Cihan University - Sulaimaniya

Clinical Biochemistry

Lab 3

Measurement of Blood Glucose

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Academic Year 2023-2024

Introduction

- **Glucose (C6H12O6)** is a ubiquitous **fuel molecule in biology**.
- It is oxidized through a series of enzyme-catalyzed reactions to **form carbon dioxide** and **water**, yielding the universal energy molecule **ATP**.
- Due to its importance in metabolism, glucose level is a key diagnostic parameter for many **metabolic disorders.**
- Increased glucose levels of glucose **(Hyperglycemia)** have been associated with **diabetes mellitus**, **hyperactivity of thyroid, pituitary and adrenal glands**.
- Decreased levels of glucose **(Hypoglycemia)** are found in **insulin secreting tumors**, **myxedema**, **hypopituitarism and hypoadrenalism.**

Measuring blood glucose is used for the diagnosis of:

- Carbohydrate metabolism disorders
- Monitoring of treatment in diabetes mellitus
- Neonatal hypoglycemia
- Idiopathic hypoglycemia
- Insulinoma.

Glucose Measurements

Glucose Measurement Methods

- Glucose is measured in **whole blood, plasma, serum, cerebrospinal fluid, pleural fluid, and urine** for a variety of diagnostic and management purposes.
- The standard clinical laboratory analysis of glucose is performed on **plasma or serum** derived from a **phlebotomy specimen**.
- Most measurements of glucose employ enzymatic methods:

1. **Glucose Oxidase**
2. **Hexokinase**
3. **rarely, Glucose Dehydrogenase**

Specimen Considerations

- ✓ Glycolysis causes plasma glucose to decline over time while the plasma or serum is in contact with cells.
- ✓ The decline can be 10 mg/dL (average 5%–7%) per hour.
- ✓ A specimen is appropriate for glucose analysis if serum or plasma is separated from the cells **within 30 minutes.**
- ✓ Until separation has been performed, it is recommended that specimens be kept on **ice.**
- ✓ Whole blood tends to give approximately **10% to 15%** lower glucose readings than plasma.
- ✓ Fluoride and iodoacetate have been used as inhibitors of glycolysis to preserve blood that cannot be separated rapidly.
- ✓ **Note: Whole blood and hemolysis are not recommended for use as a sample.**

Blood Glucose Tests

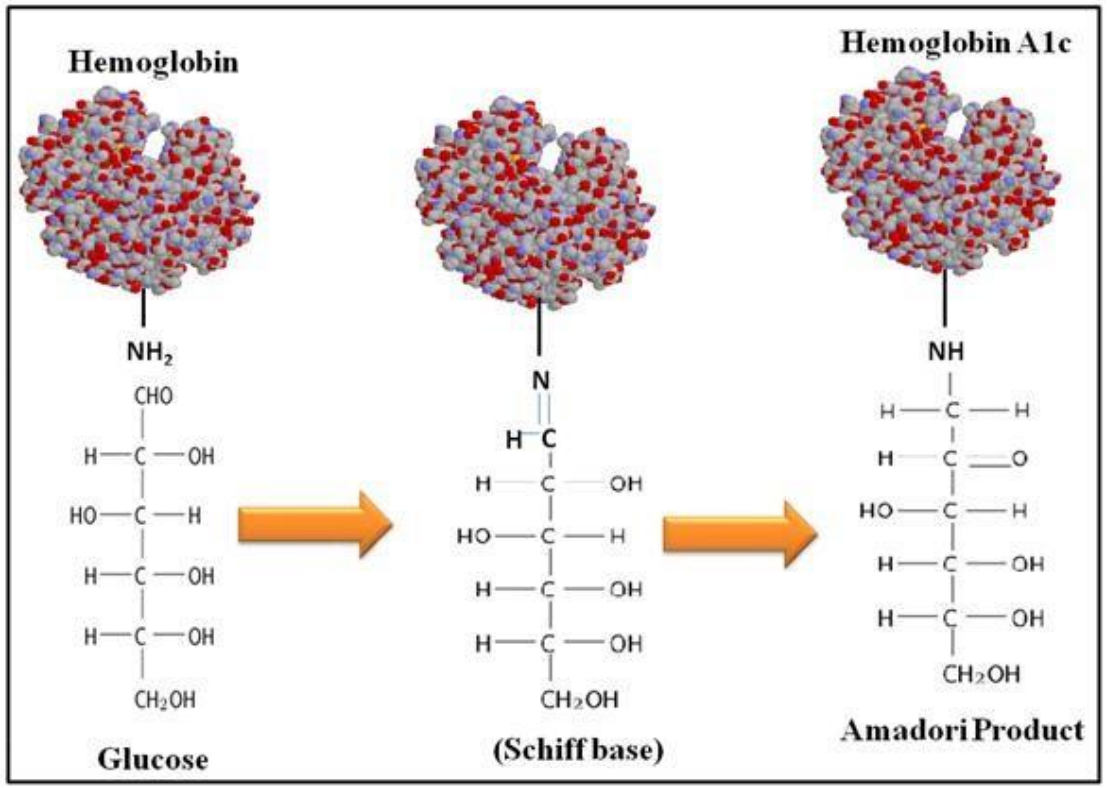
- **Random Blood Test (RBS):** measures blood glucose regardless of when you last ate.
- **Fasting Blood Sugar (FBS):** measures blood glucose after you have not eaten for at least 8 hours. It often is the **first test done to check and diagnosis of diabetes.**
- **Glucose tolerance test (GTT):** This measures your body's ability to use sugar after drinking a standard amount in a sugary drink (75 gr). The glucose tolerance test can **be used to screen for type 2 diabetes**. evaluating an individual's apparent insulin sensitivity and insulin resistance.
- **Two Hours Postprandial Plasma glucose (2HPP):**

Measures blood glucose exactly 2 hours after you eat a meal.it is used for Screening and monitoring of diabetes.

- **Glycosylated Hemoglobin (HA1C) :** This measures your average blood sugar level over the last 2 to 3 months

Glycated Hemoglobin (HbA1C)

- **Glycated hemoglobin**, also known as HbA1c, glycohemoglobin, hemoglobin A1c, A1C, is a form of hemoglobin (Hb) that is chemically linked to glucose.
- It is formed in a non-enzymatic glycation pathway by **hemoglobin's exposure to plasma glucose.**
- **Normal levels of glucose produce a normal amount of glycated** hemoglobin.



✓ As the average amount of plasma glucose increases, the fraction of glycated hemoglobin increases in a predictable way.

HbA1C Test

A hemoglobin A1C (HbA1C) test is a blood test that shows what your average blood sugar (glucose) level was over the past **two to three months**.

Clinical Importance of HbA1C test

1. **Diagnose prediabetes.** If you have prediabetes, you have a higher risk of developing diabetes and cardiovascular disease.
2. **Diagnose type 1 and type 2 diabetes.** Along with other test like FBS is used to diagnosis of Diabetes.
3. **Monitor your diabetes treatment plan.** The result of an initial A1C test also helps establish your baseline A1C level. The test is then repeated regularly to monitor your diabetes treatment plan.

Diagnosis of Diabetes Mellitus

To diagnose diabetes, **two abnormal results** from following tests are required:

(1) Fasting Plasma Glucose or FBS test: level of **126 mg/dL (7.0 mmol/L)** or **higher** (the glucose level should be obtained after an 8-hour fast),

(2) Oral Glucose Tolerance Test (OGTT) test : a plasma glucose level ≥200 mg/dL (11.1 mmol/L) **2 hours after a glucose load** (75-g oral glucose tolerance test.

(3) Random Blood Sugar (RBS) test : a Random Glucose ≥200 mg/dL (11.1 mmol/L) with **symptoms of hyperglycemia**

(4) Hemoglobin A1c (HbA1c) test: ≥6.5%.

Tests can be performed on two different days or 2 different tests can be performed on the same day.

Diagnosis od Diabetes Mellitus

	FASTING PLASMA GLUCOSE		2-HOUR PLASMA GLUCOSE LEVEL (AFTER 75 G GLUCOSE LOAD)		HBA_{1C}
	mg/dL	**mmol/L**	**mg/dL**	**mmol/L**	**%**
Normal	<100	<5.6	<140	<7.8	
Prediabetes					5.7–6.4
Impaired fasting glucose	100–125	5.6–6.9			
Impaired glucose tolerance			140–199	7.8–11.0	
Diabetes mellitus	≥126	≥7.0	≥200	≥11.1	≥6.5

Prediabetes designates conditions in which **glucose homeostasis is abnormal**, but serum glucose levels are **not high enough to be classified as diabetes**. This group includes individuals with **impaired fasting glucose and impaired glucose tolerance.**

✓ They are also at increased risk for cardiovascular and cerebrovascular diseases.

Diagnosis of Gestational Diabetes Mellitus

Perform a 75-g OGTT, with plasma glucose measurement fasting and at 1 and 2 h, at 24–28 weeks of gestation in women not previously diagnosed with overt diabetes.

The OGTT should be performed in **the morning after an overnight fast of at least 8 h.**

The diagnosis of GDM is made when any of the following plasma glucose values are exceeded:

- **Fasting: ≥92 mg/dL (5.1 mmol/L)**
- **1 h: ≥180 mg/dL (10.0 mmol/L)**
- **2 h: ≥153 mg/dL (8.5 mmol/L)**

Methods of Glucose Analysis

- ***Method 1: Benedict's:*** (qualitative, semiquantitative):
- ***Method 2: Alkaline ferricyanide*** **(**quantitative): involves the reduction of yellow ferricyanide, $Fe(CN)_6$, to colorless ferrocyanide, $Fe(CN)_6$, by glucose in alkaline conditions.
- ***Method 3: o-Toluidine*** **(**quantitative): The o-toluidine reaction is based on the ability of many aromatic amines in acid solutions to condense with the aldehyde group of glucose to form glucosamines. O-toludine +glucose (aldhyde) heat &acidity glucosamine (colored)
- ***Enzymatic methods:*** ***Glucose oxidase coupled reaction*** (quantitative): One of the most frequently used specific glucose methods uses two coupled enzyme reactions

Glucose oxidase coupled reaction

α-D-glucose $\xrightarrow{\text{Mutarotase}}$ **β-D-glucose**

$$\beta\text{-D-Glucose} + O_2 + H_2O \xrightarrow{\text{Glucose Oxidase}} \text{Gluconate} + H_2O_2$$

$$H_2O_2 + 4\,\text{Aminoantipyrine} + \text{Phenol} \xrightarrow{\text{Peroxidase}} \text{Red Quinoneimine dye} + H_2O$$

The intensity of the color formed is proportional to the glucose concentration in the sample. It is determined by measuring the increase in absorbance at **500-550 nm.**

Fujifilm DRI-CHEM Analyzer

- **DRI-CHEM** from FUJIFILM is a **dry chemistry analyzer** which can perform multiple test parameters of Clinical Chemistry.
- It has a built-in auto-pipetting system, requires no calibration and no water, providing easy preparation and maintenance.
- The new DRI-CHEM NX500 delivers results using a simple 3-step procedure.

Simple 3-step procedure:

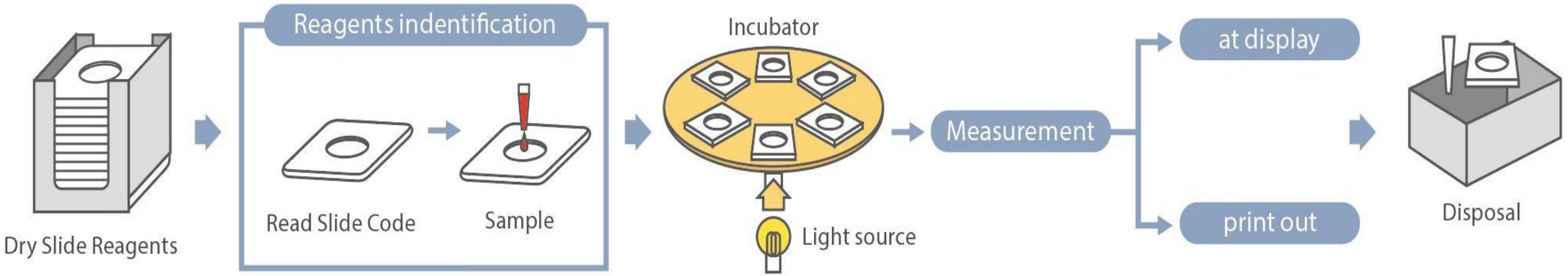
1. **Set the slide (Dry Slide Reagents).**
2. **Set the sample.**
3. **Press START.**

Introduction

Set the slide (Dry Slide Reagents)

Set the sample

Press START

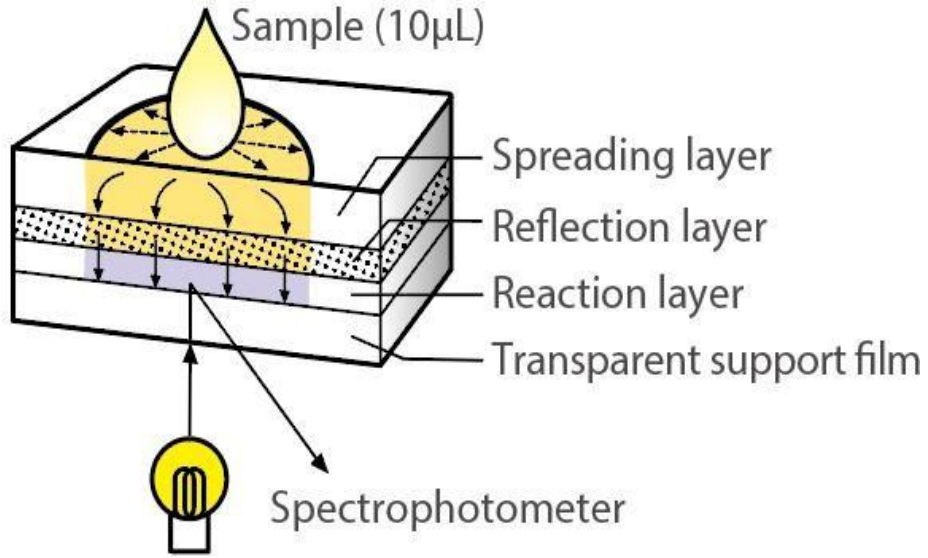


Fujifilm DRI-CHEM SLIDE methods

1. Colorimetric method slide:

(Enzymes, General chemistry, and Immunology)

This multilayered slide is composed of dry chemical ingredients needed for the reaction and other functional materials. It quantifies enzymes and chemicals using colorimetric method.

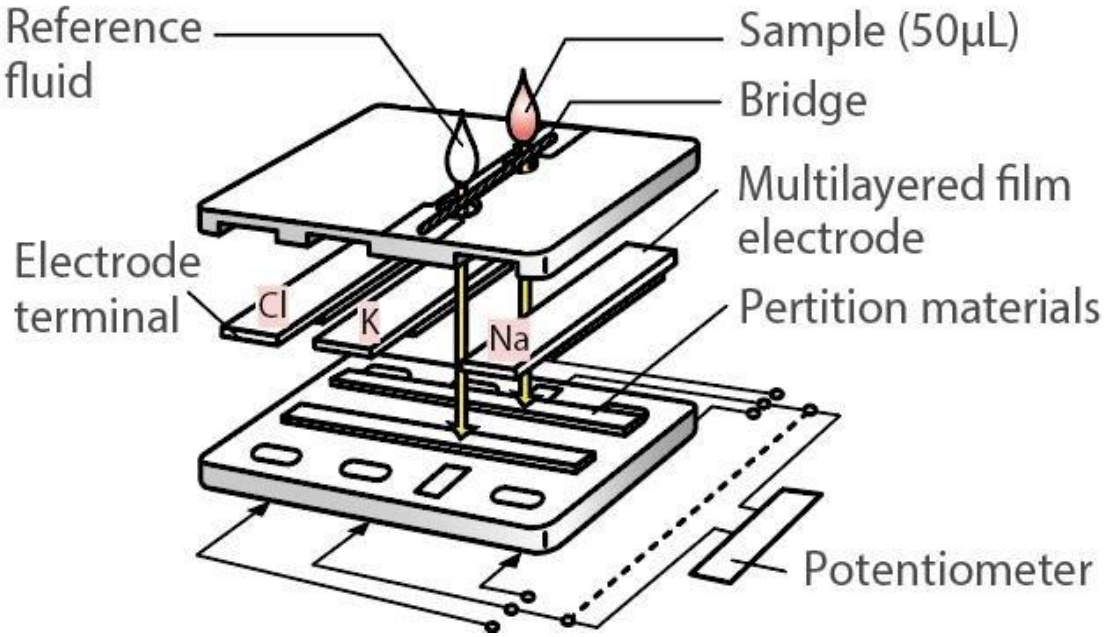


Fujifilm DRI-CHEM SLIDE

2. Potentiometric method slide (Electrolytes)

Each slide comes with an ion selective film electrode for each of Na, K, and Cl.

Slides quantify electrolytes in the sample by a potentiometric method.



Digital Glucometer

Glucometer (or glucose meter)

Glucometer is a medical tool that is used for measuring the approximate level of glucose in the blood.

Random Blood Glucose Test:

Normal: **70-140 mg/ dl**

Impaired: **140 – 200 mg/dl**

Diabetes: **> 200 mg/dl**

Working principle of a Glucometer:

Most of the glucometers are based on **electrochemical technology.**

Glucometer test strips:

In each glucometer test strip which are to be used for determining glucose in the blood, contains an **enzyme called glucose oxidase**. This **enzyme then reacts** with the glucose in the blood sample and creates an acid called **gluconic acid.**

The gluconic acid thus formed then reacts with another chemical in the testing strip called ferricyanide.

The ferricyanide and the gluconic acid then combines with each other and forms **ferrocyanide.**

iii) As soon as the ferrocyanide has been formed the device (i.e., glucometer) **runs an electronic current through the blood sample on the strip.**

This current thus generated is able to **read the ferrocyanide and identify the amount of glucose present in the blood sample on the testing strip.**

That number is then **displayed on the screen of the glucometer.**