جامعة الملك سعود
King Saud University
1957

Biochemistry team 436

Biochemistry Lab Orientation

-Doctor’s slides

-Extra notes

Lab orientation objectives

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

-General safety rules followed in Biochemistry laboratory

-Safety with laboratory equipments

-Basic emergency procedures

-Biological safety and waste disposal

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

General safety rules

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

1. Always use appropriate clothes and personal protective tools (Lab coat, safety goggles, masks, gloves, no open shoes, no eye lenses)
2. After handling chemicals, always wash your hands with soap and water.
3. During lab work, keep your hands away from your face.
4. Tie back long hair.
5. Roll up loose sleeves.
6. Know the location of the fire extinguisher, fire blanket, eyewash station, and first aid kit.
7. Keep your work area uncluttered. Take to the lab station only what is necessary.
8. It is suggested that you wear glasses rather than contact lenses.
9. Never eat or drink during a lab work.

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 equipments.
3. Unplug cords by pulling the plug not the cord.

Emergency procedure:

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

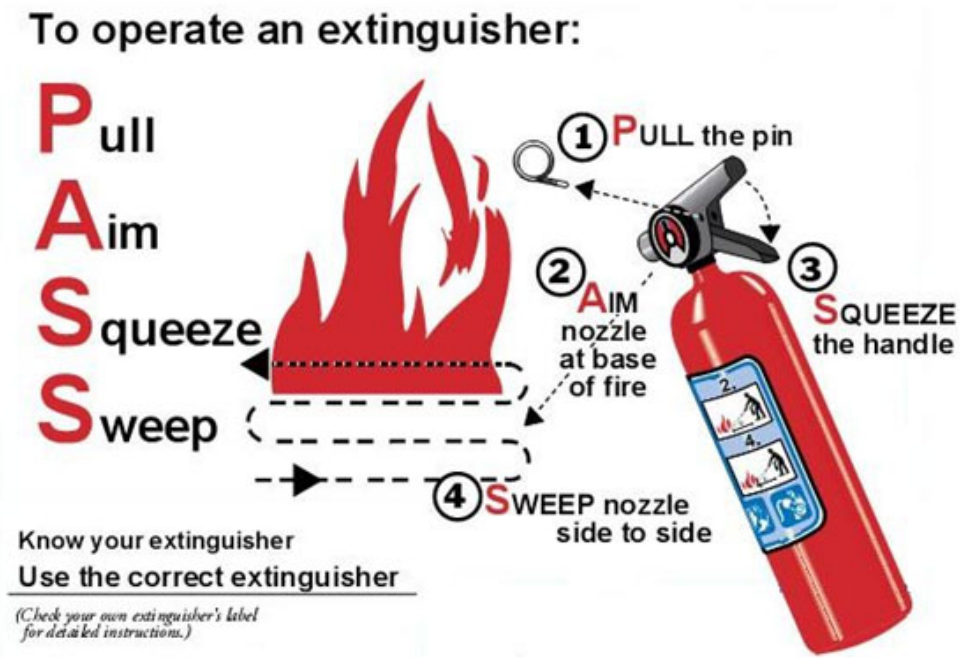
R - Rescue and relocate anyone in immediate danger .

A – Alert others by activating the buliding fire alarm .

C – Confine the emergency by closing the doors .

E – Evacuate immediately do not use elevators , use stairs .

Fire extinguisher:



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.

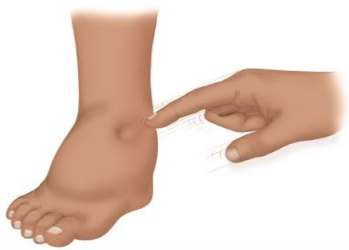
Regular waste like papers go into containers with black/white plastic bags.

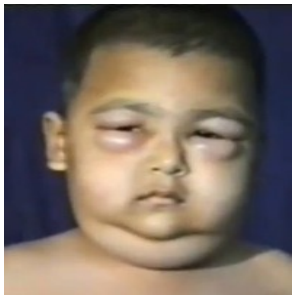
For disposal of **contaminated waste**, use containers with yellow plastic bags.

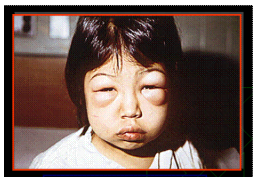
Clinical Biochemistry for Diagnosis of Diseases

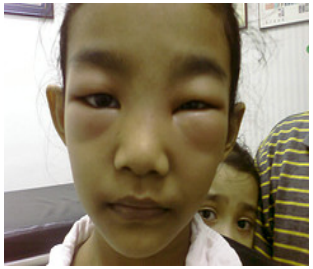
Biochemical laboratory tests are crucial tools for diagnosis of many human diseases:

Kidney diseases e.g., nephrotic syndrome

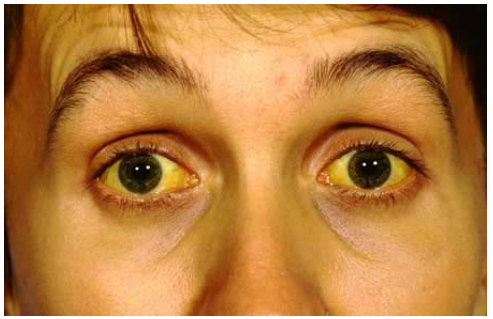








Liver diseases e.g., hepatitis and jaundice

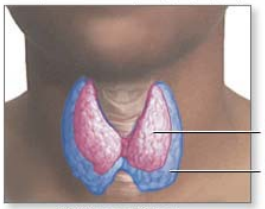


Metabolic diseases e.g., diabetes mellitus

Cancers & malignancy e.g., prostate cancer

Endocrine diseases e.g., Thyrotoxicosis

Exophthalmos (bulging eyes)



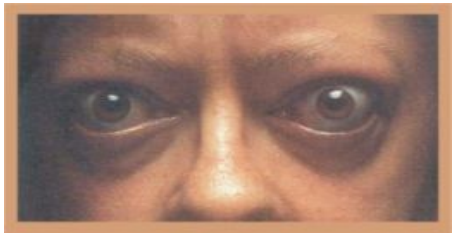
Diffuse goiter

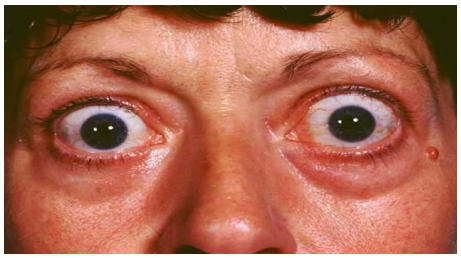
Graves' disease is a common cause of hyperthyroidism, an over-production of thyroid hormone, which causes enlargement of the thyroid and other symptoms such as exophthalmos, heat intolerance and anxiety

Normal thyroid

Enlarged thyroid

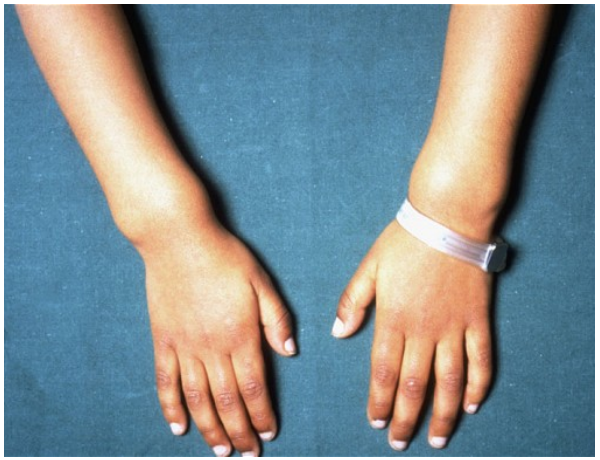
ADAM.

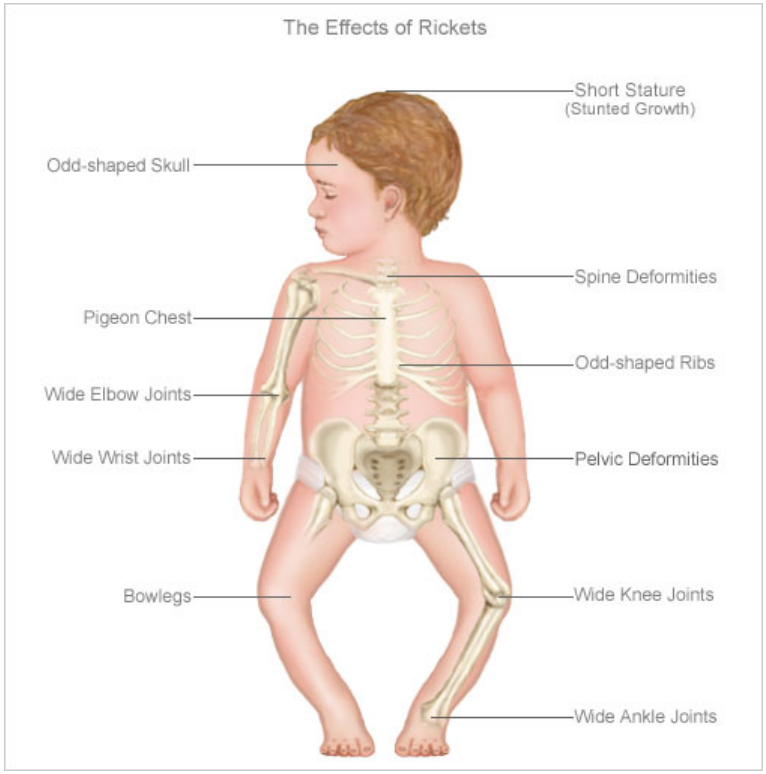




Inherited diseases e.g., (PKU) Phenylketonuria

RICKETS




The Effects of Rickets
Short Stature
(Stunted Growth)
Odd-shaped Skull
Spine Deformities
Pigeon Chest
Odd-shaped Ribs
Wide Elbow Joints
Wide Wrist Joints
Pelvic Deformities
Bowlegs
Wide Knee Joints
Wide Ankle Joints

Lap Equipment

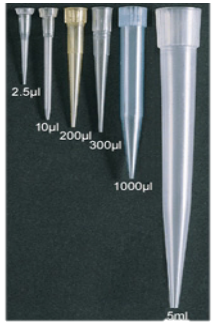
Automatic pipettes

Water bath

Eppendorf tube

Cuvettes

Tips



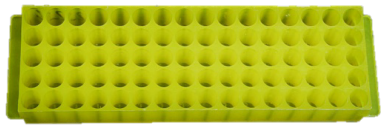
Vortex

Microcentrifuge

UV-spectrophotometer

Rack- test tube

Rack- eppendorf tube



spectrophotometers

spectrophotometers are composed of:

- Light source which works with visible wavelengths (400-700 nm
- momochromatorfilter for choosing desired wavelength
- Sample holder (cuvette)
- Detector
- Meter or recorder
- UV light

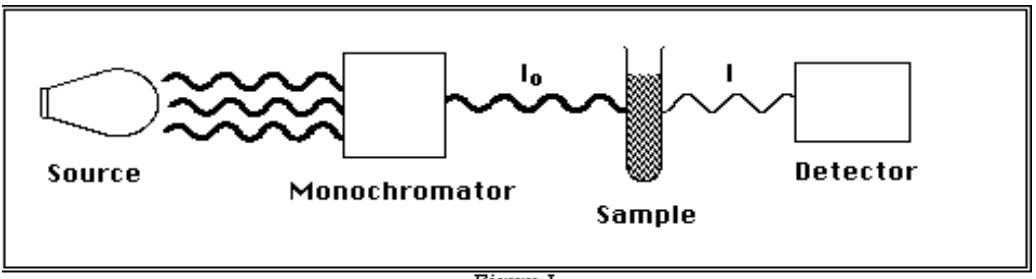


Figure I

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

Principle:

1-Lysis of nucleated cells

2-Removal of contaminants: Any substance other than DNA , e.g. Proteins

3-Measurement : UV absorbance at 260nm and 280nm

Purity of DNA solution : 260/280 ratio

DNA concentration : Absorbance at 260nm

Steps:

- **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:

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

- 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

Buffers : it makes the DNA more clear.
e.g. Lysis buffer: buffer AL
Wash buffer 1: AW1
Wah buffer 2 : AW2

DNA exraction 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

Molecular techniques using purified DNA:

A. **Amplification techniques:** PCR (Polymerase chain reaction)

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

C. Restriction Fragment Length Polymorphism (RFLP) : digestion of DNA by specific enzymes and separation of DNA fragments by electrophoresis.

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

Done by the spectrophotometer

1) **Assess the DNA purity :** Determine A260/A280 ratio
by spectrophotometer : 1.7-1.9 is accepted

2) **calculate DNA conc.** (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

It is known that when A260= 1 then the DNA concentration is 50 µg/ml so when A260 = 2.33 then the concentration is 116.5 µg/ml by cross multiplication.

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

So the Concentration is 116.5 µg/ml what if we have 0.2 ml how are we going to get the yield? So we multiply DNA concentration X the total volume of DNA so the result will be = 23.3 µg **BECAREFUL ABOUT THE UNITS.**

*= it could be any number , they will give the number in the exam

1.DNA purity:

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

thank you

girls team:
Atikah kadi
Heba Alnasser
Reem Alshathri

boys team:
Khalid Al-Qahtani
Hamad AlHumaidan