



King Saud University
College of Medicine
Clinical Biochemistry unit, Department of Pathology

Biochemistry Practical Class

Glucose Estimation In Blood & Urine

Endocrine System Block (2nd Year)

Multipurpose Lab | College of Medicine | Level 2



KING SAUD UNIVERSITY
College of Medicine
Department of Pathology
Clinical Chemistry unit

Endocrine Block

Biochemistry Practical

Practical biochemistry sessions are designed to teach the fundamental techniques used in biochemistry to medical students. In this session the students will have hands-on training in estimating glucose in blood and urine. This will enable them to understand how biochemical data is collected, analyzed and interpreted.

Plan of work:

1. Handouts will be distributed and a presentation on glucose estimation in blood and urine will be given for 15 minutes.
2. Hands-on practical session will be conducted following the presentation.

Estimation of Glucose in Blood

Principle of the method

Blood glucose is detected by a series of enzymatic reactions (oxidase and peroxidase) that ultimately form a colored product. The intensity of the color is directly proportional to the amount of glucose in blood. The color intensity is determined by measuring the absorbance at a wavelength of 546 nm using a spectrophotometer.

Procedure:

	Test	Standard	Blank
Reagent	2.5 ml	2.5 ml	2.5 ml
Blood sample	25 μ l	-	-
Standard	-	25 μ l	-
H₂O	-	-	25 μ l

- Mix and incubate for 10 min. at room temperature.
- Measure the absorbance (A) of the **test** and **standard** against **blank** at **546 nm**.

Calculation:

$$\text{Glucose conc. (mmol/l)} = \frac{A_{\text{Test}}}{A_{\text{Standard}}} \times \text{conc. of standard. (5.6 mmol/l)}$$

Normal reference values for blood glucose:

3.9-6.1 mmol/L (70-110 mg/dL)

Note: To convert mmol/l to mg/dL multiply the results by 18

Urine analysis (using dipstick):

Principle:

- Dipsticks are plastic strips impregnated with chemical reagents that 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 directly proportional to the concentration of the substance in urine.
- Color controls are provided against which the actual color produced by the urine sample can be compared. The reaction times of the impregnated chemicals are standardized.

Procedure:

- Dip the dipstick in the urine sample provided and remove it immediately.
- Wipe off the excess urine.
- Read the color produced within 60 sec. by comparing with control chart.

Item	Observation	Comment
Protein	Positive 500	Proteinuria
Glucose	Positive	hyperglycemia
Ketones	Positive +++	Ketoacidosis
pH	Positive 6.0	6.0

Criteria for the diagnosis of diabetes

1. A1C \geq 6.5 percent. The test should be performed in a laboratory using a method that is NGSP certified and standardized to the DCCT assay.*

OR

2. FPG \geq 126 mg/dL (7.0 mmol/L). Fasting is defined as no caloric intake for at least 8 h.*

OR

3. Two-hour plasma glucose \geq 200 mg/dL (11.1 mmol/L) during an OGTT. The test should be performed as described by the World Health Organization, using a glucose load containing the equivalent of 75 g anhydrous glucose dissolved in water.*

OR

4. In a patient with classic symptoms of hyperglycemia or hyperglycemic crisis, a random plasma glucose \geq 200 mg/dL (11.1 mmol/L).

A1C: glycated hemoglobin; NGSP: National glycohemoglobin standardization program; DCCT: Diabetes control and complications trial; FPG: fasting plasma glucose; OGTT: oral glucose tolerance test.

* In the absence of unequivocal hyperglycemia, criteria 1-3 should be confirmed by repeat testing.

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Categories of increased risk for diabetes*

FPG 100-125 mg/dL (5.6-6.9 mmol/L) [IFG]

2-h PG on the 75-g OGTT 140-199 mg/dL (7.8-11.0 mmol/L) [IGT]

A1C 5.7-6.4 percent

FPG: fasting plasma glucose; IFG: impaired fasting glucose; PG: post glucose; OGTT: oral glucose tolerance test; IGT: impaired glucose tolerance; A1C: glycated hemoglobin.