MED439
KING SAUD UNIVERSITY

OSPE

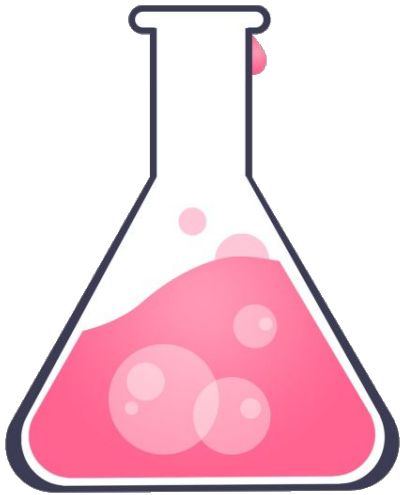
Biochemistry

The instructions for the OSPE

-Most likely it will be one station in the exam for biochemistry
-you have about 3 -4 mins per station
-**they have their own calculator**

EDITING FILE

Lab orientation



Color Index :

- important
- Examples of question may come
- ★

OBJECTIVES

You should be familiar with :

- General safety rules followed in biochemistry laboratory.
- Safety with laboratory equipment.
- Basic emergency procedures.
- Biological safety and waste disposal
- The basics of spectrophotometer and general equipment to be used In the lab during Biochemistry Practical sessions

General safety rules

Always wear appropriate clothes and personal protective tools.
-lab coat
-safety goggles
-masks
- gloves
-No opens shoes
-No eye lenses.


-Always wash your hands with soap and water after handling chemicals.

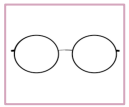
-during lab work, keep your hands away from your face.

-tie back long hair



-Roll up long 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's necessary .


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



-Never eat or drink during a lab work.

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

Safety with laboratory equipment:

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

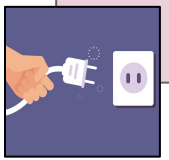
As well as injuring yourself you may cause very costly damage

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.



*Note: example for questions
What does RACE&PASS stand for?

Fire safety - R.A.C.E:

Procedures to follow in the event of a fire emergency

R – Remove or secure individuals in immediate danger.

A – Activate the alarm by pulling a fire pull station located in the corridors and calling 953.

C – Confine the fire by closing windows, vents, and doors.

E – Evacuate to a safe area

Fire safety -Extinguisher P.A.S.S:

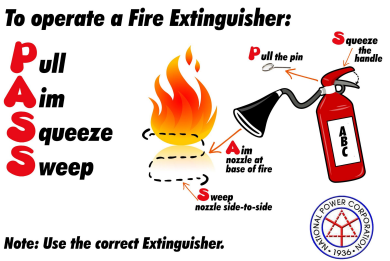
To operate an extinguisher

P – Pull

A – Aim.

S – Squeeze

S – Sweep



Note: Use the correct Extinguisher.

BIOLOGICAL SAFETY :

All biological samples are considered potentially infectious

Should be handled and processed using strict precautions

WASTE DISPOSAL :

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

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

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

Clinical biochemistry laboratories:

- Routine Biochemistry / STAT Bench Lab
- Endocrinology Lab
- Inherited Metabolic Lab
- Toxicology Lab
- Newborn screening lab
- Receiving Bench

Biochemical test profiles

Cardiac Profile
- -S.Creatinine kinase
- -S.Lactate dehydrogenase
- -S.Troponin

Renal Profile
- -Blood urea
- -S.Creatinine
- -S.Electrolytes (Na , K & Cl)

Hepatic Profile
- -S.Total proteins
- -S.Albumin
- -S.Alanine & Aspartate aminotransferases (ALT & AST

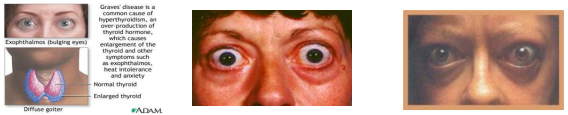
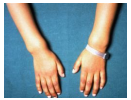
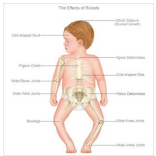
Lipid Profile
- -S.Triglycerides
- -S.Cholesterol
- -S.HDL-cholesterol
- -S.LDL- cholesterol

Bone Profile
- -S. Calcium
- -S. Phosphorus
- -S. Alkaline Phosphatase
- -S. Vitamin D

Glucose (diabetic) Profile
- -S. Fasting glucose
- -S. 2 hours postprandial glucose
- -S. Random glucose
- -S. Glycosylated hemoglobin

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 	Endocrine diseases e.g., thyrotoxicosis 
Cancer & malignancy e.g., prostate cancer 	Inherited e.g., PKU  
Skeletal disorder e.g., Rickets  	

Lap equipments

Automatic pipettes

UV- spectrophotometer

Eppendorf tube

Rack - test tube

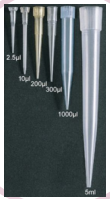
Vortex

Cuvettes

Water bath

Rack - eppendorf tube

Microcentrifuge



Tips

Spectrophotometer

Most of visible spectrophotometer 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

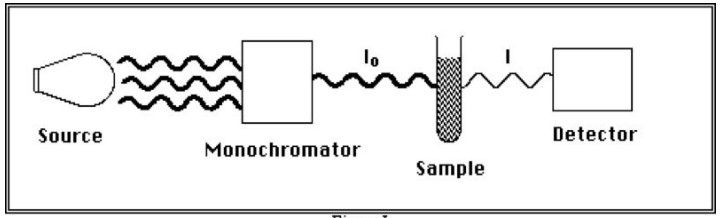
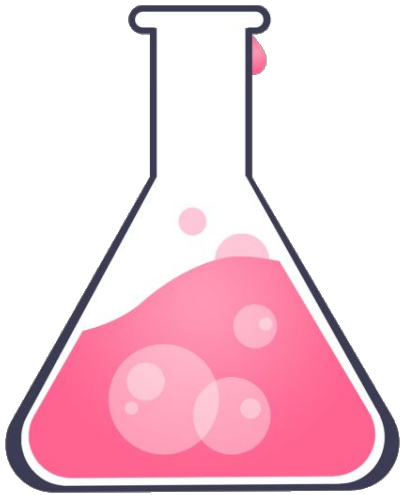


Figure I

DNA EXTRACTION



Color Index :

- important
- Examples of question may come
- ★

OBJECTIVES

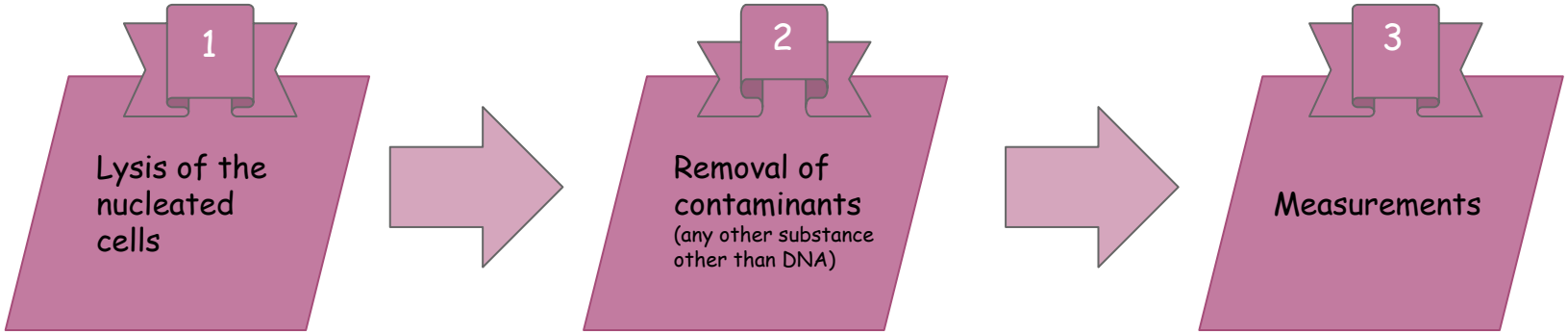
- Understand the principle behind DNA extraction and purification.
- Perform DNA extraction, purification and measurement according to the provided protocol (spin protocol).
- Interpret the results in terms of quantity, purity and yield.
- Have a knowledge about some molecular techniques and applications.

Principles

Genomic DNA is extracted from peripheral blood samples preserved in EDTA using QIAamp DNA Blood Mini Kit, spin protocol.

The principle of the test includes lysis of the nucleated cells using lysis buffer, which has high salt concentration that breaks the cellular membrane; after the lysing step, DNA is allowed to bind to the spin column membrane for separating the DNA from the cell debris; removal of the contaminants with wash buffers; and elution of pure DNA. The measurement of the purified DNA is performed by UV absorbance at 260nm and 280nm. DNA concentration is determined by measuring at 260nm, and the purity of the purified DNA is determined on the bases of 260nm/280nm ratio. A pure DNA falls in the accepted ratio, which ranges from 1.7 up to 1.9.

Thanks to Med438



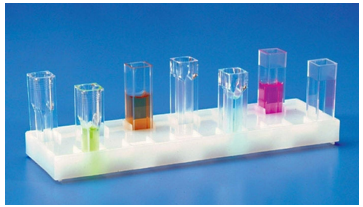
Lab equipments

Lab equipments.ال

Tools

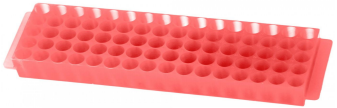
Eppendorf tube

Tips



Rack testtube

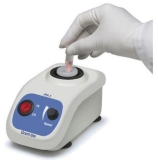
Rack Eppendorf tube



Automatic pipettes

Machines

Micro centrifuge



Vortex

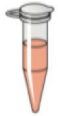
Water bath

UV Spectrophotometer

Steps

1. Lysis of nucleated cells using lysis buffer.
2. Binding of DNA to the membrane of spin column
3. Wash: using wash buffer.
4. Elution of pure DNA.

Spin Protocol of DNA Purification from Blood

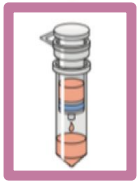


1. Pipette **20µl** protease.
2. Add **200µl** sample.
3. Add **200µl** Buffer **AL**.

protease.1,2,3 يكون موجود بالنبوب جاهز نضيف عليه عينة الدم ثم buffer AL

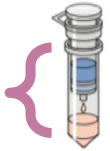
Water bath

4. Mix by pulse-vortex for 15s and incubate at **56°C** for **10 minutes**.
5. Briefly centrifuge.
6. Add **200µl** 96-100% ethanol and mix by pulse-vortex for 15s.
7. Briefly centrifuge.



Use Pipettes

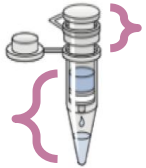
8. Apply the mixture to the Min spin column.
9. Centrifuge at **8000 rpm** for **1 minute**.



10. Discard the collection tube.
11. Place the column in a new 2ml collection tube.
12. Add **500µl** buffer **AW1**.
13. Centrifuge at **8000 rpm** for **1 minute**.

14. Place the column in a new 2ml collection tube.
15. Add **500µl** buffer **AW2**.
16. Centrifuge at **14000 rpm** for **3 minutes**.
17. Place the column in a new 2ml collection tube.
18. Centrifuge at **14000 rpm** for **1 minute**.

skip



19. Place the column in a new 1.5ml tube.
20. Add **200µl** buffer AE.
21. Incubate at room temperature for **1 minute**.
22. Centrifuge at **8000 rpm** for **1 minute**.

Add 400µl buffer AE to dilute the eluted DNA (3X dilution)

23. Quantify the DNA concentration.

After step 22. remove the upper part then add buffer AE to

After adding the buffer AE put the mixture in cuvettes tube by pipettes then into UV-spectrophotometer then do step 33

QUANTIFICATION OF THE PURIFIED DNA

measurements:

- Measure the Absorbance at 260nm and at 280nm.
- **Assess the DNA purity**= 260/280 ratio.
  (Accepted ratio: 1.7 - 1.9)

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

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

القاعده باختصار و ثابته: 3 x50µg/ml القراءة عند 260nm = DNA concentration

اذا قالوا 3 times dilution
يعني اضرب ب ٣ بس

Note: In case of diluting the eluted sample, multiplies the final concentration by the dilution factor. This can be adjusted by the spectrophotometer.

DNA YIELD:

DNA Yield (µg) = DNA Volume x final DNA Conc.

مو شرط يكون 0.2 بس على الغالب يكون هو

باختصار وثابت: =0.2ml x concentration µg/ml

يكون µl نحوله ل ml
بإننا نغير الفواصل مثلا
200µl > 0.2ml
ضروري نسوي هالتحويله

Example:

If you have
- **Volume of DNA solution:** 0.2 ML (200 microliter)
- **Final DNA Conc.**: 30µg/ml

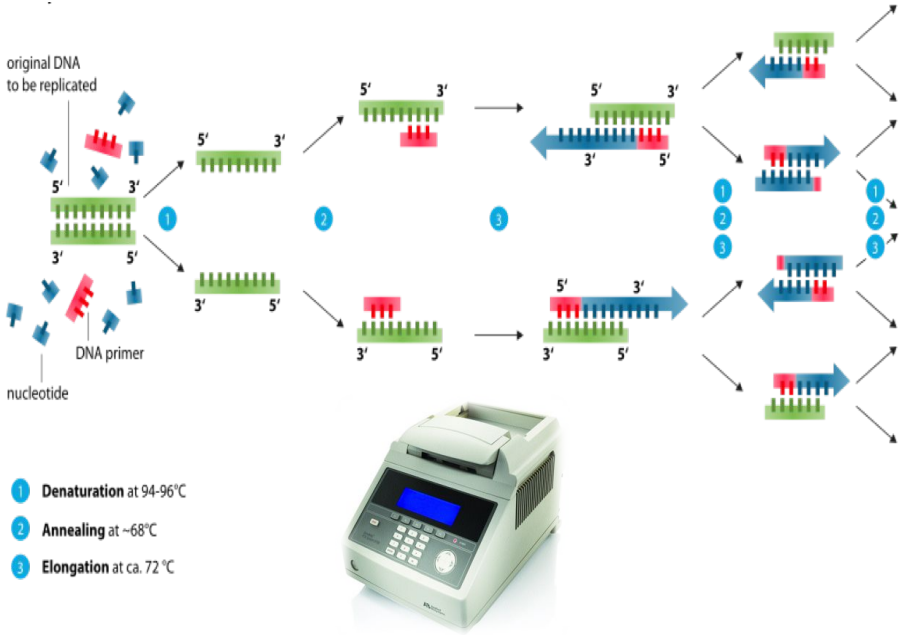
Then, the yield (µg) = **0.2ml x 30** µg/ml
= **6.0** µg

Molecular Technique and Applications

Note:- Almost all molecular biology techniques can be utilized for diagnosis and research

a. Amplification techniques:

e.g. Polymerase Chain Reaction (PCR)



Applications of PCR:

- Comparison of a normal gene with a mutant form of the gene.

- Detection of low-abundance nucleic acid sequences.

- Forensic analysis of DNA samples.

- Prenatal diagnosis.

SUMMARY

1 How to calculate the purity ?

260nm/280nm ratio

Example:
If you have
- **A260 = 0.50** **-A280 = 0.35**
Then
Answer: 0.50/0.35 = 1.428 (it's not pure because DNA ratio should be between 1.7-1.9)

2 How to calculate DNA conc ?

260nm x 50

Example:

If you have
- A260 = 0.50
Then
Answer: 0.50 x 50 = 25 µg/ml

3 How to calculate DNA yield ?

0.2 x final DNA conc

Example:

We will use the conc for the previous question
- conc = 25 µg/ml
Then
Answer: 0.2ml x 25 µg/ml = 5 µg

QUIZ

The answers

★ Explain what does PASS represent ?

★ Identify the devices in the picture ?

★ Calculate the DNA conc & the purity ?

If A260 = 0.5 , A280 = 0.29

★ Calculate the DNA yield

-If the volume of DNA solution 200µl (0.2 ml)
-final DNA conc 20µg/ml

Team members

Biochemistry team wishes you all the best

Girls Team :

1. Alia Zawawi
2. Nada Babilli
3. Rania Aqil
4. Reem alamri
5. Reema Alomar
6. Reem Alqahtani
7. Renad Alhumaidi
8. Samar Almohammedi
9. Shaden Alobaid
10. Budoor Almubarak
11. Sumo Abdulrahman
12. Noura Alsalem
13. Lama Alahmadi
14. Sadem Alhazmi

- Nuha Alkudsi
- Norah Alsheikh
- Muneerah Alssdhan
- Mayasem Alhazmi

Boys Team :

- Mansour albawardi
- Hassan alshurafa
- Abdulrahman almbki
- Mohammed alsayari
- Abdullaziz alomar
- Nawaf alghamdi
- Abdulaziz alrabiah
- Saud alrasheed
- abdullah almazroo
- Hamad almousa

Team leaders

- Shatha Aldhohair
- Mishal Althunayan

Made by :

Biochemistry439@gmail.com