Biochemistry orientation lab L 1

DOCTOR'S NOTE

VERY IMPORTANT!

EXTRA INFORMATION

OBJECTIVES

The students should be able to understand & 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

GENERAL SAFETY RULES

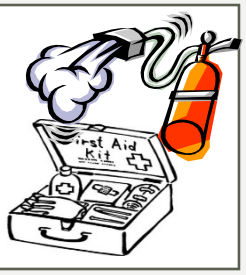
VERY IMPORTANT

- 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, always wash your hands with soap and water.
- During lab work, keep your hands away from your face.

Tie back long hair.



- Roll up loose sleeves.
- Know the location of the fire extinguisher, fire blanket, eyewash station, and first aid kit.
- Keep your work area uncluttered. Take to the lab station only what is necessary.

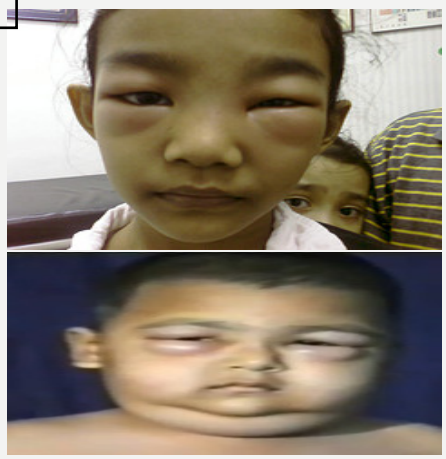
- It is suggested that you wear glasses rather than contact lenses.
- Never eat or drink during a lab work.

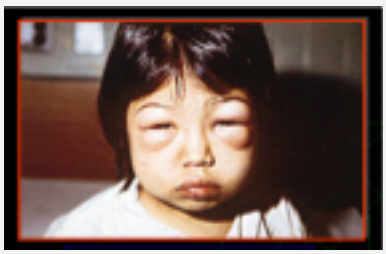
*Just read it

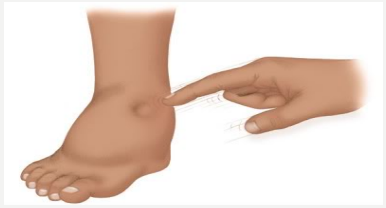
CLINICAL BIOCHEMISTRY FOR DIAGNOSIS OF DISEASES

- Biochemical laboratory tests are crucial tools for diagnosis of many human diseases:

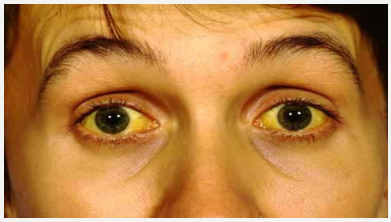
Kidney diseases e.g., nephrotic syndrome



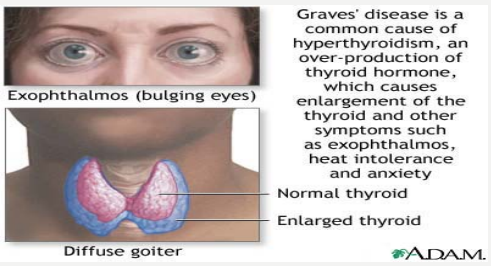


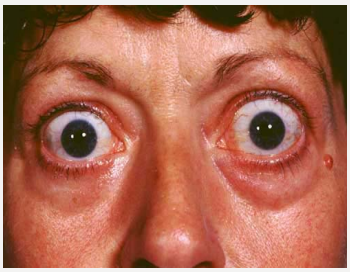


Liver diseases e.g., hepatitis and jaundice



Endocrine diseases e.g., Thyrotoxicosis





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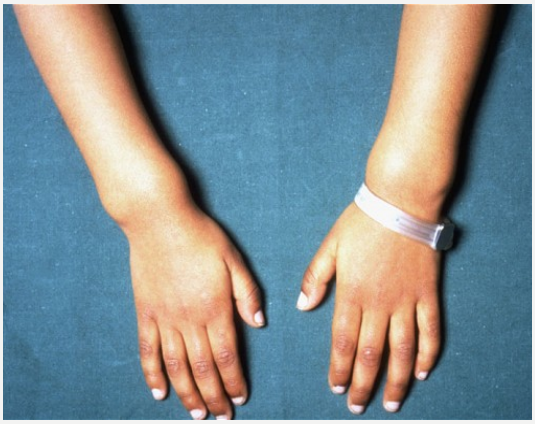
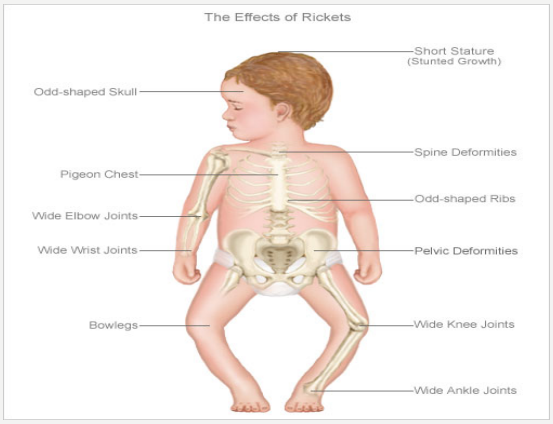
Cancers & malignancy e.g., prostate cancer.

Inherited diseases e.g., PKU

Metabolic diseases e.g., diabetes mellitus

Skeletal diseases e.g., rickets





Very important!!

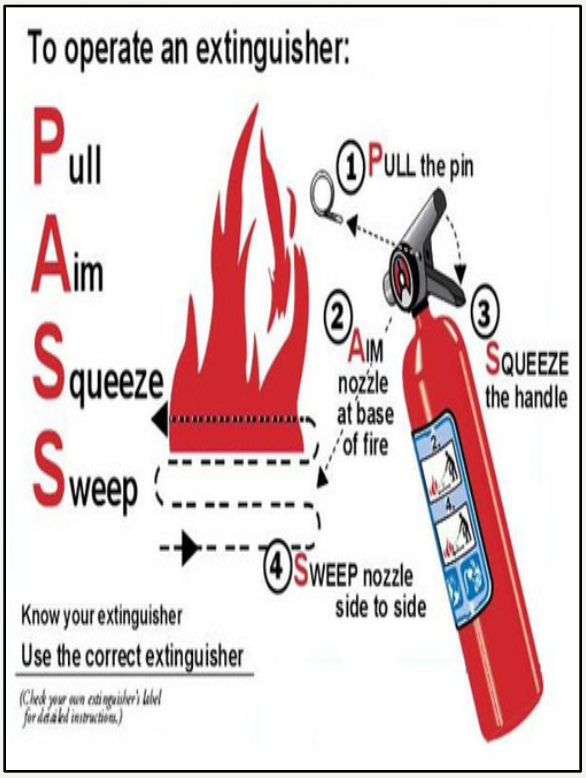
Safety with laboratory equipments:

Never use any laboratory equipment unless you are trained & have been authorized to do so As well as injuring yourself you may cause very costly damage

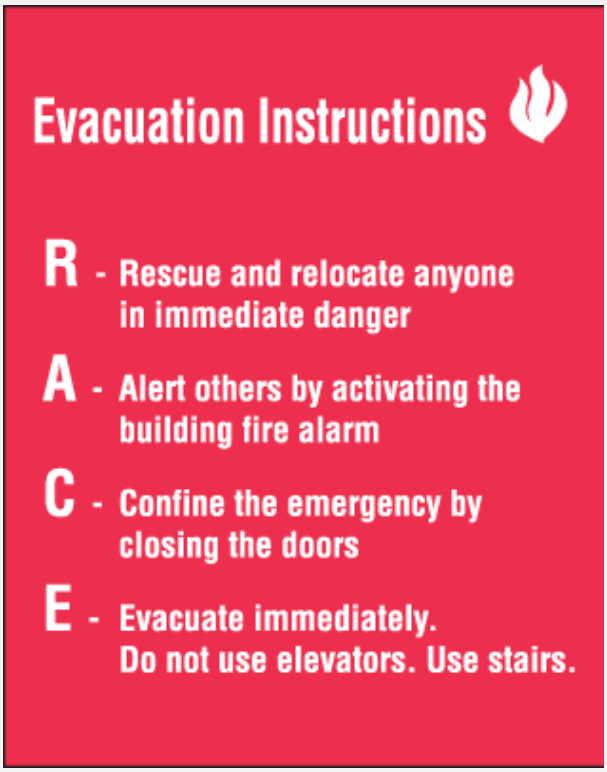
Electrical safety:

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

Fire extinguisher



Emergency procedures



Very important!!

Biological safety:

All biological samples are considered potentially infectious
Should be handled and processed using strict precautions.

Waste disposal :

All sharp objects such as needles, scalpels and even broken glassware go into yellow-red sharps container

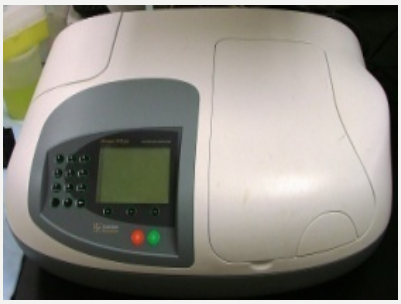
For disposal of contaminated waste, use containers with yellow plastic bags

Regular waste like papers etc go into containers with black/white plastic bags

*Just read it

LAP EQUIPMENT

Microcentrifuge



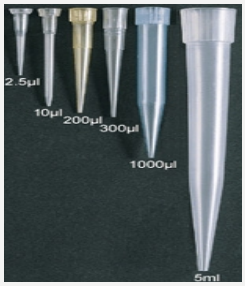
UV-spectrophotometer

Water bath

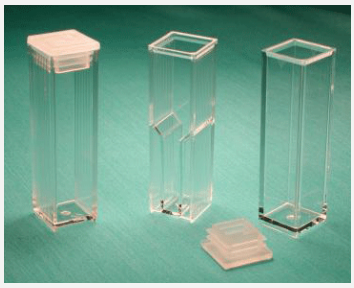
Vortex

Automatic pipettes

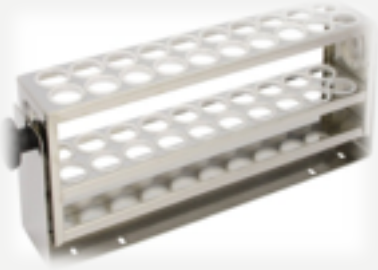
Eppendorf tube



Tips



Cuvettes



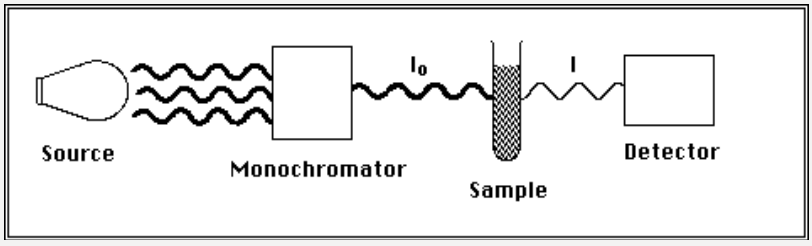
Rack- test tube

Rack- eppendorf tube

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
- UV light



- **Sample : Nucleated cells**

- **Principle:**

1. **Lysis of nucleated cells.**

2. **Removal of contaminants:** Any substance other than DNA, e.g., proteins

3. **Measurements:** UV absorbance at 260nm and 280nm

Purity of DNA solution: 260/280 ratio

DNA concentration: Absorbance at 260nm

- **Steps:** **memorize them in order**
  - **Lysis** of nucleated cells using lysis buffer.
  - **Binding** of DNA to the membrane of spin column.
  - **Wash:** using wash buffer.
  - **Elution** of pure DNA

MEASUREMENTS NOTICE THAT WE HAVE 3 MEASUREMENTS ALL OF THEM ARE IMPORTANT!

- Measure the Absorbance at **260nm (is for DNA)**
- Measure the Absorbance at **280nm**.(is for protein)

- **Assess the DNA purity:** 260/280 ratio

  (Accepted ratio: **1.7 - 1.9**) No unit

- **Calculate DNA Conc.:** Provided A260 = 1.0, DNA is 50 µg/ml, unknown DNA Conc. can be calculated by cross multiplication

A260 = 1.0	DNA conc. = 50 µg/ml
A260 = 0.5	DNA conc. ?

Note:- In case of diluting the eluted sample, multiplies the final concentration by the dilution factor.

This can be adjusted by the spectrophotometer.

- pure sample of DNA has a range of 1.7 to 1.9 at 260/280 and is relatively free from protein contamination .
- A DNA preparation that is contaminated will have a 260/280 ratio lower than 1.7

1.DNA purity:

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

So 2.33/1.3= 1.79

*Team 36

المعطيات اللي راح تكون في السؤال:
قيمA260
الثابت يحفظ
A260=1.0 يقابله DNA concentrations =50 ug/ml
المطلوب :
Concentration of A260 اللي أعطيت بالسؤال
الوحدة * µg/ml
المقصود بDilution
مثلاً يقولكIt's diluted twice
تروح تأخذ قيمة الConcentration اللي طلعتها
وتكتب الوحدة 2تضربها في
قال لكDiluted 3 times
تضرب في ٣ وتطلع الجواب والوحدة

DNA YIELD

*Very important

DNA Yield = DNA Volume x final DNA Conc.

Example:

If you have

Volume of DNA solution: 200µl (0.2 ml)

The volume given in µl so you will divide it by 1000

هنا ممكن تتسألون هل قيمة التركيز التي تضرب في الحجم احط القيمة اللي قبل مانضرب في عامل التخفيف

ولا بعد الضرب ؟ ع حسب اذا أصلاً جاء لك بالاختبار انه تخفف حط القيمة بعد التخفيف وتضرب بالحجم في حال لم يذكر أنه تخفف بتحط التركيز الأصلي وتضرب في الحجم

Final DNA Conc.: 30 µg/ml

Then, the yield (µg) = 0.2 ml x 30 µg/ml

= 6.0 µg

هنا كيف صارت µg

شطبتml مع **ml**

ضروري كتابة الوحدة

Very important!!

DNA APPLICATIONS

Purified DNA can be used for:

1. Molecular diagnosis of diseases.	2. Forensic applications.	3. Molecular biology research.
• (e.g., sickle cell anemia)	• (e.g., paternity testing)	

Molecular techniques using purified DNA:

a. Amplification techniques:	b. Southern blotting.(DNA) Northern Blotting(ss mRNA) Western Blotting (Protein)	c. Restriction Fragment length polymorphism (RFLP).
Polymerase **C**hain Reaction (PCR)		

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WISHING YOU THE BEST OF LUCK ...