RAMAIAH
College of Arts, Science
& Commerce

सत्यमेव जयते
Department of Biotechnology
Ministry of Science & Technology
Government of India

STANDARD OPERATING PROCEDURES (SOP) MANUAL

DEPARTMENT OF MICROBIOLOGY

Principal's Message

It gives me immense pleasure to present the **Standard Operating Procedures (SOP) Manual of the Department of Microbiology** developed under the **DBT-STAR College Programme** at M.S. Ramaiah College of Arts, Science and Commerce – Autonomous.

This manual is intended to provide a structured and standardized framework for laboratory practices, ensuring **quality, safety, consistency and effective hands-on learning** for our undergraduate students. The SOPs have been developed to support practical training, promote good laboratory practices and encourage students to develop confidence in experimental techniques.

I appreciate the efforts of the faculty members and laboratory staff of the Department of Microbiology in developing and documenting these procedures. I am confident that this manual will serve as a valuable resource for students and faculty and contribute to strengthening the culture of **experiential learning, scientific inquiry and laboratory excellence** at the institution.

Dr. Pushpa H
Principal
M.S. Ramaiah College of Arts, Science and Commerce
Bengaluru

About DBT-STAR College Scheme at MSRCASC

The **Department of Biotechnology (DBT), Ministry of Science and Technology, Government of India**, has been supporting **M.S. Ramaiah College of Arts, Science and Commerce (MSRCASC)** under the **DBT-STAR College Scheme since February 2022**.

The scheme aims to strengthen undergraduate science education by promoting **hands-on experimental learning, practical training, scientific skills and exposure to modern laboratory techniques**. At MSRCASC, the programme has contributed to strengthening laboratory infrastructure, enhancing practical-oriented learning and encouraging students to develop scientific aptitude and research-oriented skills.

The Department of Microbiology, along with other participating science departments, undertakes practical and experiential learning activities under the scheme. The development of **Standard Operating Procedures (SOPs), laboratory manuals, practical protocols and appropriate laboratory documentation** forms an important component of strengthening and standardizing undergraduate laboratory training.

The DBT-STAR College initiative at MSRCASC reflects the institution's commitment to providing students with **quality practical education, experiential learning and a strong foundation in scientific investigation**, thereby bridging the gap between theoretical knowledge and laboratory-based application.

M.S. Ramaiah College of Arts, Science and Commerce

DBT-STAR College Programme

Department of Microbiology

STANDARD OPERATING PROCEDURE (SOP)

SOP No.: MSRCASC/DBT/MIC/SOP-01
Title: Preparation and Sterilization of Microbiological Culture Media

1. Objective

To establish a standardized procedure for the preparation, dispensing and sterilization of microbiological culture media used for undergraduate practical teaching and laboratory exercises.

2. Scope

This SOP is applicable to the preparation of commonly used microbiological culture media in the Department of Microbiology for teaching and practical activities involving appropriate educational microorganisms.

The SOP covers:

- Preparation of culture media according to the manufacturer's instructions or approved laboratory formulation.
- Adjustment of pH, where required.
- Dispensing of media.
- Sterilization using an appropriate validated method.
- Labelling, storage and quality checking of prepared media.

3. Principle

Microbiological culture media provide the nutrients and environmental conditions required for the growth and study of microorganisms.

Prepared media must be appropriately sterilized before use to prevent contamination and ensure reliable experimental results. The sterilization method shall be selected according to the composition and manufacturer's instructions for the particular medium.

4. Responsibilities

Faculty/Faculty Coordinator

- Ensure that the SOP is followed during practical sessions.
- Verify that appropriate media and materials are available.
- Ensure that students receive suitable safety instructions.
- Review records and observations.

Laboratory Staff/Technical Assistant

- Prepare or assist in the preparation of media.
- Operate laboratory equipment according to approved equipment SOPs.
- Maintain preparation, sterilization and storage records.
- Report equipment malfunction or deviations.

Students

- Follow instructions provided by the faculty.
- Use appropriate personal protective equipment.
- Handle media, glassware and equipment responsibly.
- Record observations and report any contamination or abnormality.

5. Materials and Reagents

The materials will depend on the culture medium being prepared and may include:

- Commercially prepared dehydrated culture medium or an approved laboratory formulation
- Distilled/deionized water
- Appropriate laboratory-grade reagents
- pH adjustment reagents, where applicable
- Sterile containers or suitable laboratory glassware
- Labels and markers

6. Equipment Required

- Laboratory balance
- Measuring cylinder/flask
- Beakers or appropriate media preparation vessels
- Magnetic stirrer/hot plate, where required

- pH meter, where required
- Autoclave or other validated sterilization equipment
- Appropriate protective equipment
- Refrigerator/storage facility, where required

7. Procedure

7.1 Preparation

- Verify the name, batch number and expiry date of the culture medium or reagents.
- Refer to the manufacturer's instructions or the approved institutional formulation.
- Calculate the required quantity based on the volume of medium needed.
- Measure the required quantity accurately using an appropriate balance.
- Add the medium components to the required volume of distilled/deionized water.
- Mix thoroughly until the components are adequately dissolved.
- Adjust the pH, if specified for the particular medium.
- Dispense the medium into appropriate containers, leaving sufficient headspace where necessary.
- Close containers appropriately but do not compromise the sterilization process.

7.2 Sterilization

- Select the sterilization method appropriate for the particular medium.
- For media suitable for autoclaving, use the approved autoclave SOP and the manufacturer's recommended sterilization conditions.
- Ensure that containers are properly arranged to permit effective sterilization.
- Do not overload the sterilization chamber.
- Complete the sterilization cycle according to the validated laboratory procedure.
- Allow the sterilized material to cool safely before further handling.
- Where a medium contains heat-sensitive components, sterilize the base medium separately and add heat-sensitive components using an approved aseptic procedure, where applicable.

7.3 Labelling and Storage

Each prepared medium shall be labelled with:

- Name of the medium
- Date of preparation

- Batch/lot number, where applicable
- Expiry/use-by date, where established
- Storage conditions
- Initials/signature of the person responsible

Store the prepared medium under the conditions specified for that medium.

8. Quality Control

Before use, the prepared medium should be checked for:

- Correct labelling
- Appropriate appearance
- Absence of visible contamination
- Appropriate pH, where applicable
- Sterility, where required by the practical or laboratory quality-control procedure
- Proper storage conditions

Any batch showing contamination, abnormal appearance or other significant deviation shall not be used until appropriately evaluated.

9. Safety Precautions

- Wear appropriate laboratory coat and other required PPE.
- Follow laboratory biosafety and chemical-safety practices.
- Handle hot media and sterilized containers carefully to prevent burns.
- Follow the approved SOP for autoclave operation.
- Never open pressurized sterilization equipment until the cycle has been completed and the equipment indicates that it is safe to open.
- Avoid unnecessary exposure to aerosols or spills.
- Wash hands after completion of laboratory work.
- Report accidents, spills or equipment malfunction immediately to the faculty/laboratory staff.

10. Waste Disposal

Unused or contaminated microbiological material shall be handled according to the laboratory's approved biological-waste management procedure and applicable institutional requirements.

Liquid and solid waste shall be segregated appropriately. No microbiological material shall be disposed of directly into general waste without appropriate treatment.

11. Expected Outcome

Properly prepared and appropriately sterilized culture media should be suitable for the intended undergraduate microbiology practical and should provide reproducible experimental conditions.

12. Troubleshooting

Observation/Problem	Possible Cause	Corrective Action
Medium does not dissolve properly	Inadequate mixing or incorrect preparation	Recheck preparation instructions and mixing
Abnormal color/appearance	Incorrect preparation, overheating or reagent issue	Verify formulation and preparation conditions
Visible contamination	Inadequate sterilization or post-sterilization contamination	Review sterilization and aseptic handling
pH outside specified range	Incorrect pH adjustment	Recheck pH meter and preparation procedure
Excessive precipitation	Incorrect heating, concentration or storage	Review manufacturer's instructions and storage conditions

13. Records and Documentation

The following records should be maintained:

- Culture Media Preparation Register
- Media Preparation Date
- Medium Name and Batch/Lot Number
- Quantity Prepared
- Sterilization Record
- Quality-Control Observation
- Storage Details
- Name/Initials of the person responsible
- Deviations and corrective actions, where applicable

Maintaining equipment and SOP records is consistent with DBT's broader laboratory quality expectations, which include appropriate SOPs, equipment validation/calibration and recording deviations.

14. References

- Manufacturer's instructions for the respective culture medium.
- Approved laboratory practical manual of the Department of Microbiology.
- Institutional laboratory safety and waste-management procedures.

Student Learning Outcome

After performing the practical associated with this SOP, the undergraduate student should be able to:

- Explain the purpose of microbiological culture media.
- Select an appropriate medium for a given educational practical.
- Follow standardized media-preparation procedures.
- Understand the importance of sterilization and contamination control.
- Maintain appropriate laboratory records.
- Apply basic microbiological laboratory safety practices.

SOP-02: Safe Operation and Use of Autoclave

SOP No.: MSRCASC/DBT/MIC/SOP-02
Title: Safe Operation and Use of Autoclave

1. Objective

To establish a standardized procedure for the safe and effective operation of the autoclave for sterilization of microbiological media, laboratory materials and other suitable items used in undergraduate practical training.

2. Scope

This SOP applies to faculty members, laboratory personnel and students authorized to use the autoclave in the Department of Microbiology.

It covers:

- Preparation of materials for autoclaving
- Loading and unloading
- Selection of an appropriate validated sterilization cycle
- Safe operation
- Post-cycle handling
- Documentation and routine checks

3. Principle

An autoclave uses **pressurized saturated steam** to achieve moist-heat sterilization. The combination of temperature, pressure and exposure time provides effective destruction of microorganisms and their spores when an appropriate validated cycle is used.

The sterilization cycle must always be selected according to the **type and quantity of material, autoclave manufacturer's instructions and the laboratory's validated operating procedure**.

4. Responsibilities

Faculty/Faculty In-charge

- Ensure that only trained and authorized persons operate the autoclave.
- Ensure that the equipment is maintained and serviced as required.
- Verify that appropriate sterilization cycles are used.

- Ensure that safety instructions are displayed and followed.

Laboratory Staff/Technical Assistant

- Conduct routine pre-use checks.
- Assist students and faculty during operation.
- Maintain autoclave operation and maintenance records.
- Report malfunction or abnormal operation immediately.

Students

- Use the autoclave only under supervision.
- Follow the approved procedure and safety instructions.
- Do not operate the equipment independently unless formally authorized.
- Report any abnormality immediately.

5. Materials and Equipment

- Autoclave
- Appropriate autoclave-safe containers
- Autoclave bags, where applicable
- Heat-resistant gloves
- Appropriate laboratory coat and PPE
- Water as specified by the equipment manufacturer
- Autoclave indicator/sterilization indicator, where applicable

6. Pre-Operation Safety Checks

Before starting the autoclave:

- Check that the autoclave is clean and free from visible damage.
- Check the water level or water supply according to the manufacturer's instructions.
- Inspect the door/gasket and locking mechanism.
- Ensure that the pressure gauge and temperature display are functioning.
- Check that the drain is not blocked.
- Confirm that the intended material is suitable for autoclaving.
- Ensure that the selected sterilization cycle is appropriate for the load.
- Do not use the autoclave if any safety feature appears defective.
- Report equipment malfunction to the laboratory in-charge.

7. Preparation of Materials

7.1 Culture Media

- Use suitable heat-resistant containers.
- Do not completely seal containers during sterilization unless the equipment and container system is specifically designed for that purpose.
- Allow sufficient headspace to prevent overflow.
- Label containers appropriately.

7.2 Glassware

- Use only heat-resistant laboratory glassware suitable for autoclaving.
- Arrange glassware securely.
- Avoid tightly packed or sealed containers.

7.3 Biological/Contaminated Waste

- Use appropriate autoclavable bags or containers.
- Do not overfill bags.
- Ensure that waste is segregated according to the laboratory's waste-management procedure.
- Follow applicable institutional biosafety requirements.

8. Loading Procedure

- Arrange materials so that steam can circulate freely.
- Do not overload the chamber.
- Place liquid containers securely.
- Use appropriate secondary containment for liquid loads where required.
- Ensure that materials do not obstruct the chamber door or safety mechanisms.
- Close and lock the door according to the manufacturer's instructions.

9. Operating Procedure

- Switch on the autoclave according to the manufacturer's operating instructions.
- Select the **validated cycle appropriate to the material being sterilized**.
- Confirm the selected cycle before starting.
- Start the sterilization cycle.
- Monitor the equipment display/gauges during operation.
- Do not attempt to open or bypass the safety lock during a cycle.

- Do not leave the equipment unattended if the manufacturer's instructions or institutional procedure require monitoring.
- If an alarm, unusual noise, leakage or other abnormal condition occurs, stop the operation if it is safe to do so and inform the laboratory in-charge.

10. Completion of Cycle and Unloading

- Wait until the sterilization cycle has completely finished.
- Confirm that chamber pressure has returned to a safe level.
- Do not attempt to open the door while the chamber is pressurized.
- Open the door carefully according to the manufacturer's instructions.
- Allow hot materials to cool sufficiently before handling.
- Use appropriate heat-resistant gloves and PPE.
- Exercise particular caution when handling sterilized liquids because they may remain extremely hot.
- Inspect the load for leakage, breakage or other abnormalities.
- Transfer sterilized materials to the appropriate storage or next stage of laboratory work.

11. Quality Control

Appropriate sterilization indicators should be used according to the laboratory's quality-control programme.

Records should include, where applicable:

- Date of operation
- Type of load
- Selected cycle
- Operator
- Indicator result
- Any deviation or abnormality

For critical laboratory applications, periodic validation and monitoring should be carried out according to the equipment manufacturer's recommendations and institutional requirements.

12. Safety Precautions

- Only trained/authorized personnel should operate the autoclave.
- Never attempt to open a pressurized chamber.
- Never bypass or disable safety interlocks.

- Never autoclave sealed containers.
- Do not autoclave materials that are incompatible with steam or pressure.
- Exercise extreme caution when opening the chamber and handling hot liquids.
- Wear appropriate PPE.
- Keep combustible, volatile or incompatible materials away from the autoclave unless specifically approved for the equipment.
- Report burns, spills, breakage or equipment malfunction immediately.
- Do not attempt repairs unless authorized and qualified.

13. Emergency Procedure

In case of:

- **Steam leakage:** Move away from the equipment and inform the laboratory in-charge.
- **Equipment malfunction:** Stop operation if it is safe to do so and disconnect/use emergency controls according to the manufacturer's instructions.
- **Burn injury:** Provide immediate first aid and report the incident according to institutional procedures.
- **Broken glass:** Do not handle directly; use appropriate tools and follow laboratory waste procedures.

The laboratory's emergency contact information should be displayed near the autoclave.

14. Waste Disposal

Materials requiring sterilization shall be segregated and treated according to the laboratory's approved waste-management procedure.

After sterilization, waste shall be handled and disposed of according to applicable institutional and regulatory requirements.

15. Routine Maintenance

- Clean the chamber as recommended by the manufacturer.
- Check the gasket and door mechanism regularly.
- Maintain the required water quality and level.
- Ensure that drains and filters are functioning properly.
- Maintain service and preventive-maintenance records.
- Arrange periodic servicing and calibration/validation as recommended.

16. Records and Documentation

The following records should be maintained:

Autoclave Operation Register

Date	Load Description	Cycle	Operator	Indicator Result	Remarks

Additional records:

- Preventive maintenance
- Repairs/service
- Calibration/validation, where applicable
- Deviations/incidents
- Corrective actions

17. Expected Outcome

The autoclave should provide effective and reproducible sterilization of suitable laboratory materials when operated using an appropriate validated cycle and according to the manufacturer's instructions.

Students should understand the principles of moist-heat sterilization and demonstrate appropriate laboratory safety practices.

18. References

- Autoclave manufacturer's operation and maintenance manual.
- Institutional Laboratory Safety Manual.
- Department of Microbiology Laboratory Manual.
- Applicable DBT biosafety guidelines and institutional biosafety procedures, where relevant.

Student Learning Outcomes

After completion of the practical associated with this SOP, students should be able to:

- Explain the principle of moist-heat sterilization.
- Identify materials suitable and unsuitable for autoclaving.
- Describe the essential steps involved in safe autoclave operation.

- Recognize the hazards associated with pressurized steam.
- Follow appropriate laboratory safety procedures.
- Maintain basic autoclave operation records.

SOP-03: Aseptic Handling and Transfer of Microorganisms

SOP No.: MSRCASC/DBT/MIC/SOP-03
Title: Aseptic Handling and Transfer of Microorganisms

1. Objective

To establish a standardized procedure for the safe and aseptic handling, transfer and inoculation of microorganisms used in undergraduate microbiology practicals.

2. Scope

This SOP applies to faculty members, laboratory staff and students performing routine undergraduate microbiology practicals involving **approved, non-pathogenic teaching microorganisms**.

It covers:

- Preparation of the work area
- Aseptic handling of cultures
- Transfer of microorganisms between appropriate laboratory media
- Prevention of contamination
- Post-practical decontamination

3. Principle

Aseptic technique is a set of practices used to prevent the unwanted introduction of microorganisms into cultures, media and laboratory materials and to minimize exposure of laboratory personnel and the environment to microorganisms.

Proper aseptic technique helps maintain the purity of cultures and ensures reliable experimental results.

4. Responsibilities

Faculty/Faculty In-charge

- Ensure that students receive appropriate training before performing the practical.
- Confirm that only approved teaching cultures are used.
- Supervise student activities where required.

- Ensure compliance with institutional biosafety procedures.

Laboratory Staff/Technical Assistant

- Prepare the required materials and cultures.
- Ensure that the work area and equipment are suitable for use.
- Maintain culture and laboratory records.
- Report contamination, spills or incidents.

Students

- Follow the instructions provided by the faculty.
- Wear appropriate PPE.
- Maintain aseptic practices throughout the practical.
- Immediately report spills, breakage, contamination or other incidents.

5. Materials and Equipment

Depending on the practical:

- Approved teaching microbial cultures
- Sterile culture media
- Appropriate sterile transfer instruments
- Sterile tubes, plates or other culture vessels
- Marker/labels
- Appropriate disinfectant
- PPE
- Approved laboratory equipment for aseptic work

6. Preparation of Work Area

- Wear the required laboratory coat and PPE.
- Keep the work area clean and free from unnecessary materials.
- Disinfect the work surface using the institutionally approved disinfectant.
- Allow the disinfectant to remain for the recommended contact time.
- Arrange required materials before beginning the practical.
- Avoid unnecessary movement and conversation during aseptic work.
- Ensure that only the cultures and materials required for the practical are present.

7. Aseptic Handling Procedure

- Verify the identity and label of the culture before beginning.
- Check the condition of the culture container and media.
- Use only sterile or appropriately decontaminated equipment.
- Open culture containers only when necessary and for the minimum practical duration.
- Avoid contact between sterile surfaces and non-sterile objects.
- Keep sterile materials protected from unnecessary environmental exposure.
- Transfer the culture using the approved sterile technique demonstrated by the faculty.
- Close culture containers promptly after transfer.
- Label all inoculated materials immediately.
- Return cultures to the appropriate designated location.

8. Prevention of Cross-Contamination

The following practices shall be followed:

- Use sterile equipment for each transfer.
- Do not place sterile instruments on unclean surfaces.
- Keep culture vessels closed except when actively performing the required transfer.
- Avoid touching sterile surfaces.
- Clearly label all cultures.
- Do not interchange caps or lids between culture vessels.
- Handle one culture at a time where practicable.
- Disinfect the work area after completion.

9. Handling of Culture Spills

In the event of a spill:

- Stop work immediately.
- Inform the faculty/laboratory staff.
- Restrict access to the affected area.
- Follow the laboratory's approved biological-spill response procedure.
- Allow appropriate contact time for the approved disinfectant.
- Clean the area using the designated procedure.
- Dispose of contaminated materials according to the approved waste-management procedure.

- Record the incident where required.

Students should **not attempt to manage a significant biological spill independently**.

10. Post-Practical Procedure

- Ensure that all cultures and materials are appropriately labelled.
- Transfer cultures to their designated storage/incubation area under supervision.
- Decontaminate reusable equipment according to the relevant laboratory SOP.
- Dispose of contaminated disposable materials according to the approved waste-management procedure.
- Disinfect the work surface.
- Remove PPE appropriately.
- Wash hands thoroughly after completing laboratory work.
- Record observations and relevant details in the practical record.

11. Quality Control

The following should be checked where applicable:

- Correct identification and labelling of cultures
- Sterility of media before inoculation
- Integrity of sterile materials
- Absence of unintended contamination
- Appropriate documentation of cultures and practical observations

Unexpected contamination should be reported to the faculty/laboratory staff and investigated before results are interpreted.

12. Safety Precautions

- Only approved teaching microorganisms shall be used for routine undergraduate practical's.
- Students shall not handle clinical, unknown or potentially pathogenic isolates unless specifically authorized under an appropriate institutional biosafety procedure and facility.
- Appropriate PPE shall be worn.
- Eating, drinking and personal activities unrelated to laboratory work are prohibited in the laboratory.
- Mouth pipetting is strictly prohibited.

- Used cultures and contaminated materials shall be appropriately decontaminated before disposal.
- Hands shall be washed after laboratory work.
- Any exposure, spill, injury or accidental release shall be reported immediately.

13. Waste Disposal

All contaminated materials shall be segregated and managed according to the laboratory's approved biological-waste management procedure.

Reusable materials shall be appropriately decontaminated before cleaning or reuse.

14. Records and Documentation

The following records should be maintained where applicable:

- Culture/strain register
- Practical record
- Media preparation record
- Decontamination/waste record
- Incident/spill record
- Equipment usage record

15. Expected Outcome

Students should be able to:

- Explain the importance of aseptic technique.
- Handle approved teaching microorganisms responsibly.
- Perform routine transfers without introducing avoidable contamination.
- Recognize potential sources of contamination.
- Follow appropriate laboratory safety and waste-management practices.

16. Troubleshooting

Observation	Possible Cause	Corrective Action
Unwanted contamination	Break in aseptic technique	Review work practices and repeat using sterile materials

No growth after inoculation	Non-viable culture, unsuitable medium or procedural error	Verify culture, medium and procedure
Mixed/unexpected growth	Cross-contamination	Review culture handling and equipment sterilization
Label confusion	Inadequate labelling	Label cultures immediately after transfer
Repeated contamination	Environmental or procedural issue	Inform faculty/lab in-charge and review aseptic workflow

17. References

- Department of Microbiology, MSRCASC – Approved Laboratory Manual.
- Institutional Laboratory Safety Manual.
- Applicable institutional biosafety procedures.
- Manufacturer's instructions for relevant laboratory equipment.

Student Learning Outcomes

On completion of the practical associated with this SOP, students should be able to:

- Explain the importance of aseptic technique in microbiology.
- Identify common sources of contamination.
- Demonstrate appropriate handling of approved teaching cultures.
- Apply basic aseptic practices during microbial transfer.
- Follow laboratory biosafety and waste-disposal procedures.
- Maintain appropriate experimental records.

SOP-04: Gram Staining of Bacterial Cells

SOP No.: MSRCASC/DBT/MIC/SOP-04

1. Objective

To establish a standardized procedure for demonstrating the Gram reaction and cellular morphology of suitable bacterial teaching cultures.

2. Scope

This SOP is applicable to undergraduate Microbiology practicals involving approved, non-pathogenic teaching cultures.

3. Principle

Gram staining is a differential staining technique that broadly differentiates bacteria based on differences in their cell-envelope characteristics. The procedure involves application of a primary stain, mordant, decolourizing step and counterstain.

4. Responsibilities

Faculty: Demonstrate the technique, ensure appropriate cultures are used and supervise students.

Laboratory Staff: Provide materials, maintain stains and ensure appropriate waste handling.

Students: Follow the demonstrated procedure, use PPE and record observations accurately.

5. Materials and Equipment

- Clean microscope slides
- Approved teaching bacterial cultures
- Sterile transfer equipment
- Gram-staining reagents
- Distilled water
- Microscope
- Appropriate PPE
- Approved disinfectant
- Biohazard/biological-waste containers

6. Procedure

- Clean and label the microscope slide.
- Prepare a suitable bacterial smear using the approved teaching culture.
- Allow the smear to dry appropriately.
- Fix the smear using the approved laboratory procedure.
- Apply the Gram-staining reagents sequentially according to the department's approved practical protocol.
- Wash/rinse the slide at the specified stages.
- Allow the slide to dry appropriately.
- Examine under the microscope using the appropriate objective.
- Record Gram reaction, cellular morphology and arrangement.
- Dispose of the used material according to the laboratory waste procedure.

Note: The exact reagent exposure times shall follow the approved departmental practical manual and the validated laboratory protocol.

7. Quality Control

- Use properly prepared slides.
- Ensure staining reagents are within their approved use period.
- Include an appropriate control culture where required.
- Examine staining quality and cellular morphology.
- Repeat the preparation if staining is ambiguous or technically unsatisfactory.

8. Safety Precautions

- Wear laboratory coat and appropriate PPE.
- Handle stains and chemicals carefully.
- Avoid direct contact with cultures and staining reagents.
- Do not mouth-pipette.
- Handle slides carefully to prevent breakage.
- Decontaminate used materials appropriately.

9. Waste Disposal

Used slides, cultures and contaminated disposable materials shall be handled according to the approved microbiological waste-management procedure.

10. Troubleshooting

Observation	Possible Cause	Corrective Action
Poor staining	Old culture or staining error	Repeat using a suitable culture and approved protocol
Excessive decolourization	Incorrect decolourization	Review technique
Inadequate decolourization	Insufficient decolourization	Review technique
Uneven smear	Poor smear preparation	Prepare a uniform smear
Weak staining	Reagent deterioration	Check reagent quality

11. Expected Outcome

Students should be able to demonstrate Gram staining and interpret the Gram reaction and basic morphology of suitable teaching bacteria.

12. Records

Students shall record:

- Culture used
- Date
- Staining observations
- Microscopic observations
- Diagram, where required
- Faculty/laboratory observations, where applicable

13. References

- Department of Microbiology, MSRCASC Laboratory Manual.
- Standard undergraduate Microbiology practical references.
- Institutional Laboratory Safety Manual.

SOP-05: Serial Dilution and Enumeration of Microorganisms

SOP No.: MSRCASC/DBT/MIC/SOP-05

1. Objective

To establish a standardized procedure for performing serial dilution and estimating the viable microbial population in suitable teaching samples.

2. Scope

This SOP is intended for undergraduate practical training using approved non-pathogenic teaching cultures or appropriately prepared educational samples.

3. Principle

Serial dilution reduces the concentration of microorganisms in a sample in a systematic manner. An appropriate dilution may subsequently be used for enumeration based on the approved practical method.

4. Materials and Equipment

- Approved teaching culture/sample
- Sterile dilution blanks
- Sterile tubes
- Sterile transfer equipment
- Appropriate culture medium
- Incubator
- Colony-counting facility or manual counting method
- PPE

5. Procedure

- Label all dilution tubes/containers clearly.
- Arrange the dilution series before beginning.
- Prepare the required dilution series using sterile materials.
- Mix each dilution appropriately according to the departmental practical protocol.
- Transfer the required volume from one dilution to the next using sterile technique.
- Select the appropriate dilution(s) for enumeration.

- Inoculate the selected dilution(s) onto the approved culture medium using the designated method.
- Incubate under the conditions specified in the approved teaching protocol.
- Select suitable plates for counting according to the departmental laboratory criteria.
- Count colonies and record the observations.
- Calculate the estimated microbial concentration using the approved calculation method.

6. Quality Control

- Use sterile dilution materials.
- Maintain accurate labelling.
- Include appropriate controls where required.
- Avoid cross-contamination between dilution levels.
- Repeat counts where results are inconsistent.

7. Safety Precautions

- Use only approved teaching materials.
- Wear PPE.
- Maintain aseptic technique.
- Treat all cultures and used materials as laboratory biological material.
- Follow the laboratory's approved decontamination and waste procedures.

8. Waste Disposal

All used cultures, media, tubes and contaminated disposable materials shall be segregated and decontaminated/disposed of according to the approved laboratory procedure.

9. Troubleshooting

Problem	Possible Cause	Corrective Action
No colonies	Incorrect dilution or inoculation	Review dilution and inoculation procedure
Excessive colonies	Dilution too low	Use an appropriate higher dilution
Inconsistent counts	Uneven mixing or technical variation	Review technique and repeat
Contamination	Break in aseptic technique	Review sterile handling

10. Expected Outcome

Students should be able to perform serial dilution, obtain suitable plates for enumeration and calculate an estimated microbial population using the prescribed practical method.

11. Records

- Sample/culture identification
- Dilution series
- Plate identification
- Colony counts
- Calculations
- Observations
- Date and student/faculty details

12. References

- Department of Microbiology, MSRCASC Laboratory Manual.
- Standard undergraduate Microbiology practical references.
- Institutional Laboratory Safety Manual.

SOP-06: Isolation of Microorganisms from Environmental Samples

SOP No.: MSRCASC/DBT/MIC/SOP-06

1. Objective

To establish a standardized procedure for demonstrating the isolation of microorganisms from appropriately selected environmental samples for undergraduate practical learning.

2. Scope

This SOP applies to supervised undergraduate practical's using environmental samples selected and approved by the Department of Microbiology.

Samples and organisms shall be restricted to those appropriate for the laboratory's facilities, biosafety procedures and educational objectives.

3. Principle

Environmental samples may contain diverse microorganisms. Selective or general-purpose culture media and appropriate isolation techniques can be used to demonstrate microbial diversity and obtain distinguishable colonies for educational observation.

4. Responsibilities

- Faculty shall select appropriate samples and supervise the practical.
- Laboratory staff shall prepare materials and ensure appropriate decontamination and disposal.
- Students shall follow aseptic and safety procedures.

5. Materials and Equipment

- Approved environmental samples
- Appropriate culture media
- Sterile sampling/transfer materials
- Petri plates or suitable culture vessels
- Incubation facility
- Disinfectant
- PPE

6. Procedure

- Select and label the environmental sample.
- Record sample source, date and relevant environmental information.
- Prepare the sample according to the approved undergraduate practical protocol.
- Use sterile materials for sample handling.
- Inoculate the appropriate culture medium using the designated teaching method.
- Incubate under the conditions specified in the approved departmental protocol.
- Observe colony characteristics without unnecessary opening or manipulation.
- Record relevant observations.
- Where further study is part of the approved practical, follow the corresponding departmental SOP.

7. Quality Control

- Maintain accurate sample identification.
- Include appropriate controls where required.
- Check media sterility.
- Maintain proper labelling throughout the experiment.
- Document unexpected contamination.

8. Safety Precautions

Environmental samples are inherently variable and may contain organisms that are not suitable for routine undergraduate handling.

Therefore:

- Use only samples approved by the Department.
- Do not culture unknown environmental isolates beyond the scope of the approved teaching practical.
- Do not perform identification or manipulation of potentially hazardous organisms without appropriate institutional authorization and facilities.
- Wear appropriate PPE.
- Follow institutional biosafety procedures.

9. Waste Disposal

All environmental sample materials, cultures and contaminated disposables shall be decontaminated and disposed of according to the approved laboratory procedure.

10. Expected Outcome

Students should understand environmental microbial diversity and demonstrate the basic principles of sample collection, culture-based isolation, observation and documentation.

11. Records

- Sample source
- Date and time of collection
- Culture medium
- Practical method
- Observations
- Colony characteristics
- Photographs, where appropriate
- Disposal/decontamination record

12. References

- Department of Microbiology, MSRCASC Laboratory Manual.
- Institutional Laboratory Safety Manual.
- Applicable institutional biosafety procedures.

SOP-07: Maintenance and Preservation of Approved Microbial Teaching Cultures

SOP No.: MSRCASC/DBT/MIC/SOP-07

1. Objective

To establish a standardized procedure for the identification, maintenance, documentation and preservation of approved microbial teaching cultures used in undergraduate practical training.

2. Scope

This SOP applies to approved teaching cultures maintained by the Department of Microbiology.

It does not cover clinical, hazardous or regulated microorganisms unless specifically authorized under the appropriate institutional biosafety framework.

3. Responsibilities

Faculty/Faculty In-charge

- Approve cultures for teaching use.
- Maintain oversight of culture records.
- Ensure appropriate storage and periodic review.

Laboratory Staff

- Maintain culture records.
- Monitor storage conditions.
- Report contamination or loss of culture integrity.

Students

- Handle cultures only under supervision.
- Follow the relevant aseptic-handling SOP.

4. Culture Register

The culture register should contain, where applicable:

- Culture/strain identification
- Source
- Date received/prepared

- Storage location
- Relevant reference number
- Date of last verification
- Responsible faculty/laboratory staff
- Remarks

5. Maintenance Procedure

- Verify the identity and status of the culture before use.
- Use the approved maintenance medium and method.
- Follow the appropriate aseptic-handling procedure.
- Store cultures under the approved conditions for the particular teaching strain.
- Inspect cultures periodically for evidence of contamination or deterioration.
- Document any transfer or maintenance activity.
- Immediately report suspected contamination.

6. Preservation

Where long-term preservation is required, the Department shall use an approved preservation method appropriate to the teaching culture and available laboratory facilities.

The method shall be documented in the laboratory's culture-maintenance records.

7. Quality Control

- Verify culture identity as appropriate.
- Monitor culture condition.
- Maintain clear labelling.
- Avoid unnecessary repeated subculturing.
- Review cultures periodically.

8. Safety Precautions

- Only approved teaching cultures shall be maintained under this SOP.
- Use appropriate PPE.
- Maintain aseptic technique.
- Do not remove cultures from the laboratory without authorization.
- Report spills, contamination or loss immediately.

9. Disposal of Unwanted Cultures

Cultures no longer required shall be decontaminated and disposed of through the approved microbiological waste-management procedure.

10. Records

Maintain:

- Culture register
- Maintenance record
- Storage record
- Contamination/deviation record
- Disposal record

11. Expected Outcome

The Department should maintain a documented, traceable collection of appropriate teaching cultures in suitable condition for undergraduate practical training.

12. References

- Department of Microbiology, MSRCASC Laboratory Manual.
- Institutional Laboratory Safety Manual.
- Applicable institutional biosafety procedures.

SOP-08: Antibiotic Sensitivity Testing of Approved Teaching Cultures

SOP No.: MSRCASC/DBT/MIC/SOP-08

1. Objective

To provide a standardized undergraduate teaching procedure for demonstrating the principle of antimicrobial susceptibility testing using approved teaching strains.

2. Scope

This SOP is restricted to supervised educational practicals using approved teaching strains and laboratory-approved antimicrobial discs/reagents.

It is **not intended for clinical diagnosis or therapeutic decision-making**.

3. Principle

Antimicrobial susceptibility testing demonstrates the response of a bacterial culture to selected antimicrobial agents under standardized laboratory conditions. The resulting growth-inhibition pattern can be observed and recorded according to the approved teaching protocol.

4. Materials and Equipment

- Approved teaching bacterial strain
- Appropriate culture medium
- Sterile Petri plates
- Sterile transfer equipment
- Approved antimicrobial discs
- Incubation facility
- Measuring scale/ruler or appropriate measurement facility
- PPE

5. Procedure

- Confirm the identity and suitability of the approved teaching strain.
- Prepare the inoculum according to the departmental practical protocol.
- Inoculate the approved culture medium uniformly using the designated teaching method.
- Apply the selected antimicrobial discs using sterile technique.
- Incubate under the conditions specified in the approved departmental protocol.

- Observe the resulting growth pattern.
- Measure zones of inhibition where applicable.
- Record observations accurately.
- Interpret the results only according to the approved educational reference method.

6. Quality Control

- Use an approved teaching strain.
- Verify media quality.
- Check antimicrobial disc integrity and storage conditions.
- Maintain appropriate labelling.
- Include appropriate control procedures where required.
- Do not interpret results beyond the scope of the teaching exercise.

7. Safety Precautions

- Use only approved teaching strains.
- Wear laboratory coat and appropriate PPE.
- Follow aseptic handling procedures.
- Do not use clinical isolates unless specifically authorized under an appropriate institutional procedure.
- Do not use results for clinical or therapeutic decisions.
- Decontaminate all cultures and contaminated materials after the practical.

8. Waste Disposal

All used cultures, plates, discs and contaminated materials shall be handled according to the approved microbiological waste-management procedure.

9. Expected Outcome

Students should understand the basic principle of antimicrobial susceptibility testing, perform the approved teaching exercise and accurately record and interpret observations within the scope of the practical.

10. Records

- Culture/strain used
- Medium
- Antimicrobial agent/disc
- Date

- Observation
- Zone measurement, where applicable
- Interpretation according to teaching reference
- Faculty verification, where applicable

11. References

- Department of Microbiology, MSRCASC Laboratory Manual.
- Institutional Laboratory Safety Manual.
- Current approved teaching reference for antimicrobial susceptibility testing.

SOP-09: Safe Operation of Laminar Air Flow / Biosafety Cabinet

SOP No.: MSRCASC/DBT/MIC/SOP-09

1. Objective

To establish a standardized procedure for the safe and appropriate use of laminar airflow equipment and/or biosafety cabinets used for microbiology practical teaching.

2. Scope

This SOP applies to authorized users of the equipment available in the Department of Microbiology.

Important: A laminar airflow cabinet and a biosafety cabinet are not interchangeable. The actual equipment installed in the laboratory should be identified in the final institutional SOP.

3. Responsibilities

Faculty/Faculty In-charge

- Ensure that users are appropriately trained.
- Confirm that the equipment is suitable for the intended activity.
- Ensure that maintenance and certification records are maintained.

Laboratory Staff

- Conduct routine checks.
- Maintain equipment-use records.
- Report malfunction and arrange servicing as required.

Students

- Use the equipment only under supervision and according to departmental instructions.

4. Pre-Use Checks

Before use:

- Check the equipment for visible damage.
- Confirm that the work area is clean.
- Verify that required equipment indicators are functioning.
- Check the status of maintenance/certification records where applicable.

- Ensure that unnecessary materials are removed from the work area.
- Confirm that the equipment is appropriate for the planned activity.

5. Operating Procedure

- Switch on the equipment according to the manufacturer's instructions.
- Allow the equipment to reach its specified operating condition before beginning work.
- Disinfect the work surface using the approved disinfectant.
- Arrange materials to maintain an organized workflow.
- Avoid blocking air grilles or airflow paths.
- Perform the approved aseptic work using appropriate technique.
- Avoid unnecessary rapid movements within the work area.
- Keep the work area free from clutter.
- On completion, remove materials appropriately.
- Decontaminate the work surface.
- Switch off the equipment according to the manufacturer's instructions.

6. Safety Precautions

- Do not use the equipment for activities for which it is not designed.
- Do not obstruct airflow.
- Do not use open flames inside the cabinet unless specifically permitted by the manufacturer and institutional procedure.
- Do not rely on the cabinet as a substitute for appropriate microbiological containment.
- Follow the manufacturer's instructions.
- Report equipment malfunction immediately.
- Students shall use the equipment only under faculty/laboratory supervision.

7. Cleaning and Maintenance

- Clean and disinfect the work surface after use.
- Maintain equipment-use records.
- Arrange periodic maintenance as recommended by the manufacturer.
- Maintain applicable certification/validation records.
- Do not undertake unauthorized repairs.

8. Documentation

Maintain:

- Equipment usage register
- Cleaning record
- Maintenance record
- Certification/validation record, where applicable
- Fault/repair record

9. Expected Outcome

Students should understand the purpose of controlled-airflow equipment and demonstrate appropriate safe and aseptic laboratory practices.

10. References

- Manufacturer's operation and maintenance manual.
- Institutional Laboratory Safety Manual.
- Department of Microbiology Laboratory Manual.
- Applicable institutional biosafety procedures.

SOP-10: Segregation and Disposal of Microbiological Laboratory Waste

SOP No.: MSRCASC/DBT/MIC/SOP-10

1. Objective

To establish a standardized procedure for segregation, collection, decontamination and disposal of microbiological laboratory waste generated during undergraduate practical activities.

2. Scope

This SOP applies to microbiological waste generated in the Department of Microbiology during teaching and laboratory activities.

3. Types of Laboratory Waste

Depending on the practical, waste may include:

- Used culture plates and tubes
- Contaminated disposable materials
- Used microbiological cultures
- Contaminated gloves and other disposable PPE
- Broken contaminated glassware
- Sharps, where generated
- Non-contaminated general laboratory waste

4. Responsibilities

Faculty/Faculty In-charge

- Ensure that students understand waste segregation.
- Ensure appropriate institutional waste-management practices.

Laboratory Staff

- Maintain designated waste containers.
- Ensure appropriate treatment and disposal.
- Maintain relevant records.

Students

- Segregate waste immediately at the point of generation.

- Never dispose of biological waste in general waste containers.
- Follow laboratory instructions.

5. Waste Segregation

Waste shall be segregated **at the point of generation** using the designated containers provided by the institution.

Containers shall be:

- Clearly labelled
- Appropriate for the type of waste
- Securely positioned
- Replaced before overfilling

The exact colour coding and disposal route shall follow the **current institutional waste-management system and applicable regulations**.

6. Handling of Microbiological Waste

- Identify the waste type before disposal.
- Place waste directly into the appropriate designated container.
- Do not compress or manually sort contaminated waste.
- Handle sharps only through designated sharps containers.
- Close waste containers appropriately.
- Decontaminate microbiological waste using the approved institutional method before final disposal, where applicable.
- Maintain records as required.

7. Spill Management

In case of a biological spill:

- Stop work and inform the faculty/laboratory staff.
- Restrict access to the affected area.
- Follow the approved biological-spill response procedure.
- Use the designated disinfectant and procedure.
- Dispose of cleanup materials appropriately.
- Record the incident where required.

8. Safety Precautions

- Wear appropriate PPE.
- Never handle contaminated waste with bare hands.
- Never recap or manipulate contaminated sharps unnecessarily.
- Do not place biological waste in general waste.
- Wash hands after handling laboratory waste.
- Report injuries, spills or exposure immediately.

9. Documentation

Maintain, where applicable:

- Waste-generation/disposal records
- Decontamination records
- Spill/incident records
- Sharps disposal records
- Waste collection records

10. Expected Outcome

Students and laboratory personnel should be able to:

- Identify different categories of laboratory waste.
- Segregate waste correctly at the point of generation.
- Understand the importance of decontamination.
- Follow institutional biosafety and waste-management procedures.

11. References

- MSRCASC Institutional Laboratory Safety and Waste-Management Procedures.
- Department of Microbiology Laboratory Manual.
- Applicable Government of India waste-management and biomedical-waste requirements.
- Applicable DBT biosafety guidance, where relevant.