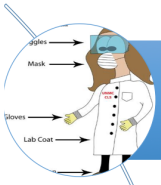
General safety rules : Important!!

-Lab safety is everyone's responsibility
- Lab safety policy and procedures must be strictly followed



Always use appropriate clothes and personal protective tools (Lab coat, safety goggles, masks, gloves, no open shoes, no eye lenses)

after handling chemicals wash your hands with soap and water.

During lab work keep your hands away from your face.

tie back long hair.

Know the location of the fire extinguisher, fire blanket, eyewash station, and first aid kit.

It is suggested that you wear glasses rather than contact lenses.

Take to the lab station only what is necessary.

Never eat or drink during a lab work.

role up loose sleeves

Keep your work area uncluttered.

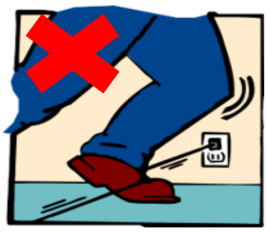
Safety with laboratory equipments:

Never use any laboratory equipment unless you are trained & have been authorised to do so

As well as injuring yourself you may cause very costly damage

Electrical safety :

1)Lay electrical cords where no one can trip on them
2)Be sure your hands and your lab area are dry before using electrical equipment
3)Unplug cords by pulling the plug and not the cord

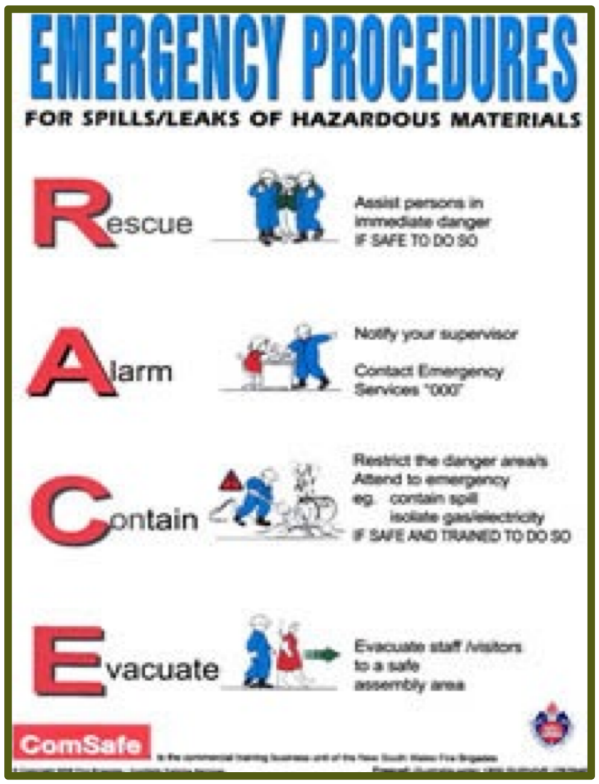


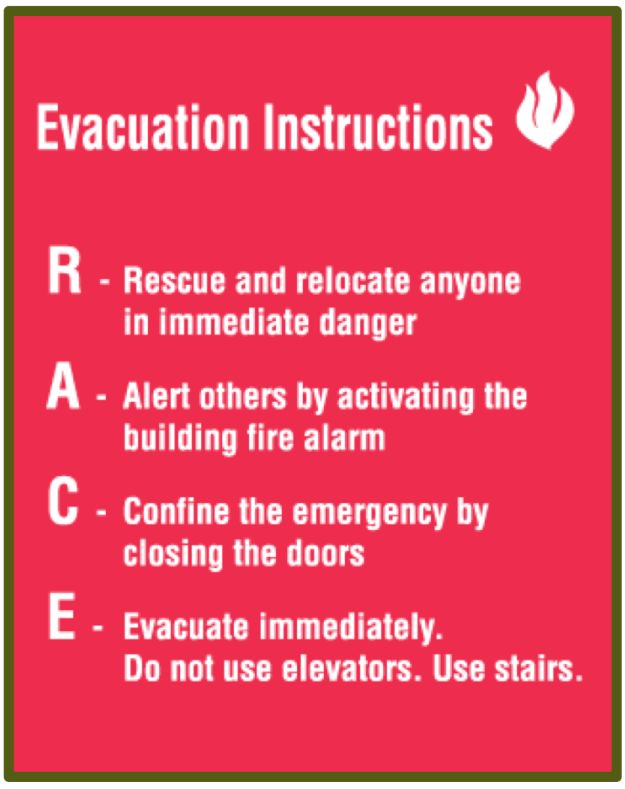
biological safety:

All biological samples are considered potentially infectious → should be handled and processed using strict precautions

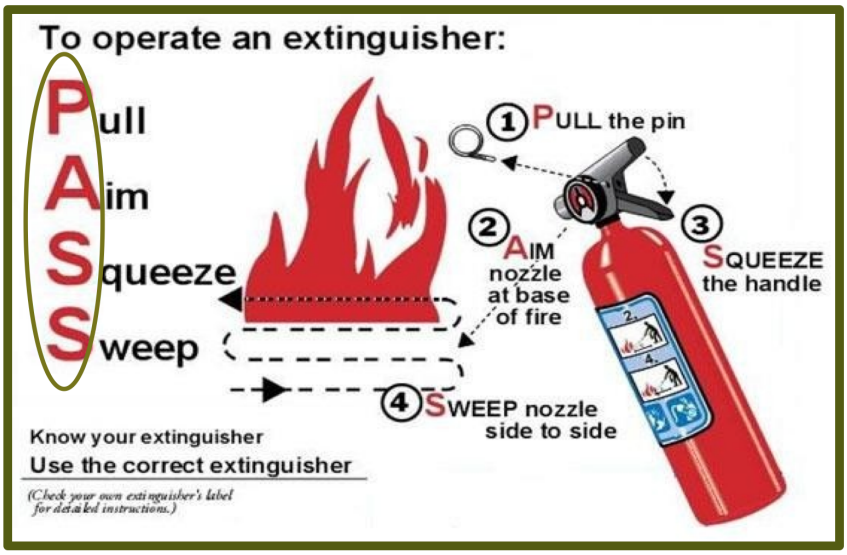
Emergency procedures:

The emergency procedure is a plan of actions to be conducted in a certain order or manner, in response to an emergency event.





Fire extinguisher (طفاية الحريق):



Waste disposal :

Regular waste like papers use containers with black/white plastic bags

Sharp objects such as needles,scalpels and even broken glassware yellow-red sharps container

Contaminated waste use containers with yellow plastic bags

باختصار الجهاز فيه لمبه تسلط الضوء على أنبوبة العينة اللي فيها المادة المراد قياس تركيزها طبعا كل ماده لها لون معين و كل لون له طول موجي معين لما يقع الضوء الابيض على المحلول يمتص جميع الاطوال الموجية ما عدا الخاصة بلونه و يرجع يعكسها من الناحية الأخرى اللي بيكون فيها مستقبل لهذا الضوء و بعمليه حسابيه خاصه يقوم بها الجهاز يعطي الطول الموجي للضوء المنعكس ثم بعمليه حسابيه او بالرسم البياني مع التركيز يعطيكي تركيز المادة الموجودة في الأنبوب

Spectrophotometer:

Most of visible spectrophotometers are composed of:

-Light source which works with visible wavelengths (400-700) nm

-Monochromator filter for choosing desired wavelength

-Sample holder (cuvette)

-Detector

-Meter or recorder

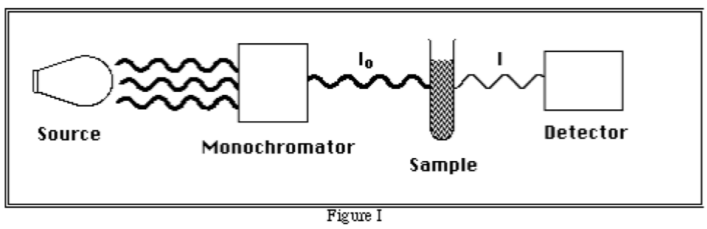


Figure I

Lab equipments:

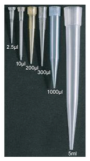
Autonomic pipettes

vortex

Eppendorf tube

Microcentrifuge

UV-spectrophotometer



Tips

Water bath

Cuvettes

Rack- eppendorf tube

Rack- test tube

The principle of this experiment:

1- Lysis of cells.

2- Removal of contaminants.

3- Isolation of pure DNA.

DNA isolation and purification : Important!!

The application of this experiment:

1- Molecular diagnosis of diseases (e.g. sickle cell anemia)

2- Forensic applications (e.g. paternity testing)

3- Molecular Biology research and techniques: Very important!!

a. Amplification techniques: PCR (Polymerase chain reaction)

is a technology in molecular biology used to amplify a single copy or a few copies of a piece of DNA generating thousands to millions of copies of a particular DNA sequence.

b. Southern blotting: detecting specific genes by hybridization between target DNA sequence and the labeled probe

Hybridization of the probe to a specific DNA fragment on the filter membrane indicates that this fragment contains DNA sequence that is complementary to the probe.

c. RFLP (restriction fragment length polymorphism): digestion of DNA by specific enzymes and separation of DNA fragments by electrophoresis.

In RFLP analysis, the DNA sample is broken into pieces and (digested) by restriction enzymes and the resulting restriction fragments are separated according to their lengths by gel electrophoresis.

Determination of purity and concentration of DNA : Very important!!

1) **Purity of DNA solution** Determine A260/A280 ratio by spectrophotometer : 1.7-1.9 is accepted

2) **Concentration of DNA** (µg/ml) :

Provided : 50 µg/ml when A260=1.0

How to calculate unknown concentration of DNA:

A260=1.0 DNA concentration = 50 µg/ml

A260=2.33 DNA concentration = ?

DNA concentration = 2.33 x 50 / 1 = 116.5 µg/ml

ركزوا ع الوحدات

3) **Yield of DNA** (µg) :

DNA concentration x Total volume of DNA (Total Sample Volume)

= 116.5 µg/ml x 0.2 ml

= 23.3 µg

What do we mean by A 260/280 ratio? when we did the practical, the spectrophotometer sends UV light and the DNA absorbs it at 260 nm and the proteins absorb the UV light at 280 nm so let's say that the UV light absorbed at 260 nm is 2.33 and at 280 nm is 1.3 so **the ratio is: the absorbance at 260 nm / the absorbance at 280 nm** So 2.33/1.3= 1.79

ملاحظة : أي شي باللون الرصاصي إضافة لزيادة الفهم

Lab orientation objectives :

The students should be able to understand and become familiar with :

1)General safety rules followed in Biochemistry laboratory

2)Safety with laboratory equipments

3)Basic emergency procedures

4)biological safety and waste disposal

5)The basics of spectrophotometer and general equipments to be used in the lab during Biochemistry practical sessions

DNA extraction and purification :

The students should be able to understand and become familiar with :

1)Understand and follow the policy and procedure for lab safety
2)Understand the principle for extraction and purification of DNA
3)Determine the purity and concentration of the isolated DNA
4)Identify different applications and uses of purified DNA