DEPARTMENT OF BIOTECHNOLOGY

ISLAMIAH COLLEGE (AUTONOMOUS), VANIYAMBADI

LABORATORY MANUAL

INSTRUMENTS

STANDARD OPERATING PROCEDURE

STANDARD OPERATING PROCEDURE		
SOP No.	IC/DBT/LAB/SOP-001	Version No. 1
SOP Title	Biotechnology Laboratory Safety Rules and Regulations	
Department	Department of Biotechnology, Islamiah College (Autonomous), Vaniyambadi	

1. Objective:

Biotechnology laboratory are equipped with many things that can harm you if used carelessly. You will be learning many procedures throughout the course – none as important as the safety procedures and expectations. Successful biotechnicians follow safety guidelines, pay attention to the work being completed and use common sense when using equipment and chemicals.

2. Introduction:

A wide range of biological agents and chemical materials are being used in biotechnology laboratories from harmful microorganisms to hazardous chemicals. The hazards associated with these subjected materials vary depending on their properties and mode of handling and usage. The risks involved include chemical hazards, biohazards, radiation hazards, injuries and adverse health effects. This calls for utmost care in handling of biological components, chemicals and materials from the time of start to end of each experiment during biotechnology practicals. The objective is to prevent any type of mishaps inside laboratory which requires a proactive approach in identifying problems and putting control measures in laboratorywhile performing the experiments.

3. Purpose and Scope:

This SOP explains the general rules and regulation guidelines involved in biotechnology laboratory for the students of academic and research.

4. Responsibility:

- Students, subjected technical Staff and professors are responsible for the laboratory safety guidelines process.
- Department Head and Professors will ensure the implementation of this SOP.
- Department Head and Principal will monitor compliance to this SOP.

5. Procedure:

This biotechnology laboratory safety manual is compiled to be used as a binding document for all the students and staff of biotechnology to ensure safe work conduct and practices inside the laboratory.

Procedures and Rules within this Manual are formulated for three reasons:

1) To avoid health risks and accidents for our personnel.
2) To be in a position to act appropriately in case of emergencies.
3) To minimize the environmental burden and risks caused by our work.

This manual cannot cover all circumstances where safety procedures must be applied. it is intended to set up a framework of how should work in biological laboratories. The avoidance of safety risks for the personnel at the institutional level requires knowledge of possible hazards in our environment; chemicals, biological agents or radioactivity. In this regard students and staff of biotechnology are required tofamiliarize themselves with safe practices for applied laboratory operations.

Laboratory Rules and Regulations:

Students in biotechnology laboratory must have an orientation before starting thepractical's. They should be giveninstructions as to how to operate major equipment including microscope, analytical balance and pH meter. Theorientation should also include potentials for fire, broken glasses, spill of chemicals, cuts with sharp tools likescalpel, blades, etc. handling hazardous chemicals, UV, disposal of wastes like contaminated media and cleaningup of spills.

Good laboratory work habits will help you a grand success. Follow the following guide lines very strictly. These will protect you and your experiments.

Dos and Don'tsinside laboratory:

1. Student should wear cleanlab-coat every time before you enter a laboratory.
2. Student should not deviate the instructions given by the instructor inside laboratory.
3. Shouting, playing and runninginside the laboratory is strictly prohibited.
4. Using mobile phones in lab is strictly prohibited.
5. Do not smoke, drink or eat in the laboratory.
6. Before starting work, clean the working table with disinfectants like 70% alcohol or dilute detergents likedettol or lyzol.

7. The table should have a notebook and only glass-wares and equipment that are needed.
8. In case of any incidentor injury inform your instructor immediately.
9. Without the instruction and supervision of instructor one should not touch any equipment.
10. Ensure the entries in respective log book records before taking chemicals or using instruments.
11. When culture of an organism is spilt cover the area, treat it with ethyl alcohol (after putting off the Bunsenflame) or any other disinfectant for some time and then only clean the area.
12. Turn off the Bunsen flame when it is not in use.
13. All microbial cultures should be handled with care.
14. Keep used liquid cultures, supernatants and glass-wares in autoclavable containers.
15. Discard contaminated plates and plastic containers in autoclavable bags.
16. Discard organic solvents (phenol and chloroform) in waste containers.
17. Do not pipette out broth cultures, concentrated acids and alkalies by mouth.
18. All culture tubes must be kept in upright position in baskets or stands.
19. Labelling of Petri plates, tubes, flasks, etc. should be done before starting an experiment.
20. Materials like chemicals, stains, reagent bottles, unused glass-wares, etc. must be replaced in their originalplace.
21. Tools like scalpel, forceps, inoculation needles, etc. that come in contact with cultures or agar medium (sterile)should be sterilised by making the portion that goes into tube or Petri plate, red hot on Bunsen flame, cool in 70%alcohol and flame heat it by passing over the flame to burn off alcohol.
22. Clean microscope lens before and after use.
23. Keep the doors and windows closed while inoculating or during transfer of sterile cultures.
24. Toxic chemicals should be handled with precaution and while discarding used ones they should be discardedin labelled containers. Toxic chemicals, besides organic solvents, include mercury compounds, some halogens,mutagenic chemicals, radioactive substances, etc.

25. Broken blades, sharp instruments and broken glass pieces should be disposed in separate containers.
26. First aid kits must be placed in each laboratory.
27. Portable fire extinguishers should be kept ready.
28. Use specific toxic and mutagenic chemicals under fume hood.
29. Do not expose yourself to UV rays.
30. Wear safety glasses, (UV) gloves, masks, hot gloves and rubber aprons while handling concentrated acids andbases.
31. Clean the table and inoculation chamber. Wash your hands and then only leave the laboratory.
32. Prepare a flowchart prior to each experiment.

A laboratory report should be prepared for each experiment in the following format:

1. Experiment number and title of the experiment.
2. Date of experiment.
3. Introduction and principle of the experiment.
4. Method/Procedure followed.
   (Do not copy methods from manual directly. Refer to manual and write the method on your own, the way you have conducted the experiment)
5. Place a flow chart at the end.
6. Results and discussion/conclusions.

Complete description of what you have observed, include graphs, tables, photographs etc. Each graph, table, figure, etc. should bear a title, a number and legend that contains allinformation needed to interpret data. (Eg: In gel electrophoresis, number each lane on the photograph of the gel andthe contents of each lane in the legend. Figures and tables should be placed immediately after respectiveparagraphs of description).

Basic Requirements of Biotechnology Laboratory:

These are the following instruments and materials present in the biotechnology laboratory.

Instruments and Appliances:

1. Bunsen burner or spirit lamp
2. Laminar clean air.
3. Water bath.
4. Oven.
5. Hot plate
6. Incubators.
7. Refrigerators.
8. Micropipettes
9. PCR
10. Autoclave.
11. Tripod stands with asbestos mat.
12. Centrifuge.
13. pH meter.
14. Colony counter.
15. Spectrophotometer.
16. Microscope and photomicrographic camera.
17. Balances.
18. Homogenisers.
19. Magnetic stirrer.
20. Distilled water plant.
21. Deep freeze.
22. centrifuge.
23. Rotary Shaker.
24. Electric heaters.
25. Lyophiliser.

Tools:

1. Inoculation platinum loop.
2. Transfer needles.
3. Forceps.

4. Scalpels.
5. Scissors.
6. Ocular micrometer.
7. Burette stands

Glass-Wares:

1. Test tubes.
2. Petri dishes.
3. Conical flasks.
4. Culture tubes.
5. Centrifuge tubes
6. Screw capped bottles for tissue culture.
7. Durham's tubes.
8. Beakers.
9. Funnels.
10. Separating funnels.
11. Measuring cylinders.
12. Graduated and bulb pipettes.
13. Burettes.

Others:

1. Non-absorbent and absorbent cotton.
2. Aluminium foil.
3. Wire gauze baskets.
4. Stains on staining racks.
5. Glass marking pencil.
6. Nail polish/ wax to seal slides.
7. Disinfectants, alcohol,dettol, etc.
8. Containers to keep used glass-wares.
9. pipette can.
10. Petri dish can.
11. Microbial culture media.

12. Chemicals.
13. Reagents.
14. Filter paper, etc.

STANDARD OPERATING PROCEDURE		
SOP No.	IC/DBT/LAB/SOP-002	Version No. 1
SOP Title	Autoclave	
Department	Department of Biotechnology, Islamiah College (Autonomous), Vaniyambadi	

1. Objective:

The purpose of this SOP is to provide guidance for the safe operation of Autoclave in the laboratory, commonly used for decontamination and sterilization process.

Purpose and Scope:

This procedure is applicable to all authorised users who carry out experiment and research work using autoclave. All students, Technical assistants and Instructors are to adopt the procedure.

2. Responsibility:

- Students,subjected technical Staff and professors are responsible for the laboratory safety guidelines process.
- Department Head and Professors will ensure the implementation of this SOP.
- Department Head and Principal will monitor compliance to this SOP.

3. Procedure:

3.1 General Cleaning

Clean the Autoclave from inside and outside with a cloth moistened in disinfectant solution.

Clean the Autoclave daily after completion of daily work.

3.2 Precautions

1. Check the calibration status of the pressure gauge, temperature indicator cum controller.
2. Do not open the lid of the autoclave when the cycle is running.
3. Take care during handling of the articles as they will be hot, after sterilization.
4. After completion of the cycle, open the lid of the autoclave when the pressure gauge shows “0” reading and temperature gauge shows below 90°C.
5. Heating coils should be completely submerged in water.

3.3 Operation

1. Open the lid of the autoclave by pressing the steel base at the bottom.
2. Remove both the perforated steel baskets from the autoclave.
3. Pour enough purified water into the autoclave such that the heating coils are completely submerged in water.
4. Replace the stainless steel baskets back into the autoclave.
5. Load the material to be sterilized into the basket.
6. Close the lid and clamp the screws in place.
7. Connect the main cord to the supply socket and switch it on.
8. Heater will be on and the temperature starts to increase.
9. Solenoid valve will be off when the temperature reaches 100°C and pressure gauge shows the pressure.
10. When the pressure gauge shows a reading of 15 lbs and the temperature display shows 121° C.
11. Timer will be on and time starts to decrease in minutes. The heater will be cut on and cut off to maintain the pressure at 15 lbs and the temperature at 121°C.
12. At the end of 15 minutes switch off the heater and the solenoid valve will be on to release the steam pressure. Pressure starts falling.
13. When the temperature reaches below 100°C, Solenoid valve will be off and allow the autoclave to cool for 15 minutes.
14. Unclamp the screws and unload the sterilized material.
15. Use separate autoclave for sterilization and decontamination process.

Relationship between temperature and pressure

Pressure (lb or psi)	Temperature (°C)
0	100
5	110
10	116
15	121

30	135

Recommended times for autoclaving

Volume (ml)	**Times (minutes)**
75 – 200	*20*
200 – 500	*25*
500 – 1000	*30*
1000 – 1500	*35*
1500 – 2000	*40*

	STANDARD OPERATING PROCEDURE	
SOP No.	IC/DBT/LAB/SOP-003	Version No. 1
SOP Title	**ELECTRONIC DIGITAL BALANCE**	
Department	Department of Biotechnology, Islamiah College (Autonomous), Vaniyambadi	

4. Objective:

The purpose of this SOP is to provide guidance for the safe operation of Electronic Digital Balance in the laboratory.

Purpose and Scope:

This procedure is applicable to all authorised users who carry out experiment and research work using Electronic Digital Balance. All students, Technical assistants and Instructors are to adopt the procedure.

5. Responsibility:

1. Students, subjected technical Staff and professors are responsible for the laboratory safety guidelines process.
2. Department Head and Professors will ensure the implementation of this SOP.
3. Department Head and Principal will monitor compliance to this SOP.

6. Procedure:

1. Place balance on a clean, level surface.
2. If the balance can be leveled, then do so by adjusting its legs until level
3. Plug in the balance and press the On button.
4. Calibrate as described in accompanying SOP
5. Make sure the weigh pan is clear and clean
6. Press the On/Zero button on the left to zero the balance. **Make sure it is reporting in grams.**
7. Place weigh paper or a weigh boat on the balance pan and press On/Zero again to tare the balance. **Never place chemicals directly on the balance pan without paper or a weigh boat.**

8. Using a clean, dry spatula or scoop, add chemicals to be weighted to the weigh paper/boat until the desired mass is obtained. Hold the chemical bottle directly over the weigh paper/boat to reduce mess from spill.
9. If mass of chemical goes over the desired amount, use the spatula to remove excess until desired mass is reached. Discard any excess chemical that has been removed from the chemical bottle rather than returning it to the bottle
10. Remove weigh paper/boat from balance pan. Put lid back on chemical bottle and return it to its designated location. Clean any spilled chemicals

STANDARD OPERATING PROCEDURE		
SOP No.	IC/DBT/LAB/SOP-004	Version No. 1
SOP Title	Centrifuge	
Department	Department of Biotechnology, Islamiah College (Autonomous), Vaniyambadi	

7. Objective:

The purpose of this SOP is to provide guidance for the safe operation of centrifuges and rotors in the laboratory. Centrifuges are a commonly used piece of laboratory equipment. Centrifuges spin at high speeds to separate substances with different particle sizes or densities. .

8. Purpose and Scope:

This procedure is applicable to all authorised users who carry out experiment and research work using the centrifuge. All students, Technical assistants and Instructors are to adopt the procedure.

9. Responsibility:

- ❖ Students, subjected technical Staff and professors are responsible for the laboratory safety guidelines process.
- ❖ Department Head and Professors will ensure the implementation of this SOP.
- ❖ Department Head and Principal will monitor compliance to this SOP.
- ❖

10. Procedure:

4.1 Choosing the Right Instrument

1. Choose the small microcentrifuge (Tarsons or Remi) if you are using a small volume in a microcentrifuge tube and you want to just pool a sample at the bottom of the tube.
2. Choose the larger microcentrifuge (REMI) if you want to pellet a sample. Its maximum speed is 14,000 rpm and 16,000 xg.
3. Choose the large REMI centrifuge when using 15 ml centrifuge tubes. It has a maximum of 4000 rpm and 1100 xg.

4.2 Operating a centrifuge

1. To assure that the centrifuge rotor is balanced, insert the tubes so that they are across from each other. The tubes should have the same volume for balancing the rotor.
2. Add an additional tube with water if needed for balancing.
3. Close any lids before operating the centrifuge. Turn speed and time knobs to desired setting.
4. Wait for the centrifuge to come to a complete stop before trying to open it.

4.3 Preparation of samples:

1. Select appropriate tubes or containers for rotor, sample and speed.
    a) Tube/container and rotor bottoms must match. E.g. conical bottom rotors need conical bottom tubes.
    b) Sample must be compatible with tube/container material.
    c) Tube/container must be rated for speed being used.
2. Inspect tubes and containers for cracks or flaws before using.
3. Avoid overfilling or underfilling tubes and containers, make sure to follow manufacturer limits when given.
4. Balance the tubes and make sure the lids are tightly secured.
5. Make sure the exterior of the tubes and/or containers are clean and dry prior to centrifugation.

4.4 Run centrifuge:

1. Ensure lid of centrifuge is properly closed.
2. Set run speed and time, never exceed the rotor's maximum run speed.
3. Do not leave the centrifuge until full operating speed is reached and the machine appears to be running safely.
4. Stop the centrifuge immediately if you notice any unusual noises or shaking.
5. Confirm rotor is properly seated and balanced.
6. If problems persist, discontinue use and contact supervisor.
7. Do not use centrifuge until it has be serviced by a qualified technician.
8. Make sure the rotor has come to a complete stop before opening the lid.

4.5 Spills

Check for leaks and spills after each run.

1. If you know, or suspect, a spill has occurred, keep the centrifuge cover closed for at least 30 minutes to reduce aerosolization.
2. Inspect the sample tubes/containers, safety cups/buckets, rotor and centrifuge.
3. If a spill has occurred, use appropriate decontamination and cleanup procedures for the spilled material(s).

STANDARD OPERATING PROCEDURE		
SOP No.	IC/DBT/LAB/SOP-005	Version No. 1
SOP Title	Laminar Air Flow	
Department	Department of Biotechnology, Islamiah College (Autonomous), Vaniyambadi	

11. Objective:

The purpose of this SOP is to provide guidance for the safe operation of Laminar Air Flow in the laboratory, commonly used for microbiology works.

Purpose and Scope:

This procedure is applicable to all authorised users who carry out experiment and research work using Laminar Air Flow. All students, Technical assistants and Instructors are to adopt the procedure.

12. Responsibility:

4. Students,subjected technical Staff and professors are responsible for the laboratory safety guidelines process.
5. Department Head and Professors will ensure the implementation of this SOP.
6. Department Head and Principal will monitor compliance to this SOP.

13. Procedure:

1. In front of the blower, there lies a mechanism through which air blown from the blower produces air velocity along parallel flow lines.

2. Inside the chamber one fluorescent tube and the other UV tube are fitted. Two switches for these tubes and a separate switch for regulating of air, the air flow is fitted outside the apparatus.

3. Before starting to work in the laminar flow hood, turn on the blower and wipe out the sterile area with 70% alcohol soaked piece of cotton.

4. Let the blower run continuously for 30 minutes. When this time has passed, repeat the wipe out of the sterile area with 70% alcohol soaked piece of cotton.

5. Switch on the UV light for a period of 30 minutes so as to kill the germs, if any present in the area of working space.
6. The front cover sheet of the apparatus is opened to keep the desired material inside. The air blower is set at the desired degree, so that the air inside the chamber is expelled because the air inside the chamber may be contaminated/may bring contaminants.
7. Sit properly in front of the chamber again, wipe the working table with alcohol to reduce the contaminants. All the works related to pouring, plating, streaking etc., are to be carried out in the flame zone of the burner or spirit lamp.
8. In microbiology laboratory, horizontal type of laminar air flow is used to supply the air through the filter.

	STANDARD OPERATING PROCEDURE	
SOP No.	IC/DBT/LAB/SOP-006	Version No. 1
SOP Title	**MICROPIPETTES**	
Department	Department of Biotechnology, Islamiah College (Autonomous), Vaniyambadi	

14. Objective:

The purpose of this SOP is to provide guidance for the safe operation of Micropipettes in the laboratory.

Purpose and Scope:

This procedure is applicable to all authorised users who carry out experiment and research work using Micropipettes. All students, Technical assistants and Instructors are to adopt the procedure.

15. Responsibility:

7. Students, subjected technical Staff and professors are responsible for the laboratory safety guidelines process.
8. Department Head and Professors will ensure the implementation of this SOP.
9. Department Head and Principal will monitor compliance to this SOP.

16. Procedure:

A. Choosing the Right Instrument

1. Each micropipette has a range of volumes for which it is accurate and reliable. This range is stamped on the pipette below the display window and should never be exceeded.

2. The volumes of the micropipettes in this lab are 1-10 µl, 10- 100 µl and 100-1000 µl. Proper selection of the correct pipette is very crucial to accurate pipetting.

B. Pipetting

1. Set the pipette to the desired volume by depressing the adjustment button next to the display and rotating the plunger button on the end until the correct numbers appear in the display.

   CAUTION: Do not rotate the volume in the display beyond the minimum or maximum numbers stamped on the micropipette. Do not rotate the plunger button without depressing the adjustment button first.

2. Fit an appropriate size tip on the tip holder by using a slight twisting motion when pressing the tip holder into a pipette tip to help form an airtight seal.

   CAUTION: If when aspirating a liquid, you see a drop appear at the end of the tip as you hold the plunger button down, then there is a tip leak and you should refit a new tip.
3. Pre-rinse the tip by aspirating the first volume of liquid and then dispensing it back into the sample container.
4. Aspirate by pressing the plunger button to the first stop slowly and smoothly.
5. While still holding the plunger button down, hold the pipette vertically and immerse the tip into the liquid and hold it at a constant depth below the surface of the liquid.
6. Slowly release the plunger button so that the pipette will aspirate the liquid in the sample. As the depth of liquid lowers, so must the tip of the pipette. Once the plunger button has completed been released, hold the pipette in the liquid for 1 additional second to complete aspiration.
7. Remove the pipette tip from the solution and continue to hold the pipette vertically.
8. To dispense the sample, place the tip against the inside wall of the tube that is receiving the sample at a slight angle. Press the plunger button slowly and smoothly to the first stop.
9. Wait at least one second as it dispenses and then press the plunger button further to the second stop to expel any residual liquid from the tip.
10. Keep the plunger button pushed down as you withdraw your sample from the tube so as not to re-aspirate any of the transferred sample.
11. Release the plunger button slowly and smoothly.

C. Tip Removal

1. The tip may be ejected from the tip holder by pressing the blue button at the base of the end button while holding the pipette tip over a waste container.
2. Tip changes are required only if aspirating a different liquid, sample or reagent or volume. If contamination of the tip is a concern, then a tip change is always appropriate.
3. Once done with dispensing samples, reset volume to maximum volume for proper storag

STANDARD OPERATING PROCEDURE		
SOP No.	IC/DBT/LAB/SOP-007	Version No. 1
SOP Title	Light Compound Microscope	
Department	Department of Biotechnology, Islamiah College (Autonomous), Vaniyambadi	

17. Objective:

The purpose of this SOP is to provide guidance for the safe operation of Light Compound Microscope in the laboratory, commonly used for Cell Biology and Microbiology works.

18. Purpose and Scope:

This procedure is applicable to all authorised users who carry out experiment and research work using Light Compound Microscope. All students, Technical assistants and Instructors are to adopt the procedure.

19. Responsibility:

10. Students, subjected technical Staff and professors are responsible for the laboratory safety guidelines process.
11. Department Head and Professors will ensure the implementation of this SOP.
12. Department Head and Principal will monitor compliance to this SOP.

20. Procedure:

A. Setting up the Microscope

1. Carry the microscope with both hands and hold it above the waist so that it does not hit anything during transport.
2. Place the microscope on the lab bench away from the edge. Plug it in and secure the cord so that it does not get tangled up with anyone
3. Do not touch the lens of the microscope with anything but lens paper. Use lens paper to clean the lens on the eye piece and objectives.

B. Using the Microscope

1. Turn on the microscope's light source and adjust the intensity of the light with the dial on the side of the base.

2. Rotate the objectives until the low power objective is in place and lower the stage to its lowest position with the course adjustment.
3. Secure a slide on the stage with the stage clips and center over the light source
4. Looking through the ocular lens, use the course adjustment to raise the stage until the image of the specimen comes into focus.
5. Use the fine adjustment to get the image clear.
6. If higher magnification is needed, rotate the nosepiece to the next highest objective. You should only have to adjust your fine focus with each objective.
7. Once finished with the microscope, remove the slide, rotate objectives to low power, lower the stage, unplug it, cover it and return it to storage.

STANDARD OPERATING PROCEDURE		
SOP No.	IC/DBT/LAB/SOP-008	Version No. 1
SOP Title	pH Meter	
Department	Department of Biotechnology, Islamiah College (Autonomous), Vaniyambadi	

21. Objective:

The purpose of this SOP is to provide guidance for the safe operation of pH meter in the laboratory, commonly used in laboratory for preparing and standardising the pH of various solutions and biological samples.

22. Purpose and Scope:

This procedure is applicable to all authorised users who carry out experiment and research work using the pH meter. All students, Technical assistants and Instructors are to adopt the procedure.

23. Responsibility:

- ❖ Students, subjected technical Staff and professors are responsible for the laboratory safety guidelines process.
- ❖ Department Head and Professors will ensure the implementation of this SOP.
- ❖ Department Head and Principal will monitor compliance to this SOP.

24. Procedure:

4.1 Calibration

1. Calibration must be carried out, in accordance with the manufacturer's instructions, once daily or, in case of infrequent use, at least on the day of pH testing. It must be performed before the first measurement of the day. Record in the logbook.
2. Temperature variation affects pH measurement. Calibration and testing of the sample must therefore be done at the same temperature ± 2 °C.
3. Select for calibration two buffer solutions that are within 3 pH units of the solution to be tested. Discard contaminated or cloudy standard buffers.
4. Calibration results are acceptable if the pH of the buffer solution is within 0.1 pH units of the expected value.

4.2 pH measurement

1. Before use, rinse the electrode with deionized water and blot dry with a soft, clean paper towel.
2. Transfer the electrode to the test solution.
3. Compensate for the temperature if necessary.
4. Record the pH when the reading is stable (5–20 seconds after insertion of the electrode into the solution)
5. Rinse the electrode with deionized water and store according to the manufacturer's instructions**.**

4.3 Cleaning and maintenance

1. Clean the pH meter with a soft, clean, damp paper towel after use. No solvents should be used.
2. Replace the electrode filling solution on a regular basis, according to the manufacturer's instructions. Record in the logbook.
3. Repair and service of the pH meter should be done by a qualified service technician.
4. Calibration results should be recorded on the pH calibration sheet. The records should be kept on file for a minimum of one year, after which they may be archived.
5. Record the pH reading on the same sheet as the calibration results of the day.

STANDARD OPERATING PROCEDURE		
SOP No.	IC/DBT/LAB/SOP-009	Version No. 1
SOP Title	Pipetting	
Department	Department of Biotechnology, Islamiah College (Autonomous), Vaniyambadi	

25. Objective:

The purpose of this SOP is to provide guidance for the safe operation of Pipettes in the laboratory.

26. Purpose and Scope:

This procedure is applicable to all authorised users who carry out experiment and research work using Pipettes. All students, Technical assistants and Instructors are to adopt the procedure.

27. Responsibility:

13. Students, subjected technical Staff and professors are responsible for the laboratory safety guidelines process.
14. Department Head and Professors will ensure the implementation of this SOP.
15. Department Head and Principal will monitor compliance to this SOP.

28. Procedure:

A. Choice of correct pipette and pump

Choosing the correct pipette

a. For volumes less than 0.2 ml, do not use a serological pipet. Use a P-1000 micropipette instead

b. For volumes between 0.2 ml and 1.0 ml, use a 1 ml serological pipette

c. For volumes between 1.0 ml and 2.0 ml, use a 2 ml serological pipette

d. For volumes between 2.0 and 5.0, use a 5 ml serological pipette

e. For volumes between 5.0 and 10.0 ml, use a 10 ml serological pipette.

f. For volumes greater than 10.0 ml, use a graduated cylinder.

g. The smaller the pipette, the more accurate its measurement, so choose the smallest if there is any choice.

Choosing the correct pipette pump

a. When using the 1.0 or 2.0 pipettes, use the smaller, blue pipette pump

b. When using the 5.0 and 10.0 pipettes, use the larger, green pipette pump.

B. Removing the pipette from its packaging

1. If the pipette comes in a multi-pack then open the pack from end nearest the larger bore of the pipette. Tilt the pack so that at least one of the pipettes begins to slide out of the pack. Grasp the pipette near the large bore end taking care to not touch it anywhere else.
2. If the pipette comes in a single pack, then open the pack from the end nearest the largest bore by pulling the two sides of the packaging away from each other. This will be like peeling a banana. Peel away the packaging only one-third of the way down the length of the pipette. Grasp the pipette near the end with the largest bore taking care not to touch it anywhere else.

C. Assembling the pipette and pump

1. Hold the white bottom of the pipette pump while inserting the large bore end of the pipette into the hole Insert the pipette until it is securely fastened in the pipette pump, but not so far as to damage the vacuum chamber. The proper depth is to the cotton plug , if one is present

D. Removing liquid with the pipette

2. Place the tip of the pipette into the solution and keep the tip under the surface of the liquid at all times when removing liquid. Be careful the liquid does not overflow the container due to displacement.
3. With the tip of the pipette under the surface of the liquid hold the pipette and container at eye level, gently roll the pump wheel up to pull the solution up into the pipet.
4. Pull the solution up until the bottom of the meniscus (concaved surface of the liquid) is at the volume value desired.
5. Never lay the pipette down with liquid in it. Never allow the liquid to reach the cotton plug or to enter the pump. Alert the instructor if this happens.

E. Dispensing the liquid

1. Move the pipette into the recipient container. Roll the pump wheel down (all the way) to dispense the solution from the pipet.

2. Touch the tip of the pipette to the side of the container so that adhesion pulls on any liquid on the side of the pipet.
3. Allow the solution to leave the pipet, but do not force out the last tiny bit. Pipets labeled TD manufactured allow this last drop to remain in the pipette without affecting the measurement.
4. When there is not a specific order required to dispensing solutions into a container, always add the smallest volume first and then the larger volumes progressively.

F. Removing the pipette from the pump

1. Holding onto the bottom of the pump, gently twist and pull the pipette out of the pump.
2. Discard the pipette if it is disposable or place it in the cleaning tank if it is not.

	STANDARD OPERATING PROCEDURE	
SOP No.	IC/DBT/LAB/SOP-10	Version No. 1
SOP Title	**Stereo Microscope**	
Department	Department of Biotechnology, Islamiah College (Autonomous), Vaniyambadi	

Objective:

The purpose of this SOP is to provide guidance for the safe operation of **Stereo Microscope** in the laboratory.

Purpose and Scope:

This procedure is applicable to all authorised users who carry out experiment and research work using **Stereo Microscope**. All students, Technical assistants and Instructors are to adopt the procedure.

Responsibility:

- Students, subjected technical Staff and professors are responsible for the laboratory safety guidelines process.
- Department Head and Professors will ensure the implementation of this SOP.
- Department Head and Principal will monitor compliance to this SOP.

Procedure (Operation and Maintenance mandatory):

a. Set your microscope on a table top or other flat sturdy surface where you will have plenty of room to work. Plug the microscope's power cord into an outlet, making sure that the excess cord is out of the way so no one can trip over it or pull it off of the table.

b. Switch on the light source(s). If you will be looking at a microscope slide or other transparent object, bottom lighting will work best. If the specimen you are viewing is opaque or solid (light cannot pass through it from below), use top lighting so that the light can reflect off the specimen's surface.

c. Center your specimen on the stage plate. If your specimen is thin and flat, or if its edges curl up easily, try using the stage clips to hold it in place. To do this, pull up the pointed end of one stage clip and slide it over one end of the specimen, then do the same with the stage clip on the other side. If your specimen is larger than the stage plate, you might need to turn the stage clips out so that they are hanging off the stage giving you more room to work.

d. Adjust the eyepiece(s) so that you can look through the microscope comfortably without straining your eyes. (See below for instructions on adjusting a stereo head). For light-colored translucent specimens such as salt crystals, use either the black side of the stage plate (if it is reversible) or a piece of dark-colored construction paper to provide contrast.

e. If your stereo microscope has a rotating objective turret, turn it so that the marking on the objective you want to use is facing the front of the microscope. To determine the magnification of your microscope, multiply the magnification level of the eyepiece lens by that of the objective lens. For example, on the microscope in the diagram above, the total magnification at the 2x objective is 20x (2 x 10 = 20).

f. While looking through the eyepiece(s), slowly turn the focus knob until the specimen comes into view. Once you can see the outline of the specimen, turn even more slowly to focus as sharply as possible. If you aren't able to see anything, try moving the specimen around slightly on the stage plate to make sure it is directly below the objective lens and then try focusing again. Once you have focused on the specimen, you can move it around to see its other parts. You may have to refocus slightly on each new area. Note: with a stereo microscope you will often be viewing three-dimensional specimens that have many different levels. You will not be able to focus every feature clearly at the same time.

g. When you are finished using your microscope, turn off the switch, remove the specimen, unplug the power cord, and cover the microscope with its dust cover. Store your microscope in a place where it will not be damaged from extreme hot or cold temperatures, and out of the reach of small children.

	STANDARD OPERATING PROCEDURE	
SOP No.	IC/DBT/LAB/SOP-011	Version No. 1
SOP Title	**Cooling Centrifuge C-24 REMI**	
Department	Department of Biotechnology, Islamiah College (Autonomous), Vaniyambadi	

29. Objective:

The purpose of this SOP is to provide guidance for the safe operation of **Cooling Centrifuge C-24 REMI** in the laboratory.

30. Purpose and Scope:

This procedure is applicable to all authorised users who carry out experiment and research work using **Cooling Centrifuge C-24 REMI**. All students, Technical assistants and Instructors are to adopt the procedure.

31. Responsibility:

16. Students, subjected technical Staff and professors are responsible for the laboratory safety guidelines process.
17. Department Head and Professors will ensure the implementation of this SOP.
18. Department Head and Principal will monitor compliance to this SOP.

32. Procedure (Operation and Maintenance mandatory):

a. <u>Load Samples</u>

- Select the appropriate rotor based on the volume of the sample(s) and the desired speed and temperature.
- Ensure that the centrifuge is located on a rigid, flat, level surface.
- Remove or lift cover from centrifuge. Insert sample(s) into the rotor making sure to place two samples opposite of each other to maintain balance.
- If an odd number of samples need to be spun, make a counterbalance by filling an empty tube with an equal volume of water.
- Securely screw on or close the lid of the centrifuge

b. <u>Centrifuge Operation</u>

- Set the desired speed, time, and temperature on the centrifuge.
- Press the “Start” button and wait for the instrument to ramp up to the desired speed. Note: It is normal to see a small vibration in the instrument as the speed

increases, but any large vibration in the instrument or irregular noise may indicate that something has gone wrong with the run. Press the “Stop” button and make sure that the tubes are balanced properly.

- When the run has completed and the rotor has come to a complete stop, unscrew the rotor lid and carefully remove the samples to prevent resuspension of the sediments.

c. <u>Training assessment</u>

The laboratory technician is considered skills trained in using a centrifuge when they have successfully completed the training assessment.

Appropriate use of the SOP for centrifuging samples recorded in the laboratory notebook.

	STANDARD OPERATING PROCEDURE	
SOP No.	IC/DBT/LAB/SOP-012	Version No. 1
SOP Title	**Magnetic stirrer**	
Department	Department of Biotechnology, Islamiah College (Autonomous), Vaniyambadi	

33. Objective:

The purpose of this SOP is to provide guidance for the safe operation of **Magnetic stirrer** in the laboratory.

34. Purpose and Scope:

This procedure is applicable to all authorised users who carry out experiment and research work using **Magnetic stirrer**. All students, Technical assistants and Instructors are to adopt the procedure.

35. Responsibility:

19. Students, subjected technical Staff and professors are responsible for the laboratory safety guidelines process.
20. Department Head and Professors will ensure the implementation of this SOP.
21. Department Head and Principal will monitor compliance to this SOP.

36. Procedure (Operation and Maintenance mandatory):

a. Place the magnetic stirrer on a stable well-levelled surface.
b. Place the stir bar at the bottom of a glass container.
c. Fill the glass container with the liquid to be stirred.
d. Plug the mains cable into a suitably earthed socket.
e. Check that the speed control knob is completely turned anti-clockwise.
f. Place the glass container on the centre of the magnetic stirrer.
g. Press the On/Off switch to turn the magnetic stirrer on. The switch will light green.
h. Adjust the speed control knob to a low stirring rate.
i. Continue to adjust the speed control knob until the desired stirring speed is achieved.
j. Wait until the liquid is properly mixed.
k. Completely turn the speed control knob anti-clockwise.
l. Press the On/Off switch to turn the magnetic stirrer off.

m. Manipulate another stir bar from the outside of the glass container to remove the immersed stir bar.

Maintenance:

Thoroughly wash the stir bar with distilled water after each application.

Store stir bars in pairs to maintain their magnetic strength and increase their life span.

	STANDARD OPERATING PROCEDURE	
SOP No.	IC/DBT/LAB/SOP-013	Version No. 1
SOP Title	**Stage Micrometer**	
Department	Department of Biotechnology, Islamiah College (Autonomous), Vaniyambadi	

Objective:

The purpose of this SOP is to provide guidance for the safe operation of **Stage Micrometer** in the laboratory.

Purpose and Scope:

This procedure is applicable to all authorised users who carry out experiment and research work using **Stage Micrometer**. All students, Technical assistants and Instructors are to adopt the procedure.

Responsibility:

- Students, subjected technical Staff and professors are responsible for the laboratory safety guidelines process.
- Department Head and Professors will ensure the implementation of this SOP.
- Department Head and Principal will monitor compliance to this SOP.

37. Procedure (Operation and Maintenance mandatory):

1. Place a stage micrometer on the microscope stage and using the lowest magnification (4X), focus on the grid of the stage micrometer.
2. Rotate the ocular micrometer by turning the appropriate eyepiece. Move the stage until you superimpose the lines of the ocular micrometer upon those of the stage micrometer. With the lines of the two micrometers coinciding at one end of the field, count the spaces of each micrometer to a point at which the lines of the micrometers coincide again.
3. Since each division of the stage micrometer measures 10 micrometers, and since you know how many ocular divisions are equivalent to one stage division, you can now calculate the number of micrometers in each space of the ocular scale.
4. Repeat for 10X and 40X, and 100X. Record your calculations below.
5. Using the stage micrometer, determine the smallest length (in microns) which can be resolved with each objective. This is the **measured** limit of resolution for each lens.

6. Using the calculated values for your ocular micrometer, determine the dimensions of the letter found on your microscope slide.
7. Use a millimetre ruler to measure the letter e directly and compare with the calculated values obtained through the microscope.

	STANDARD OPERATING PROCEDURE	
SOP No.	IC/DBT/LAB/SOP-014	Version No. 1
SOP Title	**Fermenter KEMI**	
Department	Department of Biotechnology, Islamiah College (Autonomous), Vaniyambadi	

Objective:

The purpose of this SOP is to provide guidance for the safe operation of **Fermenter KEMI** in the laboratory.

Purpose and Scope:

This procedure is applicable to all authorised users who carry out experiment and research work using **Fermenter KEMI**. All students, Technical assistants and Instructors are to adopt the procedure.

Responsibility:

- Students, subjected technical Staff and professors are responsible for the laboratory safety guidelines process.
- Department Head and Professors will ensure the implementation of this SOP.
- Department Head and Principal will monitor compliance to this SOP.

38. Procedure (Operation and Maintenance mandatory):

Description and Uses

Description and Uses of KEMI Fermentor can be used for batch culture with microprocessor control of pH, dissolved oxygen (DO), agitation, temperature, pump feed rate, antifoam, foam/level, and additional analog/digital inputs and outputs. It has a maximum volume of 5-L and a maximum operating temperature of 150°C.

Power Specifications

Voltage/Amperage: 230 V

Speed/Frequency: Variable speed control@50 Hz.

Vessel Pressure Range: -1 to +2.5 bar (36 psi)

Single Jacket

Pressure Range: -1 to +4.0 bar (58 psi)

Maximum Volume: 5-L

Maximum Operating Temperature: 150°C

Safety Precautions:

High Voltage/Possible Electric Shock (220-V)

Make sure that the wall outlet receptacle is properly wired and grounded, and matches the instrument's power cord and plug. • Do not touch the power cord or plug if hands or feet are wet, or if standing on a wet/damp surface, as severe electrical shock or death may result.

Excessive Weight/Possible Damage to Hands or Fingers when Moving or Repositioning Parts

Certain parts of the fermentor, such as the head plate, condenser, etc. are very heavy. Use extreme caution when handling or moving these parts during fermentor assembly/disassembly. If available, wear shoes with steel caps when using the fermentor.

Hot Surfaces/Possible Burns to Hands and/or Extremities

The fermentor is made of stainless steel and has a "jacket" that is filled with steam under pressure during the sterilization-in-place (SIP) process. As a result, the outer surfaces of the fermentor become HOT enough to cause serious skin burns if the fermentor is touched. Use extreme caution when working near the fermentor during sterilization.

Biohazard/Possible Infection from use of Pathogenic Microbes

Normal operation involves the use of many types of microorganisms, all of which can be opportunistic pathogens. Occasional use may also involve working with genetically modified microbes. Use extreme caution and good sterile techniques when working with these organisms. After use, autoclave the fermentor to kill any remaining microbes.

Required Personal Protective Equipment

Lab Coat

Hair Net

Safety Glasses or Goggles

No Open-toed or Open-heeled Shoes

Insulated Rubber Gloves

Steel-toed Shoes are recommended

Required Training

Machine & Site-Specific Training

Fire Safety & Extinguisher Training

Laboratory Safety: Core Concepts

Laboratory Safety: Spill Procedures

Blood borne Pathogens and Sharps Safety

Operation

Operation: Start-Up

1. Be sure to complete all the required training listed above, including machine and site-specific training by the Fermentation Facility manager. It is highly recommended that the 5-L KEMI Fermentor be operated under the direct supervision of the Fermentation Facility manager.
2. The Operating Manual provides a more complete description of the KEMI Fermentor. Be sure to read the manual before operating the fermentor to become familiar with its correct operation and individual component parts.
3. Inspect the fermentor to see if it is clean. If not, clean the vessel with warm soapy water. Then, rinse with tap water followed by a distilled-water rinse. If necessary, clean the underside of the head plate, impeller shaft, and paddles.
4. Once clean, fill the fermentation vessel with deionized (DI) water to a depth sufficient enough to cover the pH probe so that the probe does not dry out.
5. Turn ON the fermentor controller using the key on the front of the Control Panel.
6. Open the "Instrument Air Valve" located above the central lab bench.
7. Open the "5-L Vessel Air Valve" to sparge the fermentor.
8. Close the "Cooling Water Valve" to the heat exchanger and close the Thermostat Pressure Valve first. It should read ~1.5 bar.
9. Calibration of the pH Probe is necessary prior to sterilization and filling the fermentor with media. To calibrate the probe, perform the following:

a. Drain the fermentor vessel. This is necessary because the pH probe must be disconnected from the vessel during calibration. If the vessel is full of water, it will spill out when the pH probe is removed.

b. Connect the pH probe cable to the fermentor controller.

c. Remove the pH probe from the fermentor vessel.

d. turn ON the fermentor pH meter using the flip switch.

e. Set the temperature knob to ambient (~20°C).

f. Switch the pH meter to “Manual.”

g. Rinse the pH probe w/deionized (DI) water. Then, immerse the probe in pH 7 buffer.

h. Adjust the Δ pH knob until the pH reading is 7.0.

i. Rinse the pH probe with DI water. Then, immerse the probe in pH 4 buffer.

j. Keep the probe immersed in either the pH 4 or pH 7 buffer until just prior to filling the fermentor vessel with media to prevent it from drying out.

10. Check the pH probe O-ring for possible damage. If necessary, replace the O-ring.

11. Apply a small amount of glycerol or silicone grease to the pH probe O-ring.

12. Insert and secure the pH probe inside the fermentor.

13. Rinse the fermentor vessel with deionized (DI) water prior to filling and preparing the media. When finished, close the vessel drain valve.

14. Fill the vessel with DI water and add medium ingredients.

15. Set the agitator at ~100 rpm to dissolve and mix media ingredients. Note: The level of media should be high enough to cover the probes.

16. Check that all connections (e.g., sample port, inoculation port, etc.) to the vessel are securely closed.

Operation: Sterilization

1. Turn the “Exhaust Valve” to full left to exhaust the steam trap. This valve is above and to the left of the inlet air condenser. When the handle is up, the valve is closed; when the valve’s handle is to the left, condensate goes to the condenser; when the valve’s handle is to the right, condensate/ gas is vented to the atmosphere.

2. Close the “Inlet Air Valve.” Then, open the “Inlet Air Condensate Valve.

3. Be sure the “Inlet Air Control Valve” is closed.

4. Set the “Inlet Air Pressure Regulator” to 1.5 bar on the “Inlet Air Pressure Gauge”

5. Turn the “Sparge/Overlay Valve” fully counter clockwise to “Overlay”

6. Be sure the “Vessel Port Steam Valve” is closed. The valve sends steam to the sampling and drain ports to sterilize them prior to sampling the fermentor.

7. Turn on the “Stirring Motor” (Control Panel) during sterilization. If all the media ingredients dissolve, 100 rpm is sufficient.

8. Slowly open the “5-L Main Steam Valve” Opening the valve sends steam to through a pressure regulator to the fermentor. The pressure should go to ~40-60 psi.

9. Set sterilization time (STERILIZATION) on the Control Panel. This should be 20-25 min at 121°C for refined media and longer for media with a high suspended-solids content.

10. Turn ON the thermostat. Then, set the thermostat to the desired temperature to be used during the fermentation.

11. Hold the "Sterilization" switch up for a few seconds. The indicator will show the sterilization time in minutes. While the vessel heats up, the time will be flashing. When the vessel reaches sterilization temperature (121°C), the time on the indicator will be steady.

12. To cool down the fermentation vessel after sterilization, pressurize the vessel to avoid drawing a vacuum as follows

a. Close the "Exhaust Valve" by turning on

b. Close the "Inlet Air Condensate Valve." This valve is located below the "Sterilizable Gas Filter."

c. Open the "Inlet Air Valve"

d. Open the "Inlet Air Control Valve" a few turns.

e. Open the "Cooling Water Valve" to the heat exchanger slowly until it is fully open.

f. Turn on the cooling water to the condenser by fully opening the "Condenser Water Value."

g. Wait until the fermentor temperature falls below 80°C.

h. Close the steam supply to the sample and drain ports. Then, close the "5-L Main Steam Valve."

i. Turn the air on to 1 m3/h (positive air). Use the "Inlet Air Pressure Regulator" to decrease the air pressure to ~0.5 bar. Then, adjust the "Inlet Air Control Valve" to keep the pressure between 0.5 and 1.0 bar.

Operation: Fermentation

1. When the temperature in the vessel approaches the temperature to be used during the fermentation, reduce the flow of water to the heat exchanger by partially closing the "Cooling Water Valve." This valve should remain open just a "crack." Note: During the actual fermentation, keep the valve open slightly. This keeps cold water flowing to the heat exchanger. The exchanger performs a "balancing act" between the cold water and steam going to the fermentor to keep the fermentor temperature at the desired set point.

2. If the fermentation is to be performed aerobically, turn the "Sparge/Overlay Valve" fully clockwise to "Sparge."

3. After the medium has cooled to the desired fermentation temperature, sparge the fermentor with either sterile air or oxygen, as required, to saturate the medium with oxygen.

4. Fermentor Inoculation: Depending on the inoculum volume (~50-1,000 mL), the inoculum can be added to the fermentor using a large sterile syringe and a sterile large-bore needle. For volumes of several hundred mL and larger it is better to add the inoculum from a sterile bottle using a peristaltic pump. In either case, the inoculum is added through a sterilizable, self-sealing septum on the lid of the fermentor.

a. By peristaltic pump: Insert an 8-10 inch long piece of stainless-steel tube in one end of the #25 tubing. Insert the stainless-steel tube through a rubber stopper on a screw-cap for a 0.5-1.0 L bottle. Wrap foil around the cap. Then, autoclave the tubing and bottle assembly. After sterilization, aseptically transfer the desired volume of inoculum into the sterile bottle. Sterilize the septum on the fermentor lid with several mL of alcohol. Uncover the needle and insert it through the septum. Turn ON the pump to deliver the inoculum to the fermentor. After the inoculum has been added, turn OFF the pump, withdraw the needle and wash the septum again with alcohol.

7. Foam Control: If the medium is foamy, add a few drops of sterile antifoam using a sterile needle/ syringe assembly. Add the anti-foam through one of the sterilizable, self-sealing septa on the fermentor lid.

8. Sampling the Fermentor:

a. To periodically collect samples during the fermentation, open the "Vessel Port Steam Valve." This will send steam through the sampling port to sterilize it and prevent introduction of contaminants during the sampling procedure.

b. After 1-2 minutes, remove the cylindrical metal "sleeve" from the sampling port.

c. Position a collection vessel (e.g. a beaker or flask) below the sampling port and turn the sampling valve clockwise until the medium drains from the vessel into the sampling container.

d. When a sufficient sample has been collected, turn off the sampling valve until the drainage stops.

e. Replace the metal sleeve over the sampling port and steam the valve for ~2 min (as stated above in Step 8a).

Operation: Harvest and Shutdown

1. When the fermentation is complete, drain the fermentation contents from the fermentor into a collecting vessel by performing the following steps:

a. The pH controllers should be turned off; however, agitation should be continued to keep the cells in suspension.

b. Shut off inlet gas flow by turning "Inlet Air Control Valve" clockwise. Allow the vessel pressure to reach zero PSIG.

c. To drain and collect the contents in the fermentor, open the port by pushing the handle down.

2. After the fermentor has been drained, open one of the additional ports on the fermentor lid and fill the vessel about half full with deionized (DI) water until cover the DO and pH probes.

3. Re-seal the vessel.

4. Then re-sterilize the fermentor (as listed in the "Sterilization" procedure) to kill any remaining microbes.

5. Allow the fermentor to cool.

6. Then drain and discard the water from the vessel.

Clean-up Procedures

1. After the fermentor has been harvested, remove all attachments and all connections from the top of the head plate.

2. Fill the fermentor vessel about halfway with warm tap water.

3. Add a non-abrasive detergent and turn on the agitator (~150 rpm) for a Ten minutes.

4. Turn ON the agitator and drain the fermentor. Once drained, turn OFF the agitator.

7. Rinse the inside of the fermentor with distilled water. Then, drain again.

8. Store the probes according to the manufacturer instructions.

9. Fill the fermentor vessel with tap water.

10. Clean up the surrounding work area.

Machine Care and Maintenance

The fermentor must be properly cleaned and inspected after each use. All inspections are performed by the Fermentation Facility manager.

All regularly scheduled maintenance should be performed by the Fermentation Facility manager or a trained service technician.

Accessories

Accessory fermentor parts include pH probe, pressure gauge, pressure regulator, pressure transducer, and peristaltic pump.