces.

Minimize talking or unnecessary movements in the LAF hood area to reduce the risk of contamination.

4. Completion of Work

- Dispose of waste materials properly (e.g., pipette tips, gloves) in biohazard or appropriate waste containers.
- Wipe down all surfaces in the LAF hood with disinfectant once the work is completed. Pay attention to the back and side walls, work surface, and any equipment used.
- Remove materials carefully to avoid disturbing the airflow.
- Allow the LAF hood to run for at least 5 minutes after completion to clear out any remaining particles.

5. Post-Operation and Shutdown

- Turn off the LAF hood if it is not required for further use.
- Log usage details if required by lab protocol, including date, time, operator name, and any relevant observations (e.g., malfunction or unusual airflow).
- Clean and store reusable materials appropriately, outside of the LAF hood, if needed.

Maintenance and Troubleshooting

- Regularly scheduled maintenance should be performed according to the manufacturer's recommendations, typically involving inspection of the HEPA filters and airflow indicators.
- Do not attempt repairs unless trained and authorized.
- Report malfunctions immediately to lab management and place an "out of order" sign on the hood if necessary.

pH METER

1. Pre-Operation Checks

- Inspect the pH meter for any signs of damage, particularly the electrode.
- Check the expiration dates of the buffer solutions. Do not use expired buffers, as they may result in inaccurate calibration.
- Ensure proper electrode storage, as per the manufacturer's instructions, to maintain its functionality.

2. Preparation for Calibration

- Power on the pH meter and allow it to stabilize (usually a few minutes).
- Prepare buffer solutions (usually pH 4.0, 7.0, and 10.0) and allow them to reach room temperature if they aren't already.
- Rinse the electrode with distilled or deionized water and gently blot with a lab wipe to remove any residue without damaging the electrode.

3. Calibration Procedure

- Place the electrode in the pH 7.0 buffer solution and wait for the reading to stabilize.
- Set the pH meter to recognize pH 7.0 if it does not automatically do so.
- Rinse the electrode with distilled water, blot dry, and repeat the process with the pH 4.0 and/or pH 10.0 buffer solutions.
- Adjust the pH meter settings as needed after each buffer to ensure accurate calibration across the range.
- Complete the calibration and verify accuracy by re-measuring one of the buffer solutions if required by lab policy.

4. Sample Measurement

- Rinse the electrode with distilled water and blot dry with a lint-free tissue.

- Immerse the electrode in the sample solution. Ensure the sample covers the junction of the electrode fully.

Wait for the reading to stabilize before recording the pH value. Stabilization time may vary depending on the sample and meter.

- Rinse the electrode with distilled water between each sample to prevent cross-contamination.

5. Post-Operation and Storage

- Rinse the electrode with distilled water and gently blot dry after completing measurements.
- Store the electrode properly according to manufacturer instructions:
 1. For most glass electrodes, immerse in an electrode storage solution.
 2. Never store in distilled or deionized water, as it can damage the electrode.
- Turn off the pH meter and cover or secure it if required.

Maintenance and Troubleshooting

- Calibrate the pH meter daily or before each use if precise measurements are required.
- Check for electrode drift by regularly measuring known buffer solutions. If readings are inconsistent, the electrode may need cleaning or replacement.
- Clean the electrode periodically with an appropriate cleaning solution, especially if measuring samples that leave residues.
- Replace the electrode if readings become unstable or calibration fails frequently, as electrodes have a limited lifespan.

WEIGHING BALANCE

1. Pre-Operation Checks

- Inspect the balance to ensure it is clean and free from any residue from previous measurements.
- Check for level: Verify that the balance is level by adjusting the feet if necessary (most balances have a bubble level indicator).
- Ensure the balance is on a stable surface, away from vibrations, drafts, or direct sunlight, which can affect accuracy.
- Verify calibration status: Ensure the balance has been calibrated recently. Use calibration weights if necessary and as specified in lab protocols.

2. Powering On and Stabilization

- Power on the balance and allow it to stabilize. This may take a few minutes depending on the balance.
- Check that the display reads zero before beginning any measurements. If not, use the “tare” or “zero” function to reset the display.

3. Weighing Procedure

- Select an appropriate container for the material to be weighed (e.g., weigh boat, beaker) and place it on the balance.
- Tare the balance to zero with the container on it to exclude its weight from the measurement.
- Add the sample gradually into the container, avoiding spillage. If needed, use a spatula or scoop to carefully transfer the sample.
- Record the weight once it stabilizes and the display shows a consistent reading.
- For multiple additions, re-tare the balance after each addition if the individual weights of components are needed.

4. Removing the Sample and Post-Weighing Procedure

- Remove the container and sample from the balance carefully to prevent spillage.
- Clean the weighing pan and surrounding area if any materials were spilled. Use a clean brush or lint-free cloth.
- Turn off the balance if it will not be used again shortly.

Maintenance and Cleaning

- Clean the balance daily or after each use, especially if measuring powders or volatile substances.
- Use a soft brush or lint-free cloth to remove any residues from the weighing pan.
- Calibrate the balance periodically (as per the lab's schedule) or after moving it to a new location.

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MICROPIPETTES

1. Pre-Operation Checks

- Inspect the micropipette for any visible signs of damage, such as cracks, loose parts, or malfunctioning plunger.
- Check calibration status: Ensure the micropipette has been calibrated recently, especially if precise measurements are required.
- Select the correct pipette size based on the volume you intend to measure. Always choose the smallest pipette that can measure the intended volume accurately.
- Use compatible tips that fit snugly on the pipette to prevent leaks and ensure accurate measurements.

2. Setting the Desired Volume

- Adjust the volume by turning the volume adjustment dial on the micropipette to the required setting.
- Confirm that the volume is within the micropipette's range; using it outside its specified range can damage the equipment and lead to inaccurate measurements.

3. Pipetting Technique

- Attach a new, clean pipette tip securely onto the micropipette. Always use a fresh tip for each new sample to prevent cross-contamination.
- Hold the micropipette vertically and depress the plunger to the first stop (the soft resistance point).
- Immerse the tip slightly below the surface of the liquid (about 1-2 mm for small volumes and up to 5 mm for larger volumes) to ensure accurate aspiration.

- Slowly release the plunger to aspirate the liquid into the tip. Allow the plunger to return smoothly to the starting position; releasing it too quickly can lead to air bubbles and inaccurate measurements.
- Withdraw the pipette tip from the liquid, ensuring no liquid drips from the tip. If droplets form, repeat the aspiration step.

4. Dispensing the Liquid

- Hold the micropipette at a slight angle against the side wall of the receiving container to allow smooth liquid release.
- Depress the plunger slowly to the first stop to dispense the measured volume.
- Press down to the second stop (beyond the first point of resistance) to expel any remaining liquid from the tip.
- Remove the pipette tip from the container before releasing the plunger to prevent drawing any liquid back into the tip.
- Eject the used pipette tip into a waste container by pressing the tip ejector button.

5. Post-Operation and Storage

- Clean the micropipette with a lint-free cloth if any residue is present, especially around the nozzle.
- Return the micropipette to its designated holder or storage area. Avoid laying it horizontally, as this can cause liquid residue to enter the internal mechanism.
- Log usage or any issues if required by lab protocols, including date, operator name, and any observed problems with the micropipette.

Maintenance and Calibration

- Calibrate micropipettes periodically as per lab requirements or after any incidents where accuracy may have been compromised.
- Clean micropipette components regularly, following manufacturer recommendations, to ensure long-term accuracy.
- Replace tips after each use to avoid contamination between samples.

CENTRIFUGE

1. Pre-Operation Checks

- Inspect the centrifuge for visible damage, residue, or any objects that may interfere with operation.
- Check the rotor and buckets for signs of corrosion or wear, which could compromise safety.
- Ensure tubes or containers are suitable for the type of centrifuge and are free of cracks or damage.
- Confirm the rotor type and weight limit for your sample tubes to ensure safe and accurate operation.

2. Loading the Centrifuge

- Prepare samples by placing them in appropriate centrifuge tubes and sealing them tightly with caps.
- Balance the tubes accurately by ensuring that samples of equal volume and weight are placed opposite each other. For odd numbers of tubes, use balance tubes with an equal volume of water or another appropriate material.
- Load the tubes into the rotor or buckets, checking that each pair of tubes is directly opposite each other to maintain balance.
- Close the centrifuge lid securely and ensure the lid latch is engaged.

3. Setting Operation Parameters

- Set the appropriate speed (RPM or RCF) and time based on your protocol or sample requirements.
- For refrigerated centrifuges, set the temperature if required by your protocol.
- Double-check that all settings are correct before starting the run.

4. Starting the Centrifuge

- Stand clear of the centrifuge while it reaches full speed, and observe it briefly to ensure there are no unusual sounds or vibrations.
- Do not open the lid or attempt to stop the centrifuge manually while it is running.

5. Unloading the Centrifuge

- Wait until the centrifuge has come to a complete stop and the rotor is no longer moving before opening the lid.
- Carefully remove the tubes to avoid disturbing the separated contents.
- Inspect the tubes for leaks or spills before proceeding with sample handling.
- Clean any spills immediately if a tube has leaked.

Post-Operation and Maintenance

- Clean the rotor and interior of the centrifuge with a lint-free cloth and disinfectant, especially if a sample leaked or spilled.
- Remove any residues from the rotor, buckets, and centrifuge chamber to prevent corrosion and contamination.
- Record usage and any maintenance in the logbook, including details like speed, time, and any incidents or unusual sounds observed.
- Check and lubricate the rotor (if required by the manufacturer) and inspect regularly for signs of wear or damage.
- Store the rotor separately if recommended by the manufacturer, especially for high-speed and ultra-centrifuges.

Emergency Procedures

- In the event of a tube breakage or leakage, stop the centrifuge, clean the rotor and chamber thoroughly, and dispose of broken glass or contaminated materials according to lab protocols.

- If the centrifuge malfunctions (e.g., unusual noise, vibration, or smoke), turn off the power, unplug the centrifuge, and report the issue immediately. Tag the centrifuge as "out of service" until it can be inspected and repaired.

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MICROSCOPES

1. Pre-Operation Checks

- Inspect the microscope for visible damage, dirt, or any signs of wear on lenses and stage.
- Ensure all lenses are clean: If there is dust or residue, gently clean with lens tissue or a lint-free cloth. Avoid using regular tissues or cloths, as they may scratch the lens.
- Ensure the objective lenses are correctly aligned and check that the stage and focusing knobs move smoothly.

2. Setting Up the Microscope

- Turn on the light source and adjust the brightness to a comfortable level.
- Place the microscope slide on the stage with the sample area positioned above the light source.
- Use the stage clips (if available) to secure the slide in place.
- Select the lowest magnification objective (usually 4x or 10x) and bring it into position directly above the slide.

3. Focusing the Microscope

- Use the coarse focus knob to bring the slide into focus while looking through the eyepiece. Begin by lowering the stage, then slowly raise it until the sample is in focus.
- Adjust the fine focus knob to achieve a sharper image of the sample.
- Center the sample in your field of view, and then rotate to a higher magnification objective if needed.
- For objectives requiring immersion oil (e.g., 100x), apply a small drop of immersion oil to the slide before rotating the objective into position. Avoid using oil on non-oil objectives, as this can damage them.

4. Adjusting Illumination and Contrast

- Adjust the condenser and diaphragm to control the amount of light and enhance contrast. For higher magnifications, increase the amount of light as needed.
- Use the brightness control on the light source to achieve optimal lighting. Avoid excessive brightness to reduce eye strain and prevent damage to the sample.

5. Viewing and Recording Observations

- Observe the sample through the eyepieces, adjusting interpupillary distance if applicable.
- Record observations as per your lab protocol, noting details like magnification, field of view, and any notable characteristics.
- If using a camera attachment, capture images following lab guidelines and save them with relevant data for documentation.

6. Post-Operation and Cleaning

- Turn off the light source after use to prevent overheating.
- Lower the stage and switch to the lowest magnification objective.
- Remove the slide carefully from the stage, and clean it if necessary.
- Clean all lenses with lens tissue or a lint-free cloth. If immersion oil was used, clean it off immediately with lens tissue.
- Cover the microscope with a dust cover to protect it when not in use.
- Log any maintenance or unusual findings in the microscope logbook.

Maintenance and Calibration

- Clean the microscope lenses and stage regularly to prevent dust buildup and ensure clear observation.

- Schedule regular calibration of the microscope and any digital components (such as camera attachments) as per lab requirements.
- Check for loose or damaged parts periodically, and report any issues for prompt repair.

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