

INSTITUTO TECNOLÓGICO Y DE ESTUDIOS SUPERIORES DE MONTERREY  
CAMPUS CHIHUAHUA

# LABORATORY MANUAL

For Biotechnological Engineering Students

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## **GENERAL REGULATIONS FOR LABORATORY USE**

1. In order to enter the lab, you must wear a long sleeve lab coat, pants to the ankle, closed foot wear and have the hair tied.
2. Remember to wash your hands before and after you work on the lab.
3. Wear properly the personal protective equipment (PPE) like latex gloves, protection glasses, mask, as warrants to conduct the experiment.
4. Remain inside the lab until you finish the experiment and/or your teacher indicate it. Avoid entering and getting out unless it be really necessary.
5. Remaining in the laboratory is allowed only to persons whose activity requires it.
6. Be sure of not have any cuts, scrapes and other skin injuries, if so cover the injury as it be convenient to avoid any kind of contact with material and lab reagents.
7. Keep your belongings outside the work area, this includes the floor you occupy for working.
8. Make sure of keep the work area clean and ordered before and after you work.
9. If it is necessary work near the flame of the bunsen burner or inside the laminar flow cabinet, according to the bio-safety level needed for the experiment.
10. Food, drinks, yelling, running, playing and sitting on the tables of in the floor its prohibited inside the lab.
11. The use of celphones or any kind of electronic deviche is prohibited inside the lab.
12. It is strictly forbidden to take away
12. It is strictly forbidden to take away any kind of lab equipment / reagents / cultures / samples problem, if the instructor not tells you to do so.
13. Before pouring any type of solid or hazardous substances in the laboratory sink, check your MSDS or ask your instructor.
14. If you detect anomalies on the lab or in the laboratory equipment, please report it to your instructor.
15. If you are going to use some lab equipment, be sure to follow the instructions of the equipment's manual, register in the journal and notify your instructor. If you have any doubt he will guide you.
16. Is responsibility of all users to keep clean and tidy the lab, so before leaving you need to make sure you have correctly rejected the reagents no longer going to use, wash your gear and leave your work area clean and orderly.
17. Es important that all the material you use is perfectly labeled, including: name of the reagent / material preparation date, your full name and subject or project for which you are using.

## LABORATORY EQUIPMENT DESCRIPTION

### I. Autoclave Tuttnauer 2340 manual model Heidolph Brinkman



The instrument consists of five basic controls:

- 1) Barometer: indicates the pressure inside the chamber of the autoclave.
- 2) Timer: indicates for how long the autoclave will be working.
- 3) Thermostat: lets you select the temperature range within the autoclave's camera
- 4) Select function: selects the mode in which you'll be working the autoclave.
- 5) On/off: turns on and off the autoclave.

Using Manual autoclave Tuttnauer Model 2340

- 1) Make sure the autoclave is perfectly leveled before purging the water.
- 2) Wash the material according to the lab protocol before placing it in the tray of the autoclave.
- 3) Place on the tray the material to be sterilized (Do not exceed the limit of 3.2 kg of material, and do not place media bottles with liquid or horizontally, to prevent spills).
- 4) If you put bottles or tubes with screw cap, be sure to leave the lid slightly loose, otherwise sterilization will not be efficient. The volume should not exceed two thirds of the volume of the bottle to prevent overflows.
- 5) If you place packages, be sure to leave space between them, the material should not touch the walls of the equipment.
- 6) Fill the chamber with 400ml distilled / deionized / distilled water, close the door and select the option "FILLING".
- 7) Close the door turning the crank.
- 8) Place the thermostat to the desired temperature (115 ° C).
- 9) Place the timer 30-45 minutes for packages and instrumental, and from 45-60 minutes for liquids.
- 10) Turn the knob and place it in "sterilized" and make sure the indicator lights.

11) Once you have completed the sterilization wait till the barometer indicates "zero" psi. Then move the function knob on "dry" and placd the timer in 20 or 30 minutes. All the material is dried except liquids. Before opening the door make sure the pressure has dropped completely.

12) Open the door and remove the material using thermal gloves.

## **II. Manual Autoclave model: Allamerican 25X**



1) Use distilled / deionized / distilled water to fill the chamber to the tray level of and place the aluminum container inside the chamber of the autoclave.

2) In case you put bottles or tubes with screw cap, be sure to leave the lid slightly loose, and place them horizontally avoided. The volume should not exceed two thirds of the volume to avoid spillovers.

3) In case of placing packages, be sure to leave space between them, the material should not touch the walls of the aluminum container.

4) Place the lid making sure to place the exhaust air duct inside the aluminium container wall. Level the lid before closing the fasteners closing them in opposite pairs at the same time.

5) Place the control valve in the open position, until it begins to release steam, then you must turn it in the closed position.

6) Monitor the pressure and temperature until it reaches 15 psi. Adjust the thermostat to avoid excessive pressure.

7) Once at 15 psi you must take the time. For proper sterilization 15-30 minutes must pass. Once the time finish turn off the power.

8) Once the barometer indicates "zero" psi, open the control valve and loose fasteners.

9) Lift up the lid and take out the material using thermal gloves.

## **III. Laminar flow cabinet**

1) Before working disinfect the entier work suface with 70% alcohol.

- 2) Turn on the laminar flow at least 15 minutes before start work.
- 3) You must work with gloves and spray your hands with 70% alcohol every time you take them out of the cabinet.
- 4) Place inside the cabinet only the material that is useful and needed for the work, such material should be disinfected with 70% alcohol before introduce it into the work area.
- 5) Remove the material you no longer use. Disinfects the material before take it out of the cabinet with 70% alcohol.
- 6) When you finish working clean the work area, and disinfected the table using 70% alcohol.
- 7) Be sure to turn off the laminar flow and light.



#### **IV. Proper use of micropipettes**

- 1) Make sure you choose the right pipette for the volume needed. Exceeding the maximum volume or forced to a lower value of the minimum damage and decalibrate the micropipette.
- 2) When you put a tip, make sure that is not loose, caus this will allows the enter of air and the volume will not be right. Do not hit the tip of the micropipette.
- 3) Before taking the fluid presses the plunger and take it to the first stop without going over. Insert the tip into the liquid and release the plunger. Your tip and should be loaded with the desired volume.
- 4) To release the liquid, place the tip on the wall of the desired container (unless the methodology recommended it in another way) and gently press the plunger to the second stop. Push too quickly or hard can cause splashing the contents.
- 5) Never put the pipette in an inclined or horizontal position when making sample, as this will contaminate or damage the micropipette as well as causing changes in the volume obtained.
- 6) For viscous liquid filling and expulsion should be slower.
- 7) Avoid using a micropipette with corrosive solvents.
- 8) The ideal temperature for handling liquids with the pipette is 15 to 40 °C.
- 9) When not in use, avoid placing the pipette in a horizontal position for extended periods of time, as this will affect their calibration.

10) Avoid dropping or bumping the pipette and place it in unstable position or surfaces.

11) If you need to adjust the volume of the pipette, do it gently, and turn the volume control too vigorously or quickly you can damage it.

12) When finished using a micropipette, be sure to adjust it to the maximum volume allowed, cause leave it at lower values for long periods of time will eventually decalibrate it.



## **V. Use and management of balance**

1) You need to register in the journal before using the balance, and write in the same if an abnormality is observed in the equipment before or after use it, if so notify the manager.

2) Never weigh directly on the weighing plate. Always use filter paper or watch glass.

3) Make sure the instrument is calibrated before use it.

4) You should avoid any disruption leading to an error such as: vibration due to hitting, running instruments or blowing on the plates of the scale.

5) Wash the spatula before and after use with tap water and dry it with paper.

6) Once the balance is used, the equipment must be clean and off.

## **HANDLING AND CARE OF LABORATORY EQUIPMENT**

Within the research laboratories and Biomedicine you will find equipment that need proper management, as well as some care to keep them running properly. In this section you will find basic rules for handling the most commonly used equipment in the laboratory as well as links to the manuals and the person in charge of its management.

### **Before using any equipment:**

1. Learn about the uses and limitations of each of the instruments.
2. Read the user's manual.
3. Contact the person in charge of the equipment in case you have doubts about its use.
4. If it is the first time the equipment is used to do it in the presence of someone trained.
5. The samples must be prepared according to the equipment requirements in order to avoid disadvantages when it is used. Avoid putting in substances that can damage the equipment.
6. In case of any problem in the use of any equipment please report it to the nearest authority and the person in charge of the equipment.
7. Always register you in the journal of each equipment.
8. Once you have finished turn off and clean the equipment. In case the equipment has additional pieces keep them in place.

### **Investigation Laboratory Equipment**

| <b>EQUIPMENT</b>     | <b>Model / Brand</b>                | <b>Attendant</b>            |
|----------------------|-------------------------------------|-----------------------------|
| Electrical autoclave | All American 25X-1 serie<br>0024495 | Carlos Arzate<br>Quintana   |
| Centrifuge Eppendorf | Eppendorf 5804R                     | Carmen D.<br>González       |
| Centrifuge Galaxy    | Galaxy 20r VWR                      | Carmen D.<br>González       |
| Refrigerator -20°C   | Marvel Scientific 17 CAF            | Lucía Olivas                |
| Incubator            | Symphony VWR                        | Silvia L. Montes<br>Fonseca |
| Refrigerator 4°C     | Torrey R-14                         | Lucía Olivas                |
| Refrigerator 4°C     | LG                                  | Lucía Olivas                |
| Balance              | Ae ADAM PGW453i                     | Yazmín Reyes                |

|                    |  |                             |
|--------------------|--|-----------------------------|
| Balance            | Pionner Ohaus                            | Yazmín Reyes                |
| Thermocycler       | Agilent Technologies<br>Sure Cycler 8800 | Carmen D. González          |
| Termoblock         | Eppendorf Master Cycler<br>Personal      | Silvia L. Montes<br>Fonseca |
| Thermomixer        | Eppendorf Thermomixer<br>compact         | Carmen Daniela<br>González  |
| Bouble boiler      | Techne                                   | Cynthia González            |
| Optical microscope | PrimoStar/Carl Zeiss                     | Carlos Arzate<br>Quintana   |
| Hotplates          | Thermo Scientific/Cimarec                | Cynthia González            |

### Biomedicine Laboratory Equipment

| Equipment           | Model / Brand                         | Attendant                   |
|---------------------|---------------------------------------|-----------------------------|
| Inverted microscope | Olympus PME3                          | Silvia L. Montes<br>Fonseca |
| Balance             | Oken/FSF                              | Yazmín Reyes                |
| Spectrophotometer   | Spectronic 20D+/ Thermo<br>scientific | Lucía Olivas                |
| Potentiometer       | Hanna Instruments/ HI255              | Lucía Olivas                |
| Shaker              | Thermo scientific/ MaxQ 200           | Yazmín Reyes                |

|                          |  |                          |
|--------------------------|--|--------------------------|
| Bouble boiler            | Sheldon Manufacturing/H2O series                 | Cynthia González         |
| Bioanalyzer              | Agilent 2100/ Agilent Technologies               | Silvia L. Montes Fonseca |
| Thermocycler qPCR        | Agilent Stratagene Mx3005p/ Agilent Technologies | Carmen D. González       |
| Optical microscope       | Primo Star/Carl Zeiss                            | Carlos Arzate            |
| Fotodocumentador         | UVP BioDoc-it 210 Imaging System                 | Carmen D. González       |
| UV/Vis Spectrophotometer | Beckman Coulter/ SU730 Life Science              | Lucía Olivas             |
| Inverted microscope      | Axio Vert.A1/ Carl Zeiss                         | Silvia L. Montes Fonseca |
| Bouble boiler            | TerLab   | Cynthia González         |
| CO2 Incubator            | Symphony VWR /3074                               | Silvia L.Montes Fonseca  |
| Refrigerator -20°C       | VWR  | Lucía Olivas             |
| Refrigerator -80°C       | NorLakescientific                                | Lucía Olivas             |
| Autoclave                | Tuttanauer 2340/Heiddph                          | Carlos Arzate            |
| Autoclave                | AESA modelo CV300                                | Carlos Arzate            |

## LABORATORY REAGENTS

- The reagents must be stored as the NOM0-053-ECOL-1993 indicates.
- It is mandatory to wear personal protective equipment (gown, gloves and security glasses)
- The student must be familiar with the handling and transportation of hazardous waste (Corrosive, highly reactive, toxic, flammable, explosive, etc.)
- The arrangement of reagents and solvents will be controlled and safe (according to the manual).
- In case of preparing a reagent place it in a storage jar properly labeled with the following information: Name and full chemical formula, concentration of solution Molarity and Normality as applicable, reagent preparation date and the name of the person who elaborate it.
- It is strictly forbidden to pipette reagents with the mouth, always use a propipeter.
- When working with highly toxic substances is necessary to use a protective mask and work inside the fume extraction cabinet.
- Before using a specific compound, ensure that it is the right reagent: read the label a few times.
- Never return the leftover of the products to the jar.
- When disposing of any reagent ensure that circulate enough water on the sink.
- Do not touch with your hands, not with the mouth, chemical products. If so check the manual contingency measures.
- The acids require special care. When required to dilute them, never add water over them; on the contrary, dilute it in a water layer.
- Flammable products should not be near heat sources such as heaters, stoves, heaters, burners, etc.
- When any chemical spill you must act quickly, but without haste.
- Once you finish the practice , return the reagent bottles back to their storage place and remove the waste according to the rules of this manual.

## HAZARDOUS CHEMICALS

The chemicals are classified according to their dangerousness in:

**Explosives:** Substances and preparations which may explode under the effect of flame.

**Oxidising:** Substances and preparations which, in contact with others, particularly with flammable ones, cause a highly exothermic reaction.

**Extremely flammable:** Substances and chemical products whose flash point lower than 0 ° C, and boiling point 35 ° C or less.

**Highly flammable:** are defined as such:

- Substances and preparations which, at room temperature, in air and without energy supply, may get hot and even catch fire.
- Liquid substances and preparations with a flash point at or above 0 ° C and below 21 ° C.
- Solid substances and preparations which may easily ignite because of brief contact with a source of ignition and which continue to burn or to be consumed after removal of the fire source.
- Gaseous substances and preparations which are flammable in air at normal pressure.
- Substances and preparations which, in contact with water and damp air, release flammable gasses in dangerous quantities.

**Flammable:** Substances and preparations having a flash point equal to or greater than 21 ° C and below 55 ° C.

**Very toxic:** Substances and preparations which by inhalation, ingestion or skin penetration may involve serious, acute or chronic health risks and even death.

**Harmful:** Substances and preparations which by inhalation, ingestion or skin penetration involve risks of limited severity.

**Corrosive:** Substances and preparations which in contact with living tissue can have a destructive action on them.

**Irritant:** Non-corrosive substances and preparations which, through immediate, prolonged or repeated contact with the skin or mucous membrane may cause inflammation.

**Environmental hazards:** Substances and preparations which use present or may present immediate or delayed risks for the environment.

**Carcinogens:** Substances and preparations which by inhalation, ingestion or skin penetration can cause cancer or increase its frequency.

**Teratogenic:** Substances and preparations which by inhalation, ingestion or skin penetration can induce lesions in the fetus during its intrauterine development.

**Mutagenic:** Substances and preparations which by inhalation, ingestion or skin penetration may cause alterations in the genetic material of cells.

Some of these substances appear in the labeling of chemicals by a symbol or pictogram:



**Explosive**



**Oxidizer**



**Toxic**



**Irritant**



**Flammable**



**Corrosive**

## **USE AND HANDLING OF HAZARDOUS BIOLOGICAL INFECTIOUS WASTE**

It is a Hazardous waste any material of biological origin (blood, urine, sputum, feces, tissue, cell culture, bacterial and vegetables). Hazardous waste disposal will be as follows:

**Red bag:** petri plates previously sealed with parafilm, gloves, dressing material containers which have been in contact with hazardous waste.

**Plastic red container for sharps:** syringe needles, slides, cover slips, glass pieces, lancets, etc.

**Plastic red container for liquids:** blood

**Plastic yellow container for liquids:** urine, agarose and polyacrylamide gels.

**Yellow bag:** tissue and organs, animals.

Note: Both the bags and the boxes must not exceed 80% of its capacity. Do not forget to always use personal protective equipment to use or transport hazardous waste.

### **Hazardous biological infectious waste contingencies:**

Splashes on the skin: wash with soap and water and disinfect the area with 70% alcohol.

Splashes in the eyes: wash with water and see a doctor immediately.

Puncture: Wash with soap and water and disinfect the area with 70% alcohol. See a doctor to continue the treatment.

Spillover: Directly inactivate with chlorine undiluted by 15-20 min. Wipe with an absorbent material and dispose of it in the red bag. Finally clean the area with 70% alcohol.