



# **College of pharmacy**

## **Clinical laboratory training**

### **Fifth stage**

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### **Lecture: 2**

**Biochemical Tests: Fasting blood glucose, random blood glucose, oral glucose tolerance test, and post-prandial glucose**

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Blood for plasma glucose estimation should be taken if a patient presents with symptoms of diabetes mellitus or glycosuria or if it is desirable to exclude the diagnosis, for example because of a strong family history. The **primary reason** to measure glucose is for the diagnosis of diabetes.

Blood samples may be taken at least 10 hours after a fast, at random, as part of an oral glucose load test. **In most cases a diagnosis can be established** from either **fasting** or **random** blood glucose determinations. In equivocal cases an **OGTT** may be required.

Glucose level measured in mmol/L or mg/dL. Conversion formula for converting mmol/L to mg/dL: **Multiply mmol/L by the number 18.**

**Fasting blood glucose test** (FBG test), is a glucose test on the blood of a **fasting** person. Fasting is defined as **no caloric intake for at least 8 hours**. Fasting of at least 8 hours is required prior to the test.

**Interpretation of fasting venous plasma glucose:**

- Normal glucose: < 100 mg/dL (<5.6 mmol/L)
- Pre-diabetic: 100 to 125 mg/dL (5.6 to 6.9 mmol/L)
- Diabetic:  $\geq 126$  mg/dL ( $\geq 7.0$  mmol/L) (on more than one occasion or once in the presence of diabetes symptoms).
- Diabetes mellitus is unlikely if the fasting venous plasma glucose concentration is less than 5.5 mmol/L on two occasions.

**A random glucose test** (RBG test), also known as a **casual blood glucose test** (CBG test) is a glucose test on the blood of a **non-fasting** person. This test assumes a recent meal and therefore has **higher** reference values than the fasting blood glucose (FBG) test. Casual is defined as **any time** of day without regard to time since last meal.

Diabetes mellitus has been defined by the WHO, as a random venous plasma glucose concentration of 11.1 mmol/L or more on two occasions or

once with symptoms. Samples taken at random times after meals are **less reliable for excluding** than for **confirming** the diagnosis.

**Oral glucose tolerance test (OGTT)** the blood is taken 2 hours after the oral ingestion of the equivalent of 75 g of anhydrous glucose.

The OGTT is **only indicated** when the diagnosis is in **doubt**. Sometimes an OGTT may be required to establish the diagnosis in **equivocal** cases.

Diabetes mellitus can be diagnosed if the venous plasma glucose concentration is 7.0 mmol/L or more (fasting) and/or 11.1 mmol/L or more 2 h after the oral ingestion of the equivalent of 75 g of anhydrous glucose. The patient fasts overnight (for at least 10 hours but not more than 16 hours). **Water**, but no other beverage, is **allowed**.

**The indications for performing an OGTT to diagnose diabetes mellitus may include:**

- fasting venous plasma glucose concentration **between** 5.5 mmol/L and less than 7.0 mmol/L – this is debatable as the WHO recommends an OGTT only if fasting plasma glucose is **greater** than 6.0 mmol/L,
- random venous plasma concentration between 7.0 mmol/L and less than 11.1 mmol/L,
- a high index of clinical suspicion of diabetes mellitus, such as a patient at high risk of gestational diabetes with equivocal blood glucose results.

**The following factors may affect the result of the OGTT test:**

- **Previous diet**

No special restrictions are necessary if the patient has been **on a normal diet for 3–4 days**. However, if the test is performed after a period of carbohydrate restriction, for example as part of a weight-reducing diet, this may cause abnormal glucose tolerance, probably because metabolism is adjusted to the **‘fasted state’** and so **favours gluconeogenesis**.

- **Time of day**

Most OGTTs are performed in the **morning** and the reference values quoted are for this time of day. There is evidence that tests performed in the afternoon yield higher plasma glucose concentrations and that the accepted

'reference values' may not be applicable. This may be due to a circadian variation in islet cell responsiveness.

- **Drugs** as steroids, oral contraceptives and thiazide diuretics may impair glucose tolerance.

**A 2-hour postprandial blood glucose test** measures the blood sugar **exactly 2 hours** after the start of a meal. This test may be used to **check** or as a **screening** for diabetes and **to see how treatment for diabetes is working**. It will give you the body's glucose tolerance after 2 hours of a meal. In normal person blood glucose will be normal within 2 hours. In diabetic patients, glucose levels will remain elevated.

#### **Interpretation of 2 hours glucose (postprandial) level:**

1. Normal if glucose level <140 mg/dL.
2. Impaired glucose tolerance (IGT) if glucose level 140 to 199 mg/dL. If glucose level is >140 mg/dL and <200 mg/dL, then GTT (glucose tolerance test) is advised.
3. Diabetes mellitus is confirmed if glucose level  $\geq 200$  mg/dL
4. Advise HbA1c for further evaluation.

When the test is done on serum, the serum **should be separated** within 30 minutes of collection. The serum can be stored at 25° C for 8 hours and 72 hours at 4 °C. The plasma can be stored at 25 °C for 24 hours (with preservative **sodium fluoride**).

#### **Blood glucose**

Blood glucose concentrations may be measured using glucose testing reagent **strips**. The colour change of the strip can be assessed **visually** or by using a **portable glucose meter** and the reaction often involves an enzyme for determination of glucose, for example glucose oxidase. **Meters** should ideally be overseen by laboratory staff expert in point of care testing. Although the measurement of blood glucose concentrations involves the discomfort of several skin punctures, many motivated patients are able to adjust their insulin dose more accurately based on these results [**Self-monitoring of blood glucose (SMBG)**] than on those obtained by testing

their urine. This method of testing is also useful in the detection of hypoglycaemia. For patients who do not like blood testing, urinary glucose testing can be used, but of course **urinary glucose testing cannot** detect hypoglycaemia and is dependent on the renal glucose threshold.

Due to the imprecision of the meters and the difference in results between different manufacturers of meters, they are **not recommended for the diagnosis of diabetes** but only for the **monitoring** of treatment.

All **modern methods** for glucose analysis employ **enzymes** as reagents to increase analytical specificity. The enzymes used are the hexokinase, glucose oxidase, and glucose dehydrogenase reactions, the product concentration is proportional to glucose concentration in the sample.

A **spectrophotometer** is a popular glucose measurement **device** due to its speed and ease of use. The absorbance of standard and sample solutions are both measured. The absorbance is directly proportional to the concentration of glucose.

### **Specimen choice**

The choice of specimen used for glucose determination depends on the analytical method to be used. Serum or plasma, **free** of hemolysis, is the specimen of choice for automated enzymatic methods. **Plasma is recommended for the diagnosis of diabetes**, since the diagnostic cutoff points have been derived using plasma samples. A plasma sample drawn after an overnight fast of 8 to 16 hours is used for the diagnosis of diabetes. The plasma should be separated from the cells within 60 minutes of collection. **Serum samples are appropriate for glucose analysis.**

Glucose in whole blood at room temperature can undergo **glycolysis** at a rate of approximately 5% to 7% (10 mg/dL or ~ 0.6 mmol/L per hour). The sample should be centrifuged and removed from clot or cells as soon as possible.

**Capillary blood glucose** measurement is widely used to **monitor** glucose levels in diabetic patients. Concentrations are approximately 2 to 5 mg/dL (0.11 to 0.28 mmol/L) higher than venous blood glucose concentrations in fasting patients. However, following a glucose load, the difference can be as great as 70 mg/dL (3.9 mmol/L).