

# BIOCHEMISTRY PRACTICAL SESSIONS



## ESTIMATION OF BLOOD GLUCOSE IN DIABETES MELLITUS

❖ **Important**

❖ Extra

❖ Biochemistry Edit

# INTRODUCTION

- ❖ **Blood glucose** is normally regulated and maintained within a narrow range under various conditions by hormones e.g. **insulin, glucagon**.
- ❖ **Diabetes mellitus**: The most common disorder of carbohydrate metabolism characterized by **high blood glucose level (Hyperglycemia)** i.e. unbalance of this regulation.
- ❖ **Other signs and symptoms of diabetes:**
  - ✓ Polyuria and glucosuria
  - ✓ Polydipsia
  - ✓ Polyphagia
- ❖ Diabetes has to types:



	Type 1 Diabetes	Type 2 Diabetes
AGE OF ONSET	Usually during childhood or puberty; symptoms develop rapidly	Frequently after age 35 years; symptoms develop gradually
NUTRITIONAL STATUS AT TIME OF DISEASE ONSET	Frequently undernourished	Obesity usually present
PREVALENCE	<10% of diagnosed diabetics	>90% of diagnosed diabetics
GENETIC PREDISPOSITION	Moderate	Very strong
DEFECT OR DEFICIENCY	$\beta$ Cells are destroyed, eliminating production of insulin	Insulin resistance combined with inability of $\beta$ cells to produce appropriate quantities of insulin
FREQUENCY OF KETOSIS	Common	Rare
PLASMA INSULIN	Low to absent	High early in disease; low to absent in disease of long duration
ACUTE COMPLICATIONS	Ketoacidosis	Hyperosmolar hyperglycemic state
RESPONSE TO ORAL HYPOGLYCEMIC DRUGS	Unresponsive	Responsive
TREATMENT	Insulin is always necessary	Diet, exercise, oral hypoglycemic drugs, insulin (may or may not be necessary); reduction of risk factors (weight reduction, smoking cessation, blood pressure control, treatment of dyslipidemia) is essential to therapy

# LABORATORY TESTS FOR GLUCOSE

**Normal blood glucose range: 3.9–5.6 mmol/L (70–100 mg/dL )**

**Fasting plasma glucose:** is measurement of plasma glucose after 12 hours of fasting (no caloric intake).

**OGTT<sup>1</sup> and 2-hour post-prandial test:** Serial measurement of plasma glucose before and after a specific amount of glucose given orally (75g glucose).

**HEMOGLOBIN A<sub>1C</sub> measurement:** used to estimate glycemic control in the last 1-2 months & Recommended for the detection of type 2 DM.

**Random plasma glucose:** in Hyperglycemic crisis & In a patient with classic symptoms of hyperglycemia.

- ✓ Measurement of blood glucose is one of the most commonly performed tests in clinical biochemistry labs of hospitals.
- ✓ Hemoglobin A<sub>1C</sub> is produced due to non-enzymatic glycosylation of hemoglobin.
- ✓ HBA<sub>1C</sub> with a **Cut-off point of  $\geq 6.5\%$**  is used to diagnose diabetes.
- ✓ HBA<sub>1C</sub> + **fasting plasma glucose** are effective in **diagnosing diabetes**.

1. OGTT: Oral Glucose tolerance Test

NOTE: in OGTT we give the patient a known amount of glucose "75g" but in 2-hour post-prandial test the amount of Glucose given is unknown; u just ask the patient to eat then measure his blood glucose after 2hs.

# DIAGNOSIS OF DM

## ❖ ADA CRITERIA FOR DIAGNOSIS OF DM: IMPORTANT

	Pre-Diabetes	Diabetes
FPG*	100-125 mg/dL 5.6 mmol/L – 6.9 mmol/L	126 mg/dL and over 7.0 mmol/L and over
OGTT* after 75 g glucose load	140 mg/dL - 199 mg/dL 7.8 mmol/L – 11.0 mmol/L	200 mg/dL and over 11.1 mmol/L and over
A1c	5.7 % - 6.4%	6.5% and over
Random plasma glucose	—	200 mg/dL and over 11.1 mmol/L and over + signs and symptoms of hyperglycemia

## Q/ WHAT IS PreDiabetes “IMPAIRED GLUCOSE TOLERANCE”?

- ❖ When a person fasting plasma glucose or 2-hour postprandial glucose level is above normal but below diabetic level.

## ❖ KETONE BODIES

Produced by the liver and utilized for energy production by peripheral tissues

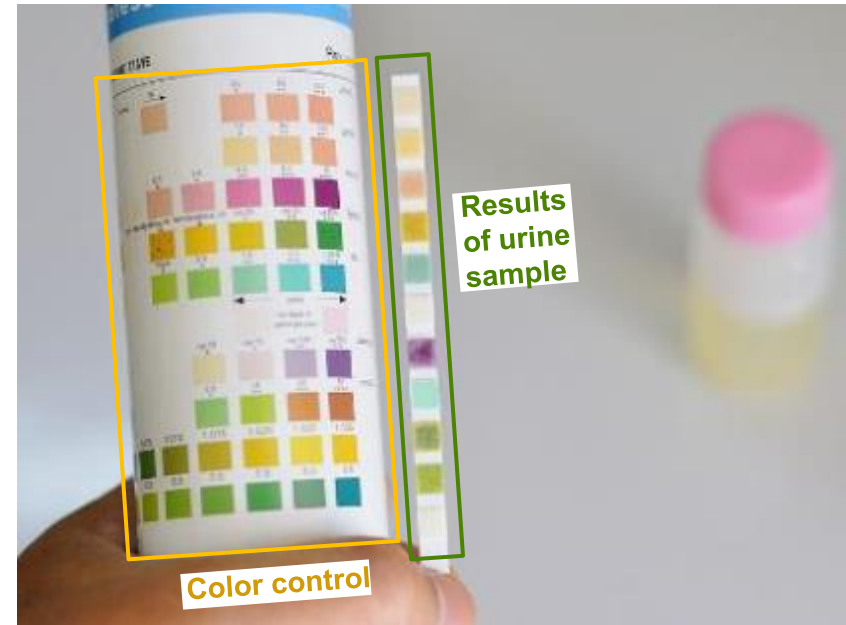
- ✓ **Acetone** (exhaled by lungs, gives characteristic “fruity” smell in DKA)
- ✓ **Acetoacetate.**
- ✓  **$\beta$ -Hydroxybutyrate.**

# URINALYSIS USING DIPSTICK

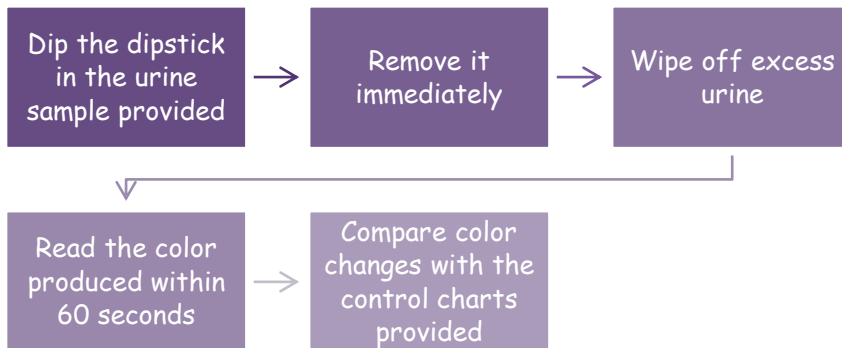
**Dipsticks** are plastic strips impregnated with chemical reagents which react with specific substances in the urine to produce color-coded visual results

They provide quick determination of pH, protein, glucose and ketones. The depth of color produced is proportional to the conc. of the substance in urine

Color controls are provided against which the actual color produced by the urine sample can be compared "You must know how to compare color changes with the control charts."



## ❖ PROCEDURE: NOT IMPORTANT



**Note**

In Physical examination of urine:  
-**Colorless** urine indicates **Polyuria**.  
-**Fruity odor** indicates the presence of **ketones**.

# BLOOD GLUCOSE ASSAY

Blood glucose is detected by a series of enzymatic reactions that ultimately form a colored product.

✓ **The intensity of color is proportional to the amount of glucose present in blood.**

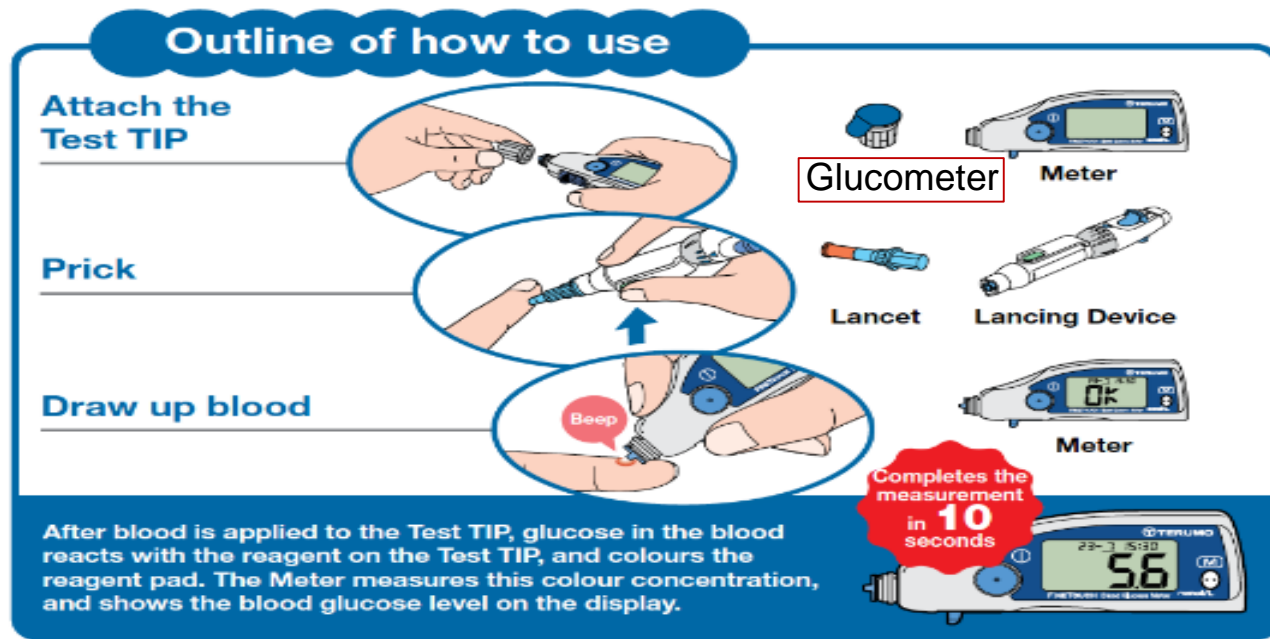
❖ **PROCEDURE:** NOT IMPORTANT

	Test tube	Standard tube	Blank tube
Reagent	2.5 ml	2.5 ml	2.5 ml
sample	25 $\mu$ l	-	-
Standard	-	25 $\mu$ l	-
H <sub>2</sub> O	-	-	25 $\mu$ l

- **Mix and incubate** for 10 minutes at room temperature then Measure absorbance of the color by spectrophotometrically at 546 nm wavelength.
- **Calculate:**

$$\text{Glucose conc. (mmol/l)} = \frac{\text{Abs of sample}}{\text{Abs of standard}} \times \text{Conc of standard (5.6 mmol/l)}$$

# MEASUREMENT OF BLOOD GLUCOSE LEVEL USING GLUCOMETER



## Benefits of self-monitoring of blood glucose level:

- ✓ It allows patients to detect their blood glucose levels without visiting a clinic.
- ✓ It helps patients to immediately confirm hypo or hyperglycemia to avoid complications.
- ✓ It facilitates patient education about diabetes and its management by giving patients more self-care responsibilities.
- ✓ It helps to promote well-being of patients.

# TEST YOUR SELF!

Q1 :- What is your Interpretation of the patient's results below:

	Patient A	Patient B	Patient C
FPG	12 mmol/L	6.5 mmol/L	10.2 mmol/L
Hb A1C	10%	6%	9%
Serum ketones	50 mg/dl	Negative	Negative
Urine Ketones	+++	Negative	Negative
Urine Glucose	+++	Negative	++
Protein	+++	Negative	+++
pH	5	7	6.5

## Patient A results



### Patient A:

- Very High FPG “**Hyperglycemia**” and this indicates that the patient is diabetic
- HbA1C is raised which shows his blood glucose level was uncontrolled for the last 2 months, this **confirms** diabetes.
- “**Ketonemia**” and “**ketonuria**” indicate that the patient has DKA.
- “**Glucosuria**” indicates the glucose filtration level is abnormally higher than the kidney threshold.
- The presence of protein in urine “**proteinuria**” is caused by nephropathy (complication of uncontrolled DM).
- Low pH of urine “**Acidic urine**” indicates metabolic acidosis due to increase production of ketone bodies.

**The diagnosis is: Diabetes ketoacidosis**

### Patient B:

- FPG is higher than normal BUT not diabetic “**Pre-diabetic**” and shows Increase risk of diabetes.
- HbA1C is raised and shows his blood glucose level was uncontrolled “high” for the last 2 months.

**The diagnosis is: Impaired Fasting Glucose “PreDiabetes”**

### Patient C:

- High FPG “**Hyperglycemia**” and this indicates that the patient is diabetic
- HbA1C is raised and shows his blood glucose level was uncontrolled “high” for the last 2 months.
- The presence of glucose in urine “**Glucosuria**” which this also indicates the patient has high blood glucose level. And the glucose filtration level is abnormally higher than the kidney threshold.

**The diagnosis is: Diabetes Mellitus with nephropathy**



# TEST YOUR SELF!

A 32-year-old male with type 1 diabetes since the age of 14 years was taken to the ER because of drowsiness, fever, cough, diffuse abdominal pain, and vomiting. Fever and cough started 2 days ago and the patient could not eat or drink water. He has been treated with insulin, On examination: he was tachypneic, his temperature was 39° C, pulse rate 104 beats per minute, respiratory rate 24 breath/minute. He also has dry mucous membranes, poor skin turgor and He was slightly confused.

The results of lab investigations are shown below:

Plasma analytes	Patient's results	Normal levels
Glucose (mmol/L)	50	4.2-6.1
Ketoacids	++++	(trace)
Arterial blood pH	6.9	7.35-7.45
WBCs count	18,000x10 <sup>9</sup>	4-11x10 <sup>9</sup>

Urine dipstick	Observation
Protein	++
Glucose	+++
Ketones	+++
pH	5.3

**Q1/What is your diagnosis?**

Diabetic ketoacidosis

**Q2/from the history given and lab investigations, what are the three most imp. findings that correlate with your diagnosis?**

Hyperglycemia, metabolic acidosis, ketonemia & ketonuria

**Q3/what is the significant of high fever and caught in this case?**

High fever + caught + high WBCs count are all indicative of infection. Infections by far are the most important precipitating factors for DKA

**Q4/How will you manage this patient?**

Immediate infusion of normal saline + intravenous insulin + bicarbonate because pH is 6.9 (low) + Serum potassium levels should be checked with potassium administration.

**Q5/mention three Precipitating factors for Such condition in diabetic patient other than The one in this case?**

1. missed insulin dose or insulin resistance      2. Trauma      3. Drugs: e.g., steroids

# TEST YOUR SELF!

An 82-year-old man with a 17-year history of Type 2 diabetes was brought by family members to the ER with fever, confusion, and an infected ulcer on the right little toe that had developed over the previous few days. He had been treated with metformin and glimepiride for the last 15 years, with inadequate diabetic control.

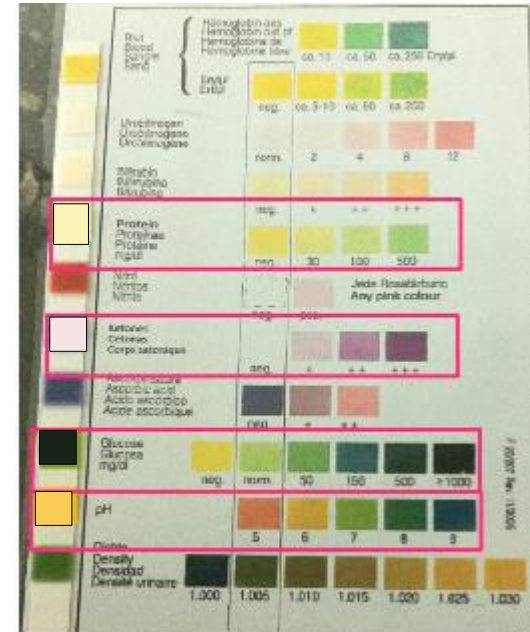
-On physical examination in ER, his blood pressure was 84/52 mmHg, pulse rate 112/min, and temperature 38.4° C. He was drowsy and disoriented to place and time. His oral mucosa was dry and skin turgor diminished.

## Q1/name two differential diagnosis?

- ✓ Hyperosmolar Non-ketotic Hyperglycemia
- ✓ Diabetic Ketoacidosis

**Laboratory evaluation:** revealed a serum glucose level of 1120 mg/dl (62.3 mmol/L), His WBC count was 18,000 x10<sup>9</sup> (80% polymorphonuclears), hematocrit and platelet counts were normal, erythrocyte sedimentation rate (ESR) 85 mm/h (normal 0-20), the serum osmolality is 366.3 Osm/kg [normal 275-295], the Urinalysis using dipstick results are shown next:

Item	Protein	Glucose	Ketones	pH
Observation	+	++++	+	6.7



## Q2/What is your final diagnosis? Justify

The patient obviously has a hyperglycemic crisis (extremely high blood glucose levels) + no acidosis + minimal, if any, ketosis + very high serum osmolality.. Thus, he fulfills all the criteria for Hyperosmolar Hyperglycaemic State (HHS).

## Q3/what is the mechanism of releasing insulin by beta-cells?

1. Glucose is transported into beta cells through facilitated diffusion of GLUT2 glucose transporters.
2. Intracellular glucose is metabolized to ATP.
3. ATP induces closure of cell-surface ATP-sensitive K<sup>+</sup> channels, leading to cell membrane depolarization.
4. Cell-surface voltage-dependent Ca<sup>2+</sup> channels are opened, facilitating extracellular Ca<sup>2+</sup> influx into the beta cell.
5. A rise in free cytosolic Ca<sup>2+</sup> triggers the exocytosis of insulin.

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