Laboratory Measurements of Hormones

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Introduction

The term 'hormone' was initially applied to molecules which were synthesized in endocrine glands, secreted into the bloodstream, and then transported to a distant target site. Today, the term hormone is used more broadly to include all chemical messengers synthesized by the body that acts by binding with high affinity to target cells within the same individual. There are over 100 known hormones, and this list is growing.

Laboratory tests are essential for the diagnosis and management of many conditions. Accurate hormonal assays play a significant role in the practice of endocrinology. A few decades ago, development of the radioimmunoassay (RIA) was awarded the Nobel Prize as a revolutionary tool for measuring peptide hormones. Various laboratory methods are used to assess endocrine problems including immunoassays and more recently, mass spectrometry. Immunoassays remain the most commonly used method to evaluate hormonal disorders. The laboratory must warrant the technical validity and reproducibility of the hormone measurements through standardized laboratory procedures and adequate quality standards and quality management. The physician/lab specialists are responsible for the interpretation of the laboratory results and the use of this medical information for patient care. Before a laboratory test is requested, clinicians should consider the aim of the test and have a clear understanding of how the result will be interpreted and how the patient's management will be affected by the result.

One of my aims of this course is the student will gain knowledge about the correlation of the assessment of endocrinology and diseases with the measurement of hormones. The Post-graduate students will explore the role of the laboratory professional in providing the laboratory results that aid in the diagnosis and monitoring of clinical disorders. Also, they will have the ability to describe the regulatory guidelines that define the practice of different hormones assay.

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Blood Collection

There are two basic types of blood collection procedures: **skin puncture** and **venipuncture**. The sites of skin puncture for adults and children is usually the finger-tip, while for infants are the heel or the great toe.

Box 1: Sample collection

Containers for investigation of blood

• Lithium heparin: most hormones.

• Peptide hormones and catecholamines tend to be less stable than other hormones and need prompt delivery to the laboratory on ice.

Containers for investigation of urine

• Acid: calcium, 5-hydroxyindoleacetic acid (5-HIAA), catecholamines.

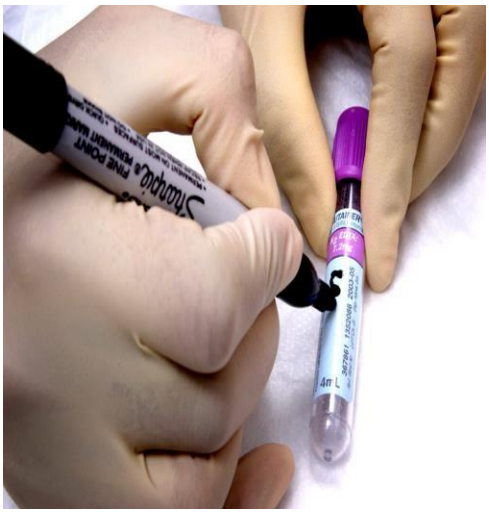
• No preservative: urinary free cortisol.

Hormones that require 9 AM collection

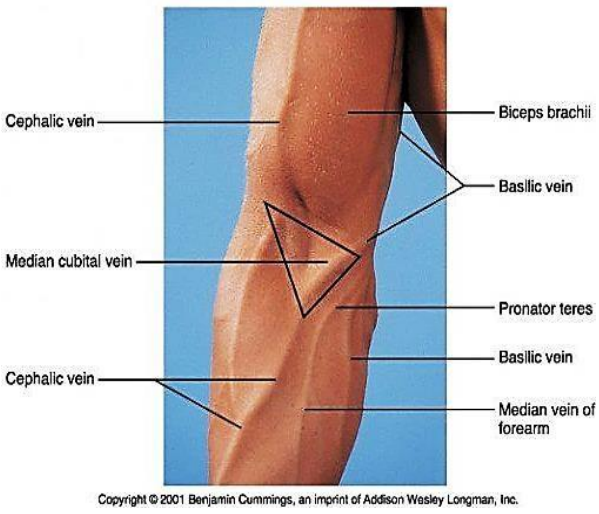
• Cortisol • Testosterone

Performance of venipuncture:

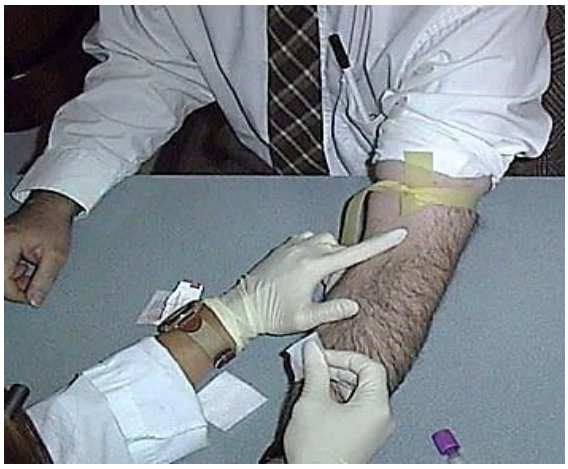
1- Label tube with the patient ID.



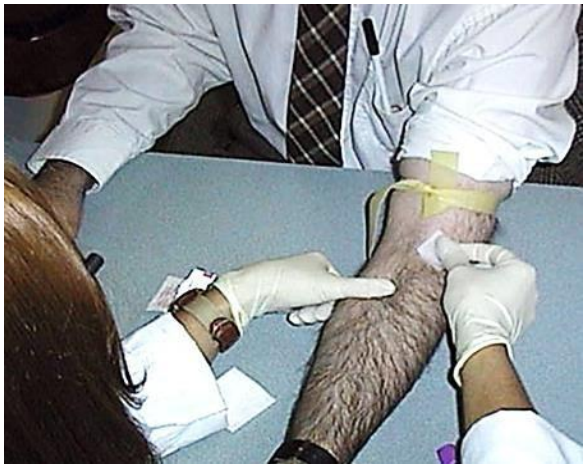
2- Determine the site of venipuncture.



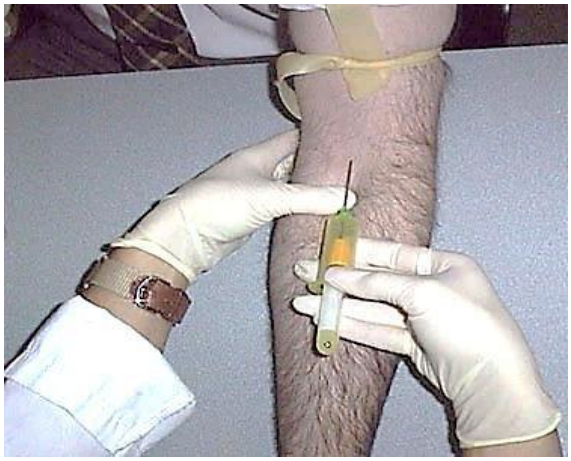
3- Put tourniquet, make the patient form a fist and locate the vein.



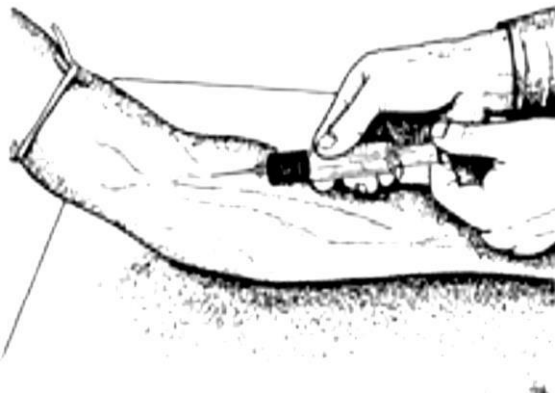
4- Clean the puncture site with a disinfectant.



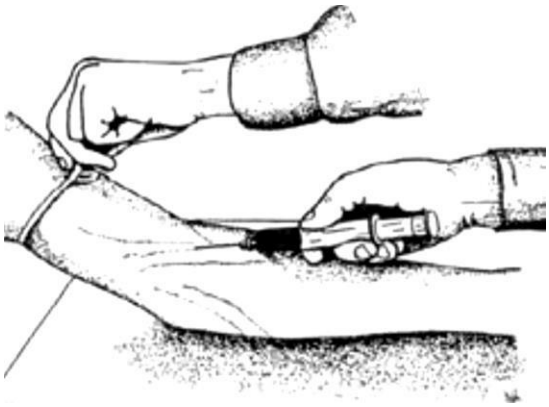
5- Insert a needle into the vein (15º-30º).



6- Aspirate the blood.

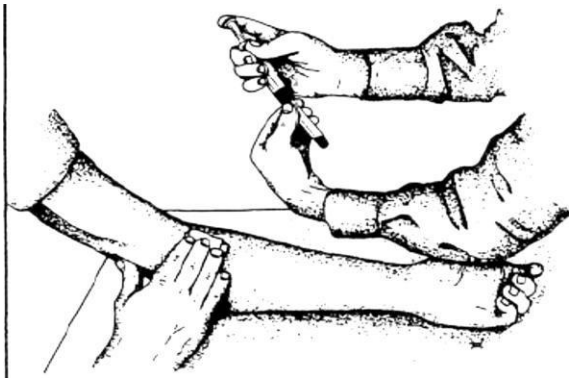


7- Release the tourniquet and allow the patient to open his hand.



8- Place a sterile pad over the site and withdraw the needle.

9- Keep the arm extended and apply mild pressure over the site to achieve hemostasis.



Needle Positioning And Failure To Draw Blood

Correct insertion technique; blood flows freely into needle.

Bevel on vein upper wall does not allow blood to flow.

Bevel on vein lower wall does not allow blood to flow.

Needle inserted too far.

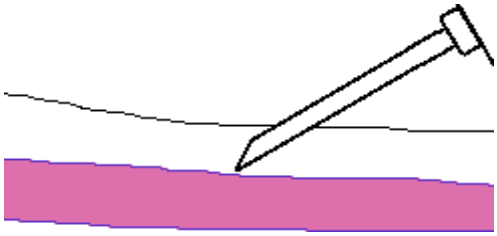
Needle partially inserted and causes blood leakage into tissue.

Collapsed.

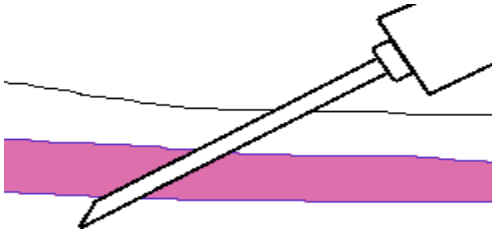
Troubleshooting guidelines

1- If an incomplete collection or no blood obtained.

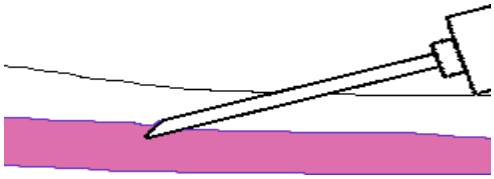
- Change the position of the needle. Move it forward (it may not be in the lumen).



- or move it backward (it may have penetrated too far).

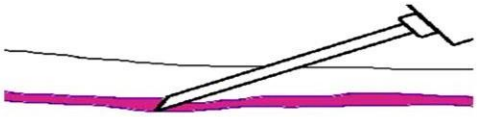


- Adjust the angle (the bevel may be against the vein wall).



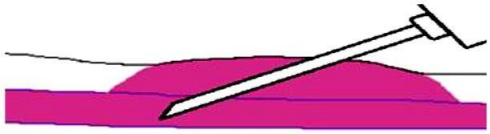
- Loosen the tourniquet. It may be obstructing blood flow.
- Try another tube. There may be no vacuum in the tube being used.
- Re-anchor the vein. Veins sometimes roll away from the needle and puncture site.
- Pre-warm the area of the vein to reduce vasoconstriction and increase blood flow.

2- If blood stops flowing into the tube.

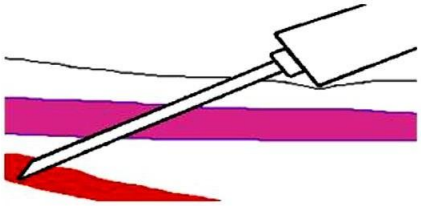


The vein may have collapsed; resecure the tourniquet to increase venous filling.
If this is not successful, remove the needle, take care of the puncture site, and redraw.

3- Problems other than an incomplete collection.



A hematoma forms under the skin adjacent to the puncture site - release the tourniquet immediately and withdraw the needle. Apply firm pressure.



The blood is bright red (arterial) rather than venous. Apply firm pressure for more than 5 minutes.

Additional considerations

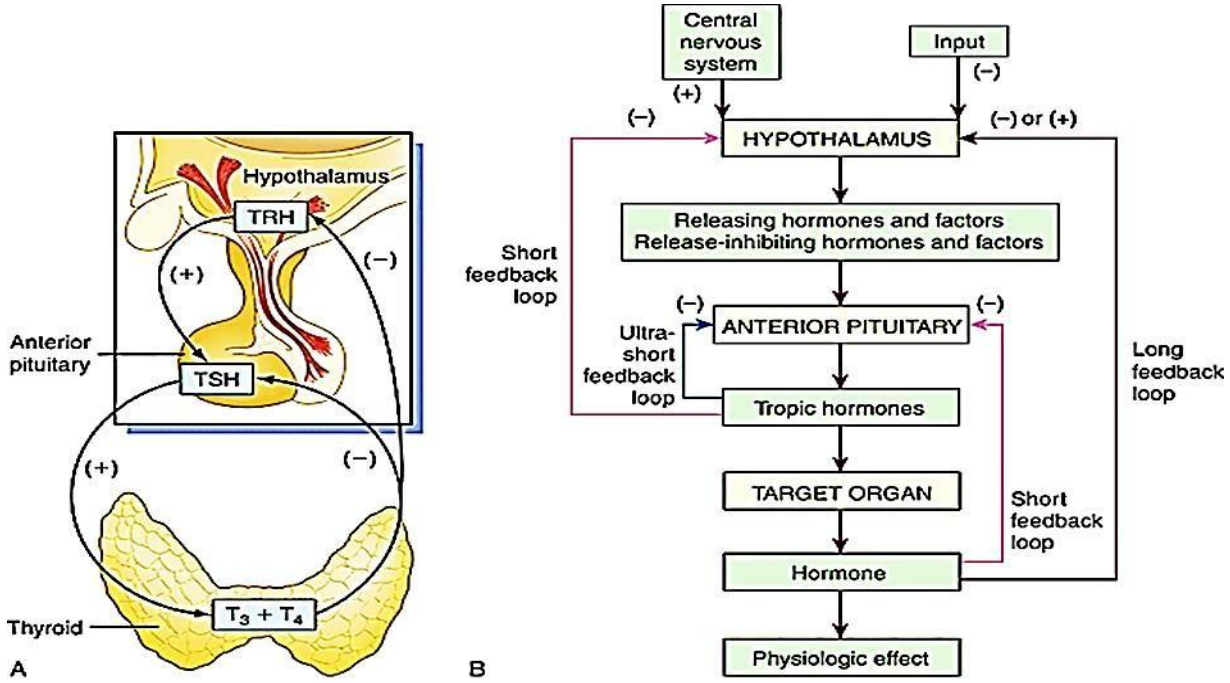
To prevent hematoma:

- Puncture only the upper-most wall of the major superficial veins.
- Remove the tourniquet before removing the needle.
- Apply mild pressure to the venipuncture site.

To prevent hemolysis which can interfere with many tests:

- Mix tubes with anticoagulant additives gently 5-10 times.
- Avoid drawing blood from a hematoma.
- Avoid drawing the plunger back too forcefully.
- Make sure the venipuncture site is dry.

Guiding Principles of Endocrine Investigation



- The initial approach to the assessment of endocrine function is the measurement of hormone levels.
- Hormone levels can be measured in plasma, serum, urine, or other biologic samples. However, urinary excretion of hormone or hormone metabolites over 24 hours, in individuals with normal renal function, maybe a better estimate of hormone secretion than one-time plasma-level measurement.
- Regulation of hormone release is a dynamic process that is constantly changing to adapt to the needs of the individual to maintain homeostasis. For example, plasma insulin levels reflect the fed or fasted state; estrogen and progesterone levels reflect the stage of the menstrual cycle.
- In addition, hormone levels can reflect the time of day during which they were obtained. For example, because of the circadian rhythm of cortisol release, cortisol levels will be higher early in the morning than in the late afternoon.
- Age, health status, gender, and sleep patterns are among the many factors that influence hormone levels.

- Diseases and 24-hour light periods like those in an intensive care unit alter the rhythm of hormone release.
- In general, disorders of the endocrine system result from alterations in hormone secretion or target cell responsiveness to hormone action.

Guiding principles that should be considered during an endocrine investigation:

1) ***Use dynamic tests*** rather than ***random (static) sampling*** when the hormone under investigation is secreted in infrequent pulses (e.g. growth hormone, GH) or levels are easily influenced by other factors (e.g. cortisol varies markedly with stress levels and has a marked circadian rhythm). Dynamic measures of endocrine function rely on the integrity of the feedback control mechanisms that regulate hormone release. They are based on either **stimulation** or **suppression** of the endogenous hormone production.

❖ **Stimulation tests**

- ✶ Designed to determine the capacity of the target gland to respond to its control mechanism (either a tropic hormone or a substrate that stimulates its release).
- ✶ Examples of these tests are the use of ACTH to stimulate cortisol release in suspected adrenal insufficiency (Addison disease) and the use of an oral glucose tolerance test (OGTT) to induce insulin release.

❖ **Suppression tests**

- ✶ Designed to determine whether the negative feedback mechanisms that control the hormone's release are intact.
- ✶ For example, the use of dexamethasone, a synthetic glucocorticoid, to see if pituitary ACTH and consequently cortisol secretion is appropriately diminished. If it is not, it implies that the adrenal cortex is overactive (Cushing syndrome).

In general

If you are suspecting a **low** level, do a **stimulation** test (to see If it stays low)

If you are suspecting a **high** level, do a **suppression** test (to see If it stays high)

**** Hormone-Receptor Measurements**

- Receptor measurements in tissue samples obtained surgically allow detection of tissue responsiveness to hormone and prediction of tumor responsiveness to hormone therapy. For example, the assessment of

estrogen and progesterone receptors (ER&PR) in breast tumors to determine the applicability of hormone therapy.

2) ***Use the correct collection method*****.**

- ACTH or insulin levels require a rapid separation of the sample and prompt freezing (–20°C).
- Urinary catecholamines require a specific acid preservative in the collection container.
- Time of sampling is also critical (e.g., cortisol requires 9 AM & 9 PM collection). Label samples carefully, including the time of collection! Check procedures with the local laboratory.

3) Do tests in the right sequence.

- ACTH levels can only be interpreted once the cortisol status is known.
- In many cases, simultaneous samples are required for interpretation, e.g. parathyroid hormone (PTH) with calcium for hypo/hyperparathyroidism; glucose with insulin for insulinoma; thyroid hormones (T3&T4) with thyroid-stimulating hormone (TSH).

4) ***Normal results may be abnormal depending on the activity of the hormone axis under investigation*****.**

- Interpretation of the absolute levels of hormones alone may be highly misleading.
- For example, a serum PTH within the normal range in the presence of hypocalcemia suggests hypoparathyroidism; normal luteinizing hormone (LH) and follicle-stimulating hormone (FSH) levels in the presence of a very low serum testosterone concentration suggest pituitary failure.
- Thus, the level of the regulatory hormone (or releasing factor) must be considered in the light of the simultaneous level of the 'target' hormone or metabolite.
- Also, target hormone excess should be evaluated with the appropriate tropic hormone to rule out ectopic hormone production, which is usually caused by a **hormone-secreting tumor**.
- Elevation of the hormone and the substrate that it regulates such as high plasma glucose and insulin levels suggests a **hormone-resistance state**.

Table 1: Interpretation of hormone levels

Pituitary hormone level	Target hormone level		
	Low	Normal	High
High	Primary failure of target endocrine organ		Autonomous secretion of pituitary hormone or resistance to target hormone action. (impaired negative feedback mechanisms)
Normal		Normal	
Low	Pituitary failure		Autonomous secretion by target endocrine organ

5) Results may vary according to the lab assay

- For example, different prolactin assays cross-react very differently with macroprolactin.
- Reference ranges also vary between labs (interpret your tests according to your local lab's normal range).
- Some individuals have a heterophile interfering antibody that affects the results of many radioimmunoassays (**Do not** discard the clinical evidence in favor of a numerical value).

6) Beware of interfering medication

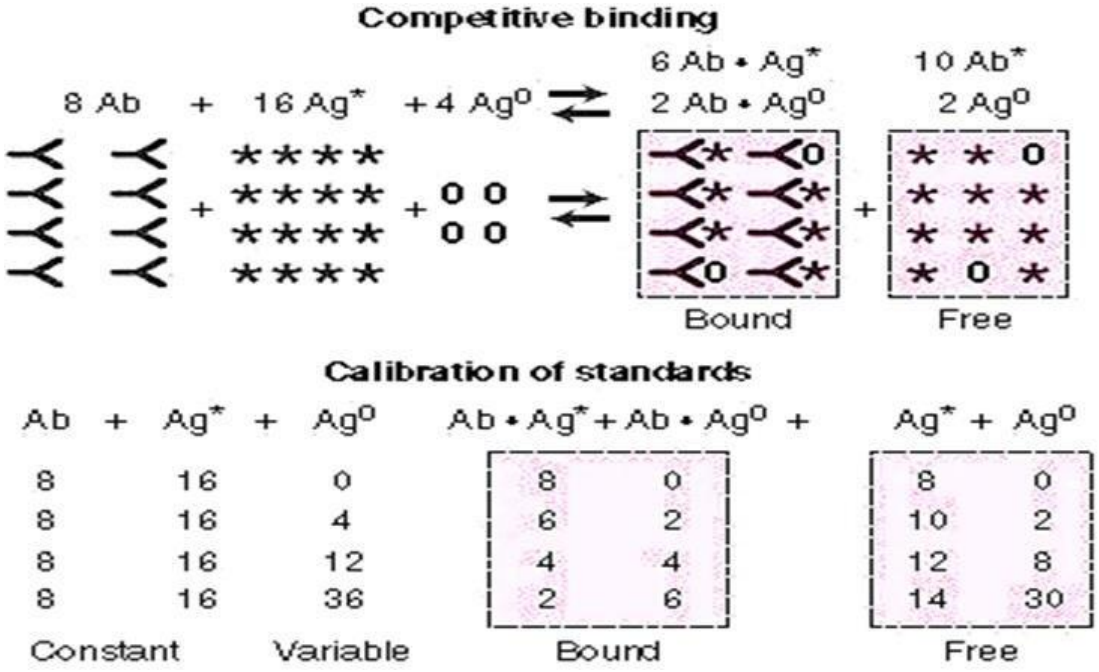
- Inhaled beclomethasone can suppress serum cortisol levels.
- Synthetic androgens and estrogens (contraceptive pills) can cause low serum testosterone/estrogen.
- Some anti-emetics and antipsychotics raise circulating prolactin levels.
- Carbenoxolone or liquorice may cause hypokalemia.
- Always ask the patient for a full medication list (including herbal remedies and other self-medication).

7) *Take a family history,* which may be required for discrimination between diabetes type 1 and 2.

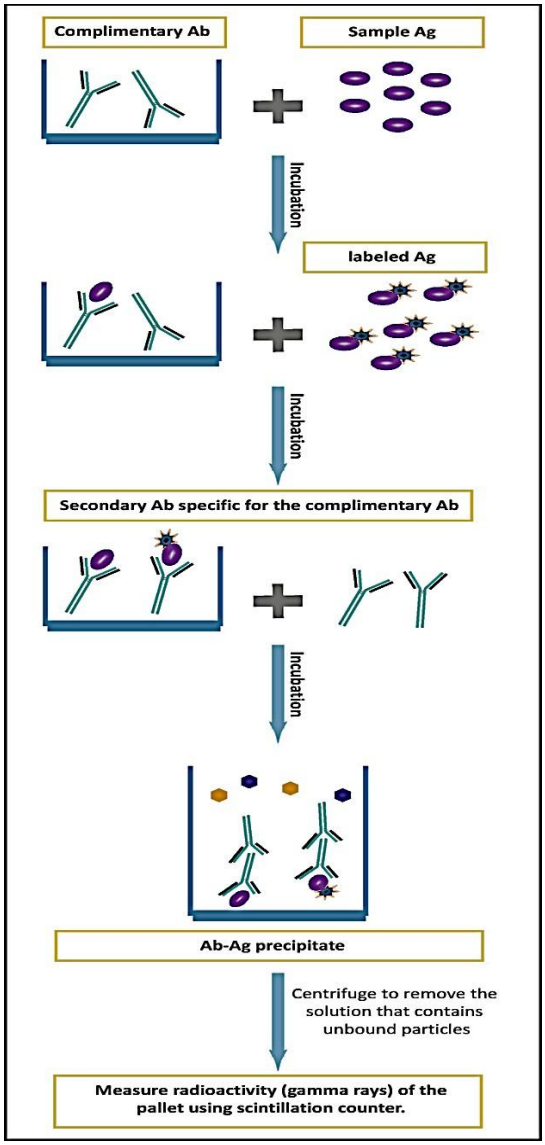
Laboratory Techniques for Hormone Measurement

- ☒ Hormones are generally measured using **radioimmunoassay (RIA)**, **enzyme-linked immunosorbent**, or **chemiluminescent enzyme assays**, although **mass spectrometry** is increasingly used.
- ☒ Immunoassay is a broad term for one of two different techniques: **true immunoassay** and **immunometric assay**.
- ☒ Both forms are based on the hormone to be measured being **antigenic** and bound by specific **antibodies** to form an **antibody-antigen complex**.
- ☒ Both forms also employ a label (radioisotope, fluorescent or enzyme tracer) to generate a quantitative signal.
- ☒ Both assays rely on comparing the patient sample with known concentrations (5-8) of a reference compound (to set up a **calibration** or **standard curve**).

1) Immunoassays (the competitive-binding assay)



a) The competitive radioimmunoassay

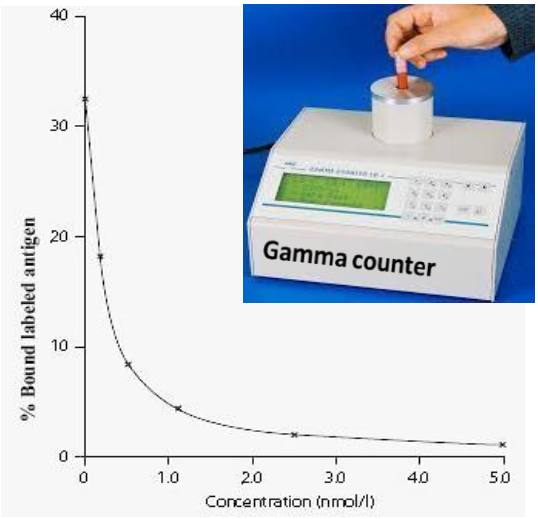


- Is based on the competitive reaction between a determined amount of antigen labeled with a radioisotope (usually ^{125}I or ^{131}I; Ag*) and an unknown amount of antigen in the sample for a limited number of binding sites on an antibody.

- The separation of the unbound components is mostly done by precipitation and subsequent centrifugation of the antigen-antibody complex, with the help of a 2^{ry} antibody and addition of a precipitation catalyst (such as polyethylene glycol).

- The concentration of the sample antigen can then be determined by measuring the radioactivity of the pellet; therefore, <u>the more sample antigen present in the sample, the less radiation is obtained.</u>

- A standard dilution series is used for calibration: % bound Ag* are plotted against known concentrations of the used antigen in a standard graph.

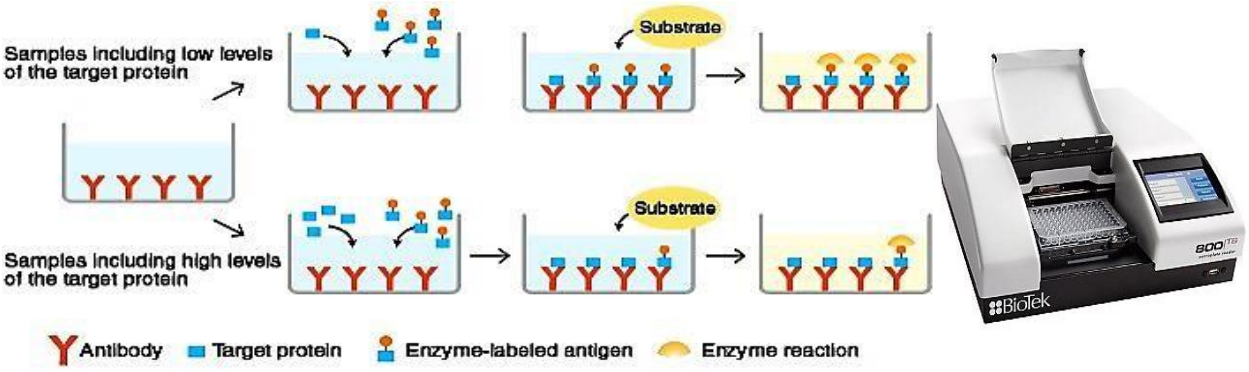


- Since the technique requires the use of radioisotopes, which has safety concerns and short shelf-life; RIA was modified and replaced by assays that use colorimetric, fluorometric, or chemiluminescent signals rather than radioactivity to quantify the analyte of interest.

- The advantages of these non-isotopic labeling technologies include biosafety, longer reagent shelf life, ease of automation, and reduced cost. However, they can be more subject to matrix interferences than radioactive detection systems. Radioactivity is not affected by changes in the analyte concentration, hemolysis, color or drugs.

b) The competitive enzyme-linked immunosorbent assay (ELISA)

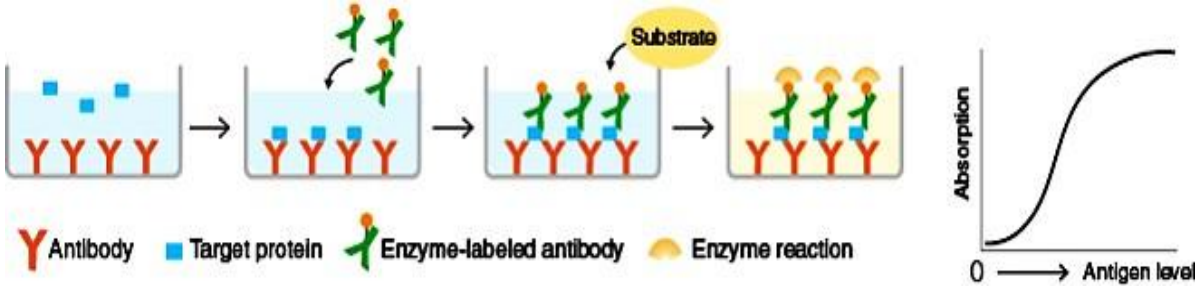
- Competitive ELISA is useful for measurement of low molecular weight targets.



- A constant amount of antibody (capture Ab) specific for a target analyte is immobilized on the surface of microplate wells (solid phase) and incubated with samples containing the target analyte and a known amount of enzyme-labeled target analyte. After the reaction, the activity of the well-bound enzyme is measured.

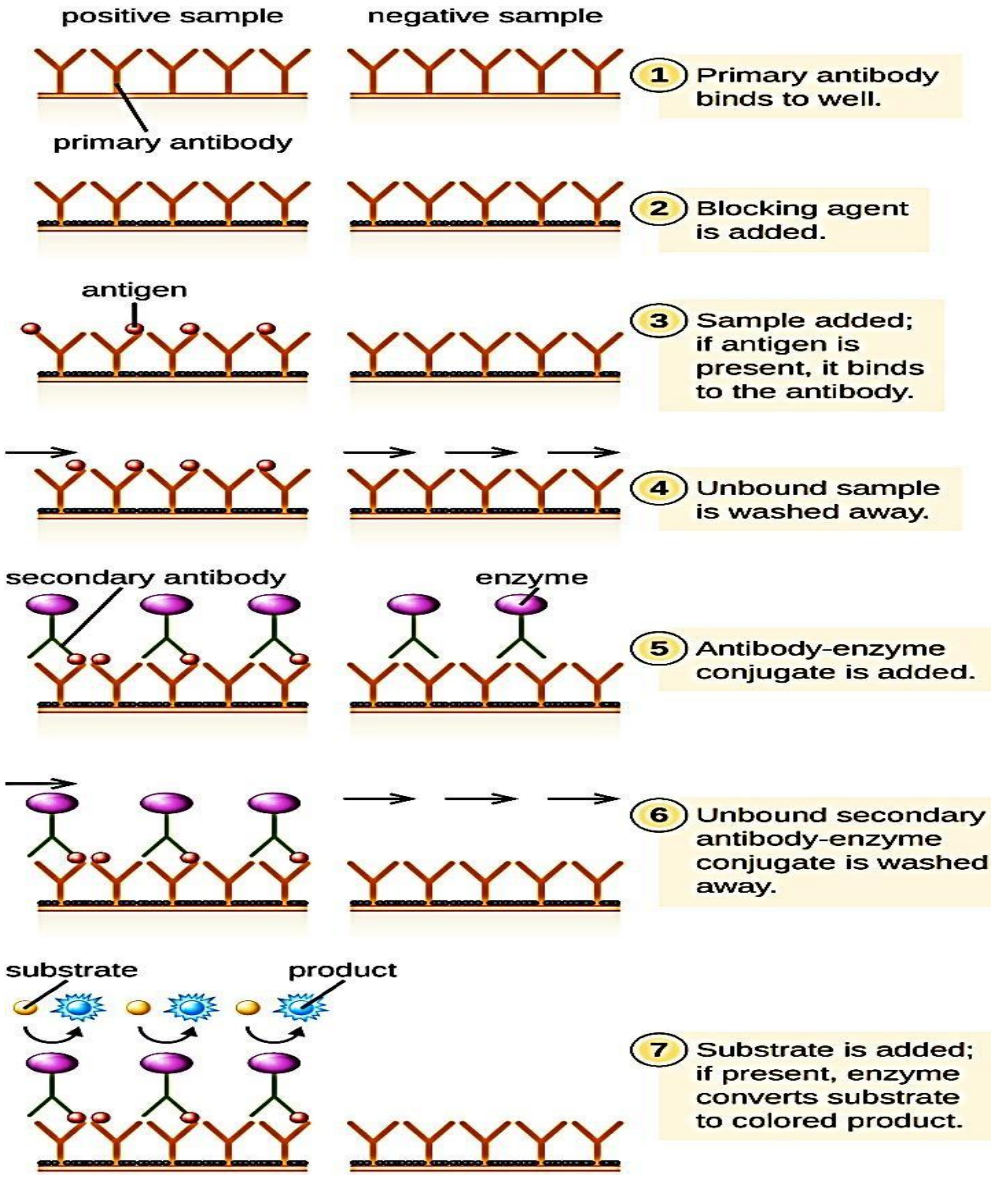
2) Immunometric assays (Sandwich ELISA)

- Used for measuring antigens containing multiple antibody-binding epitopes (i.e., protein hormones).



- A constant amount of antibody (capture or 1^{ry} Ab) to a target analyte is immobilized on the surface of microplate wells and incubated first with the target analyte.

- After incubation, the amount of hormone bound to the 1ry antibody is detected by adding another target hormone-specific antibody (detection or 2^{ry} Ab).
- This second antibody is already covalently attached (conjugated) to an enzyme such as **alkaline phosphatase** or **horseradish peroxidase** which can catalyze a **chromogenic** reaction.
- When the color reagents are added, the intensity of color that develops depends on the amount of enzyme which ultimately depends on the amount of the unknown analyte in the sample.
- The immobilized antibody and the enzyme-labeled antibody must recognize different epitopes on the target hormone.



Note:

Enzyme-linked fluorescent immunoassay (ELFA) is performed in automated instruments. A fluorogenic substrate such as 4-methylumbelliferyl phosphate is converted by alkaline phosphatase to the blue-fluorescent 4-methylumbelliferone. The intensity of fluorescence is measured by the optical scanner in the instrument; it is proportional to the target hormone concentration in the sample.

Mass spectrometry (MS)

- In some situations, immunoassays are unreliable or unavailable, commonly because antibodies lack sufficient specificity or there are difficulties with measurements at low concentrations (e.g. serum testosterone in women).
- Mass spectrometry is applied either by itself or in tandem (MS/MS) or downstream of liquid chromatography (LC/MS) or gas chromatography (GC/MS).

Reference ranges

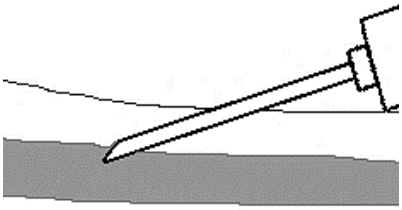
- Whenever possible, hormones are measured in molar units (e.g. pmol/L) or mass units (e.g. ng/L).
- However, this is not possible for complex hormones such as the glycoproteins TSH, LH and FSH, because they circulate in a variety of slightly different forms.
- In this scenario, international reference preparations are agreed, with potency expressed in ‘units’ (U) and their subdivisions [e.g. milliunits (mU)].

Self-Study Questions

Choose the most correct answer.

1- Regarding venipuncture, this trouble is..... and the suggested solution is ……

a. Hematoma; withdraw the needle.
b. Incomplete collection; move the needle forward.
c. Hematoma; move the needle backward.
d. Incomplete collection; adjust the angle of the needle.



2- Which of the following would be expected to alter hormone levels?

a. Changes in mineral and nutrient plasma levels
b. Pituitary tumor
c. Transatlantic flight
d. Training for the Olympics
e. All of the above

3- Samples used for RIA are cleaned:

a. Using chromatography.
b. To concentrate the analyte.
c. When the amount of the analyte is low.
d. All the above.

4- The RIA test requires:

a. Radioisotopes b. Enzyme c. Fluorescent dye d. Gold particles

5- In RIA the initial amount of antigen in the sample is …… proportional to the bound radioactivity.

a. directly b. not c. inversely

6- Why is a positive and negative control necessary in the setup of ELISA?

a. Positive controls are equal to negative controls.
b. ELISA is subject to errors; if controls fail the results are untrustworthy.
c. ELISA is a well-run test that typically does not need controls.
d. Negative and positive controls are needed to exclude all results.

7- Why is it necessary to wash the wells repeatedly during the ELISA run?

a. It is important to keep the wells clean.

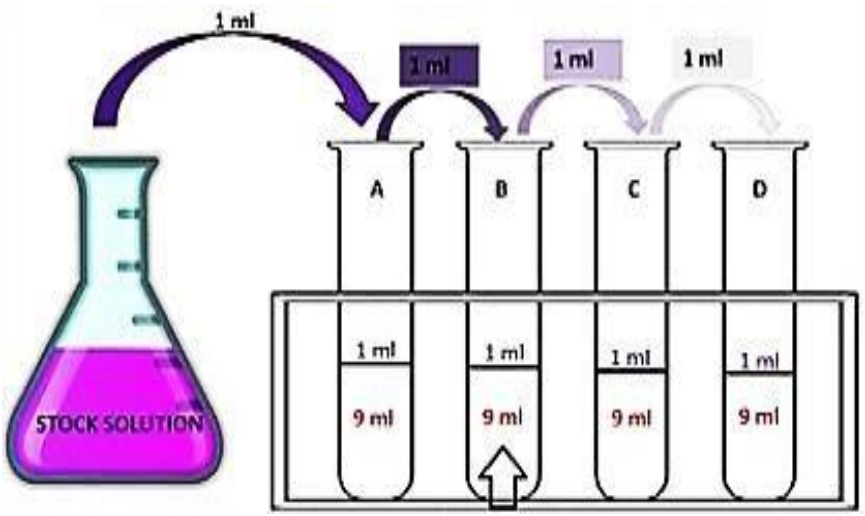
b. It is important to wash away all bound and unbound antibodies from the wells.

c. It is important to wash away the unbound antibodies.

d. Washing the wells is just solid lab practice.

8- What would be the 4th tube dilution?

a. 1/10 b. 2/10 c. 1/100 d. 1/10000

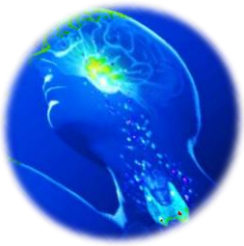


9- From what cells do antibodies originate?

a. B lymphocytes b. T lymphocytes c. RBCs d. Plasma cells

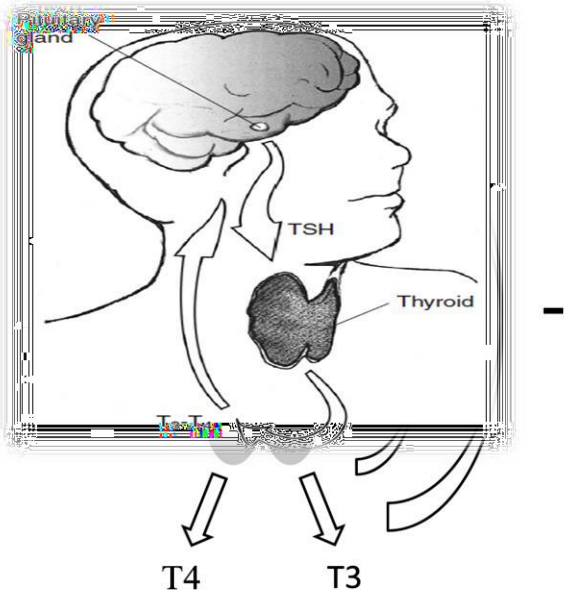
10- What is another name for antibodies?

a. Hemoglobin b. Complements c. Immunoglobulins



Quantitative Determination of the Thyroid Function Tests

Hormonal control



- The thyroid makes three hormones: Thyroxin (T4), triiodothyronine (T3) and calcitonin; only a small amount of T3 in the blood comes from the thyroid. **Most T3 is made from T4.**
- Thyroid-releasing hormone (TRH) produced **by the hypothalamus** which stimulates Thyroid-stimulating hormone (TSH), made by the **pituitary gland** in the brain, regulates thyroid hormone production.
- When thyroid hormone levels in the blood are **low**, the pituitary releases **more** TSH. When thyroid hormone levels are **high**, the pituitary **decreases** TSH production.

Tests to evaluate thyroid function include the following: **TSH, T4 and T3.**

(1) Quantitative Determination of the TSH

Purpose of the TSH test

- Evaluating the thyroid function and how well the thyroid is working.
- Diagnose and find the cause of thyroid disorders such as hyperthyroidism and hypothyroidism.
- Differentiate among primary (thyroid) from secondary (pituitary) and tertiary (hypothalamus) hypothyroidism.

Principle of the TSH test

- The test based on solid-phase enzyme immunoassay.
- This assay uses two mouse monoclonal antibodies (Mab) directed against distinct antigenic determinants on the TSH molecule. The polystyrene wells are coated with a captured antibody against TSH.
- Standards, controls, and patient samples are added to the wells the TSH present in the wells is bound to the anti-TSH antibodies.
- The HRP labeled anti-TSH is added to the wells.
- A solution of TMB is added to wells cause the development of a blue color.
- The intensity of the color is proportional to the amount of TSH present in the sample.
- The color development is stopped by the addition of stop solution, causing the blue color to change to yellow.

Sample collection and preparation

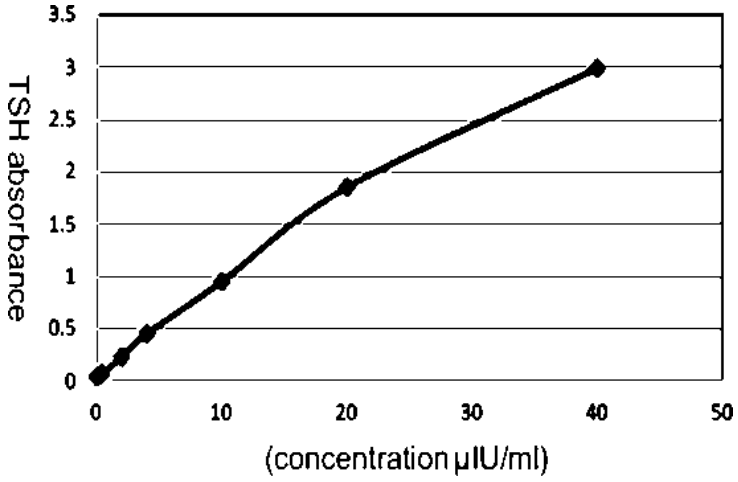
- TSH blood test is similar to other simple blood tests. Blood usually draw from a vein on the inside of the elbow.
- They will begin by cleaning the skin and may then place an elastic band around the upper arm to make the vein easier to access.
- They will insert a needle into the vein, allowing blood to flow into the connecting tube and vial.
- Once the vial contains enough blood to carry out the test, the healthcare professional will remove the needle and elastic band and place cotton wool or a bandage over the puncture site.
- After labeling the blood sample, they will send it to a laboratory for testing

The procedure of the TSH test

1. Pipet 50 µl standard, control or sample.
2. Pipet 100 µl TSH-HRP tracer.
3. Incubate 60 min. at RT.
4. Wash x 4 (300 µl).
5. Pipet 100 µl TMB.
6. Incubate 20 min. at RT.
7. Pipet 100 µl stop solution.
8. Read at 450/630 nm.

Calculation of results

- Standard curves are constructed for each assay by plotting absorbance value against the concentration of each standard.
- The TSH concentrations of patient samples are then read from the standard curve.



Abnormal values

Note: An abnormal TSH indicates an excess or deficiency in the thyroid hormone in the body, but it does not indicate the reason why so usually followed by additional testing to investigate the cause of the increase or decrease.

A) <u>Causes of a low level of TSH:</u>

- An overactive thyroid gland (hyperthyroidism) as **Graves disease**.
- Excessive amounts of thyroid hormone medication taken by those who are being treated for an underactive (or removed) thyroid gland.
- Insufficient anti-thyroid medication in a person treated for hyperthyroidism.
- Damage to the pituitary gland.
- Thyroid cancer.
- Decreased TSH concentration is observed in primary hyperthyroidism, secondary and tertiary hypothyroidism.

B) <u>Causes of a high level of TSH:</u>

- An underactive thyroid gland (1^{ry} hypothyroidism) as **Hashimoto thyroiditis**.
- TSH-producing pituitary tumor.
- A person with hypothyroidism receiving too much thyroid medication.
- A rare inherited disorder is present in which the body and/or pituitary do not respond normally to thyroid hormones, resulting in high TSH despite clinically normal thyroid function.

(2) Quantitative Determination of T4

- **T4 (thyroxin** or 3,3',5,5'-L-tetraiodothyronine) the primary secretory product of the normal thyroid gland, is secreted into the circulation in response to TSH.
- This secretion is regulated by a negative feedback mechanism involving the thyroid, hypothalamus and pituitary gland.

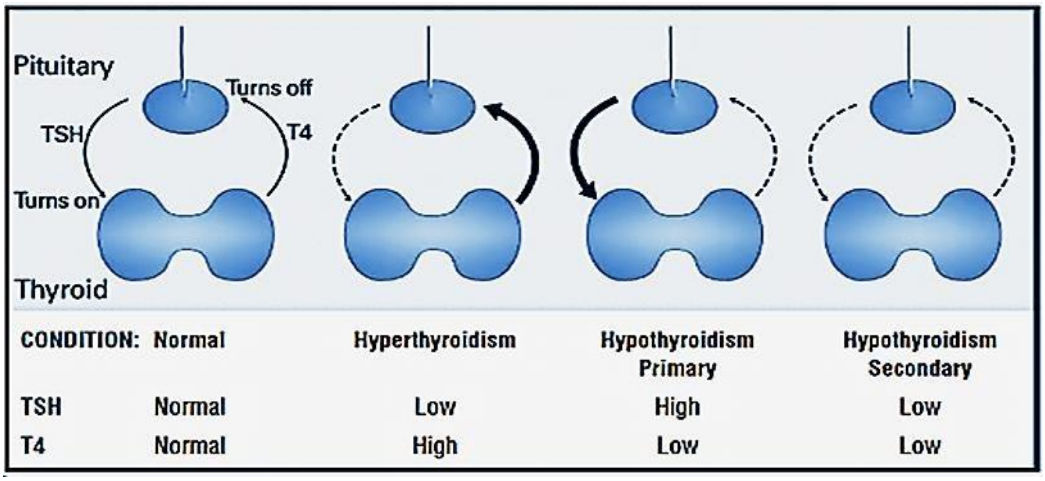
<u>T4 circulates in the blood in two forms:</u>

1) **T4 bound to proteins** that prevent the T4 from entering the various tissues that need thyroid hormone.

2) **Free T4,** which does enter the various target tissues to exert its effects. The free T4 fraction is the most important to determine how the thyroid is functioning.

<u>Purpose of the T4 test</u>

- ❖ Free T4 tests are used to help evaluate thyroid function.
- ❖ Diagnose thyroid diseases, including hyperthyroidism and hypothyroidism (Usually after discovering that the TSH level is abnormal).



Principle of the T4 test

- The T4 EIA kit is a solid-phase competitive binding enzyme immunoassay which is performed in the wells coated with captured antibody.
- A sample containing an unknown amount of the analyte to be assayed and a constant amount of T4 conjugated with HRP is added to the wells. During the incubation, T4 in the patient serum and T4-HRP, in presence of blocking agent (ANSA) compete for limited binding sites on the wells. The amount of T4-HRP that binds to the anti-T4-Ab is inversely proportional to the T4 concentration in the test sample.
- After incubation, excess enzyme-conjugated T4 is removed through a wash step and a solution of TMB (3,3',5,5' tetramethylbenzidine) is added to each well, resulting in the development of a blue color. The color development is stopped by the addition of stop solution, causing the blue color to change to yellow.

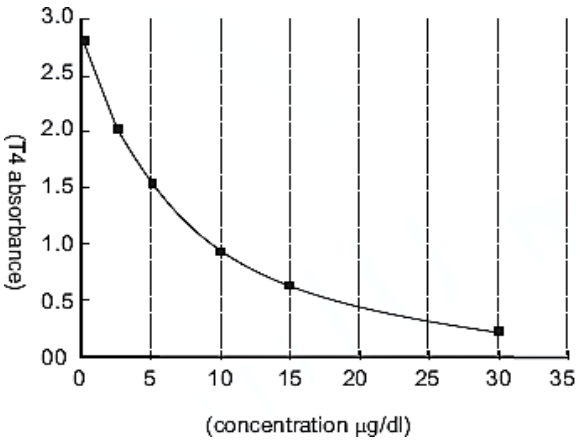
The procedure of the T4 test

All reagents should be brought to RT prior to use.

1. Pipet 25 µl standard, control or sample.
2. Pipet 100 µl T4-HRP Tracer. Mix one minute.
3. Incubate 60 min. at RT.
4. Wash x 4 (300 µl).
5. Pipet 100 µl TMB.
6. Incubate 20 min. at RT.
7. Pipet 100 µl stop solution.
8. Read at 450/630 nm.

Calculation of results

- ❖ Standard curves are constructed for each assay by plotting absorbance value against the concentration of each standard.
- ❖ The T4 concentrations of patient samples are then read from the standard curve.



(3) Quantitative Determination of T3

T3 exists in two forms in the blood:

1. **Bound T3**, the more abundant form, is attached to proteins that help transport the hormone through the body.
2. **Free T3**, the less abundant form, circulates unattached.

- ✓ The T3 total test, the most common type of T3 blood test, measures both the bound and free forms of T3 in your child's blood.
- ✓ T3 helps control many body functions including growth, body temperature, and heart rate.
- ✓ Most T3 in the blood is produced in the body where T4 (the major thyroid hormone produced by the thyroid gland) is chemically converted to T3.

Purpose of the T3 test

- Monitor the effectiveness of treatment for hyperthyroidism.
- T3 test is performed as part of an evaluation of thyroid function.

Principle of the T3 test

- The T3 EIA kit is a solid phase competitive binding enzyme immunoassay which is performed in wells coated with captured antibody.
- In this assay, a sample containing an unknown amount of the analyte to be assayed and a constant amount of T3 conjugated with HRP is added to the wells. During incubation, T3 in the patient serum and T3-HRP in presence of blocking agent (ANS) compete for limited binding sites on the wells.
- The amount of T3-HRP that binds to the anti-T3-Ab is inversely proportional to the T3 concentration in the test sample.

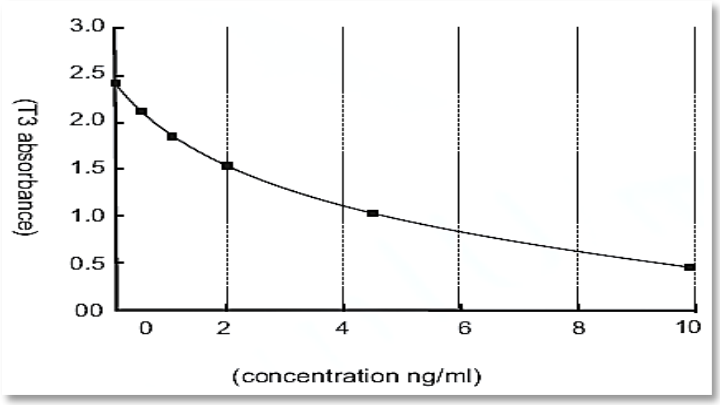
- After incubation, excess enzyme-conjugated T3 is removed through a wash step and a solution of TMB is added to each well, resulting in the development of a blue color. The color development is stopped by addition of stop solution, causing the blue color to change to yellow.

The procedure of the T3 test

1. Pipet 100 µl standard, control or sample.
2. Pipet 100 µl T3-HRP Tracer. Mix one minute.
3. Incubate 60 min. at RT.
4. Wash x 4 (300 µl).
5. Pipet 100 µl TMB.
6. Incubate 20 min. at RT.
7. Pipet 100 µl stop solution.
8. Read at 450/630 nm.

Calculation of results

- Standard curves are constructed for each assay by plotting absorbance value against the concentration of each standard.
- The T3 concentration of patient samples are then read from the standard curve.



Normal values

Test	Range	Unit
TSH	0.4 - 5	mU/L
T3	0.92 - 2.78	nmol/L
FT3	0.22 - 6.78	PmoL/L
T4	58 – 140	nmol/L
FT4	10.3 – 35	PmoL/L

<u>The following table summarizes some examples of typical test results and their potential meaning.</u>

NOTE: LABORATORY RESULTS MUST ALWAYS BE CORRELATED WITH THE CLINICAL FINDINGS OF THE PATIENT.

TSH	FREE T4	TOTAL OR FREE T3	MOST LIKELY DIAGNOSIS
Normal	Normal	Normal	Normal thyroid function (e.g., "euthyroid")
Normal or decreased	Normal or decreased	Decreased	Normal adjustment in thyroid function due to illness (nonthyroidal illness or sick euthyroid syndrome)
Increased	Normal	Normal	Subclinical hypothyroidism[1]; in a person with hypothyroidism on treatment, not enough thyroid hormone is being given
Increased	Decreased	Normal of decreased	Hypothyroidism resulting from a problem with the thyroid gland itself (primary hypothyroidism)
Normal or increased	Increased	Increased	Hyperthyroidism resulting from a problem with the pituitary gland signals (central hyperthyroidism) or from a problem with the thyroid hormone receptor (thyroid hormone resistance)
Decreased	Normal	Normal	Subclinical hyperthyroidism[2]; in a person with hypothyroidism, too much thyroid hormone is being given
Decreased	Normal	Increased	Hyperthyroidism resulting from the thyroid gland making too much active thyroid hormone T3 (uncommon, also known as T3 toxicosis)
Decreased	Increased	Increased	Hyperthyroidism resulting from the gland making too much thyroid hormones (primary hyperthyroidism)
Decreased	Decreased	Decreased	Hypothyroidism resulting from a problem with the hypothalamus or pituitary signals that govern the thyroid gland (central hypothyroidism)

Self-Study Questions

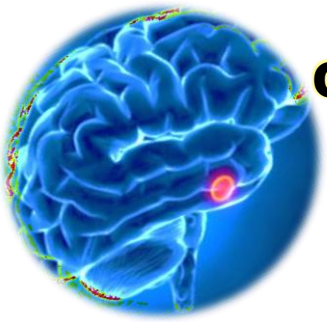
1. **When thyroid hormone levels are high, the pituitary..... TSH production.**
    a. decreases
    b. increases
    c. not change
    d. none of the above

2. **A high TSH result may mean that:**
    a. presence of Graves' disease
    b. Damage to the pituitary gland
    c. presences of Hashimoto thyroiditis
    d. An overactive thyroid gland (hyperthyroidism)

3. **A low TSH result may mean that** .....
    a. presence of Graves' disease
    b. the pituitary tumor
    c. presences of Hashimoto thyroiditis
    d. An underactive thyroid gland (hypothyroidism)

4. **One of the hormones used for determination the thyroid function is ...**
    a. LH
    b. TSH
    c. FSH
    d. GH

5. **T3 test may be ordered as part of the investigative workup when a person has symptoms suggesting ……..**
    a. hyperthyroidism
    b. Hashimoto thyroiditis
    c. Addison disease
    d. hypothyroidism

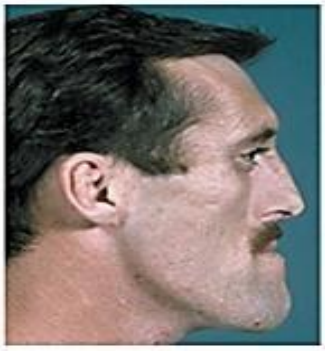


Quantitative Determination of the Growth Hormone

Growth Hormone (GH)

- Also known as somatotropin and pituitary growth hormone.
- It consists of about 190 amino acids synthesized and secreted by the **anterior pituitary**.
- Overproduction of GH can result in childhood cause **gigantism** or after puberty cause **acromegaly** whereas deficiency or defects in its binding to the receptors can contribute to **dwarfism** in childhood.

(1) Gigantism



(2) Acromegaly

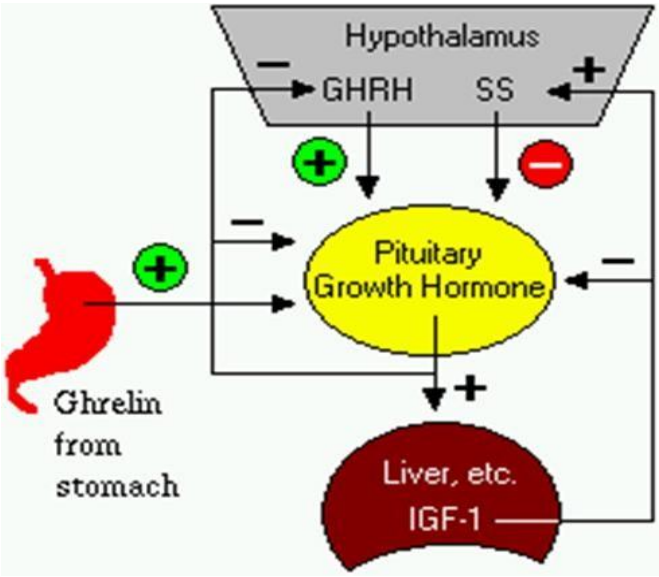
(3) Dwarfism

Hormonal control of GH

- Production induced by stress, exercise, nutrition, sleep and GH itself.
- Primary controllers:

A) Growth hormone-releasing hormone (GHRH) and Growth hormone inhibitory hormone (somatostatin; SS) produced by the **hypothalamus**.

B) Ghrelin produced from the **stomach**.



Purpose of the GH test

- ❖ Diagnose GH deficiency or excess and evaluate pituitary function.
- ❖ Monitor the effectiveness of treatment for excess production of GH.
- ❖ Diagnose and monitor the treatment of acromegaly and gigantism.

Principle of the test

- ❖ The test based on solid-phase enzyme immunoassay.
- ❖ This assay system uses two mouse **monoclonal antibodies** directed against distinct antigenic determinants on the GH molecule. The polystyrene wells are coated with a captured antibody against GH.
- ❖ Standards, controls, and patient samples are added to the wells (solid phase) and incubate. The GH present in the wells is bound to the anti-GH antibodies.
- ❖ The unbound material is removed by washing. After washing, the HRP labeled anti- GH Mab is added to the wells. After second incubation and washing, a solution of TMB is added to each well, resulting in the development of a blue color. The color development is stopped by the addition of stop solution, causing the blue color to change to yellow.

Sample collection and preparation

Patient prepare for the procedure:

- ➢ Fast for 10-12 hours before the test.
- ➢ Reduce physical activity for 10-12 hours before the test.
- ➢ If instructed by your doctor, stop taking regularly prescribed medication before the test.
- ➢ Ninety minutes before the test, rest and relax and avoid physical activity.

Usually several blood samples, drawn at timed intervals from veins in arms.

- ➢ The assay can be performed on serum or heparinized plasma samples.
- ➢ Keep samples at 2-8°C for 1-2 days; for longer periods store the sample in aliquots at -20°C.
- ➢ Avoid repeated freezing and thawing of samples.
- ➢ Do not vortex patient samples.

Note:

I) Sample rejected due to clots, hemolysis, lipemia and icterus (bilirubin).

II) Prior to assay, frozen specimens should be slowly brought to room temperature and gently mixed by hand.

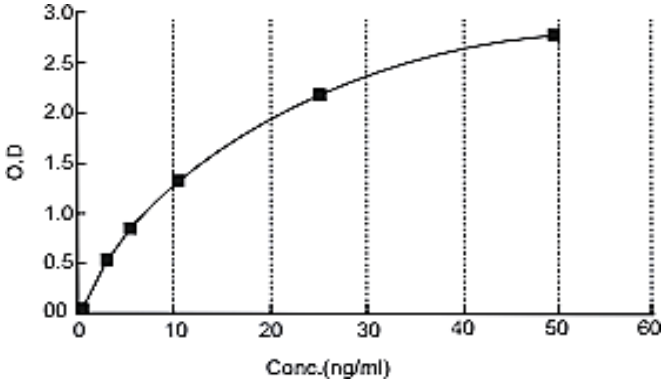
Procedure

1. Pipet 25 µl standard, control or sample.
2. Pipet 200 µl dilution buffer.
3. Incubate 30 min. at RT. then Wash x 4.
4. Pipet 50 µl an Anti-GH tracer.
5. Incubate 30 min. at RT. then Wash x 4.
6. Pipet 100 µl TMB.
7. Incubate 15 min. at RT.
8. Pipet 100 µl stop solution.
9. Read at 450/630 nm.

Note: 350 ml washing buffer used in each time.

Calculation of results

- The results can be calculated by microliter plate spectrophotometer reader or manual evaluation.
- For manual evaluation, a standard curve is constructed by plotting the absorbance (A) values obtained for each GH standard against the corresponding GH concentrations (ng/ml).
- The unknown GH concentration can then be read from the standard curve using the absorbance value of each patient specimen.



Normal values

Note: **III(** GH is released in pulses. The size and duration of the pulses vary with time of day, age and gender. A higher level may be normal if the blood was drawn during a pulse. A lower level may be normal if the blood was drawn around the end of a pulse.

➢ Because of the episodic and pulsatile secretion of GH, determination of the basal serum level of GH is difficult, the intervals of **serum GH concentration in a healthy population is defined** as follow:

- **Children:** 0 - 10 ng/mL
- **Adult males**: 0 - 4 ng/mL
- **Adult females:** 0 - 18 ng/mL

Abnormal values

A) *Causes decrease GH level:*

❖ Dwarfism.
❖ Infection or tuberculosis.
❖ Chemotherapy and radiotherapy.
❖ Traumatic brain injury.
❖ Hypopituitarism (the low function of the pituitary gland).
❖ Pituitary tumors and Parapituitary tumors.
❖ Genetic causes as Laron syndrome or pituitary transcription factor 1 defect.
❖ Pituitary apoplexy.
❖ Sheehan's syndrome.

B) *Causes of increased GH level:*

❖ Too much GH in adults (acromegaly).
❖ Abnormal growth due to excess GH during childhood (gigantism).
❖ GH resistance.
❖ Pituitary tumor.
❖ Disorders of the GH/IGF1 system.

Technical hints

➢ Avoid foaming or bubbles when mixing or reconstituting components.
➢ Avoid cross-contamination of samples or reagents by changing tips between sample, standard and reagent additions.
➢ Ensure plates are properly sealed or covered during incubation steps.
➢ Complete removal of all solutions and buffers during wash steps is necessary to minimize background.

- All samples should be mixed thoroughly and gently.
- Avoid multiple freeze/thaw of samples.
- Samples generating values higher than the highest standard should be further diluted in the appropriate sample dilution buffers.

Troubleshooting

Problem	Reason	Solution
Poor standard curve	Inaccurate pipetting	Check pipettes
	Improper standard dilution	Prior to opening, briefly spin the stock standard tube and dissolve the powder thoroughly by gentle mixing.
Low signal	Incubation times too brief	Ensure sufficient incubation times; increase to 2 or 3 hours standard/sample incubation.
	Inadequate reagent volumes or improper dilution	Check pipettes and ensure correct preparation.
	Incubation times with the TMB too brief	Ensure sufficient incubation time until blue color develops prior addition of stop solution.
Low sensitivity	Improper storage of the ELISA kit	Store your reconstituted standards at -80°C, all other assay components 4°C. Keep the TMB development solution protected from light.
Precipitate in diluent	Precipitation and/or coagulation of components within the diluent	The precipitate can be removed by gently warming the diluent to 37ºC.

Dynamic tests of GH status

- ✶ Because of the pulsatile nature of GH secretion, the measurement of random GH serum levels is unhelpful in diagnosing GH deficiency (GHD).
- ✶ Therefore, investigators have used a range of physiological and pharmacological stimuli to provoke the release of GH.
- ✶ Physiological methods have included fasting, exercise and sleep.
- ✶ An impressive number of pharmacological agents have been used to stimulate GH.

Pharmacologic agent	Side-effect
Clonidine	Tiredness
Levodopa	Nausea
Arginine HCl	Postural hypotension
Glucagon	Nausea
Insulin	Hypoglycemia

- ✶ Although the insulin tolerance test (ITT) is labor intensive, contraindicated in the elderly and in adults with seizure disorders and ischemic heart disease, can be unpleasant for the patient, and is potentially hazardous, this test remains the gold standard test for the biochemical demonstration of GHD in adults.

Test	Results
75 g oral glucose tolerance test (OGTT)	Rapid suppression of GH secretion to a nadir of < 0.3 ng/mL if normal. Remains high in acromegaly or gigantism.
Insulin tolerance test [serum glucose ≤ 40 mg/dL]	Stimulation of GH secretion: > 6.7 ng/mL, normal. 3-6.7 ng/mL, partial deficiency. < 3 ng/mL, severe GH deficiency.
Amino acid infusion (commonly arginine)	Stimulation of GH secretion (useful in patients where insulin-induced hypoglycemia is undesirable; e.g. in children)

Self-Study Questions

Complete the following questions:

1. The main role of GH on hepatocytes is to induce the formation of …….
2. The condition is caused by excessive GH after puberty. It does not increase the length of bones but does increase the growth of soft tissues ….
3. The main target organ for growth hormone that causes its long-term effect related to growth…….
4. Failure of the pituitary to stop producing growth hormone after body growth is completed results in…….

Choose the correct answer:

1. Which of the following substances are produced by the pituitary gland:

a. Luteinizing hormone
b. Growth hormone
c. Follicle-stimulating hormone
d. Adrenocorticotropic hormone
e. Prolactin
f. All of these

2. What test is used to diagnose growth hormone deficiency:

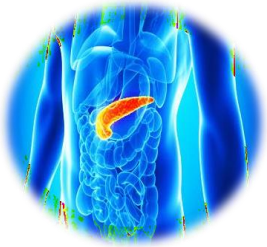
a. growth hormone stimulation test
b. pituitary test
c. lumbar puncture
d. a simple blood test

3. Which of the following is Growth hormone inhibiting hormone?

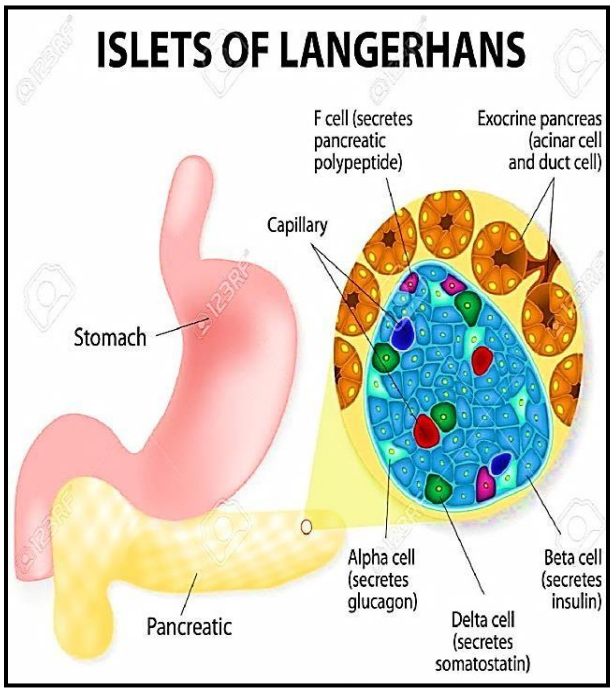
a. FSH
b. TRH
c. GHRH
d. Somatostatin

4. The growth hormone produced by the pituitary gland is known as:

a. somatotropin
b. prolactin
c. Luteinizing hormone
d. follicle-stimulating hormone

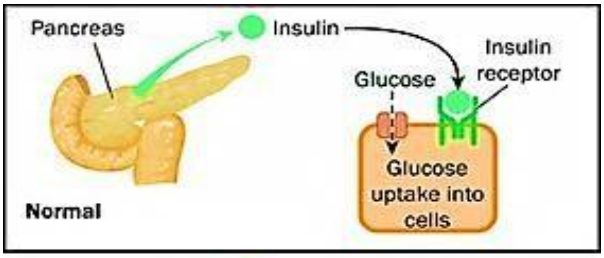


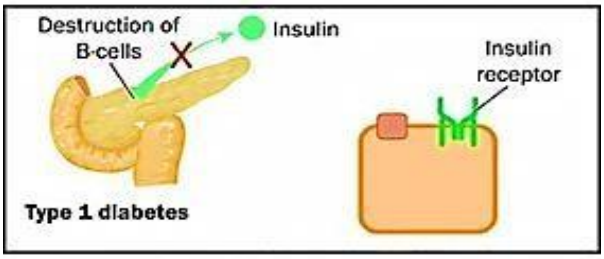
Quantitative Determination of Insulin

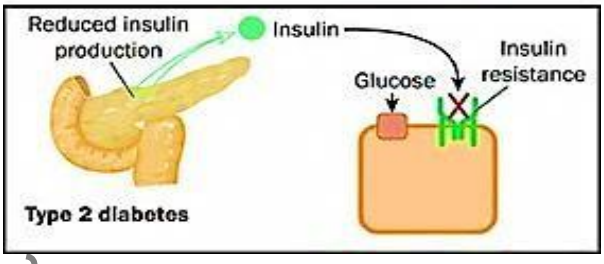


- Insulin is made by specialized areas within the pancreas called **islets of Langerhans**.

- Plays a role in keeping glucose at the right levels. If glucose levels are too high or too low cause serious health problems.
  - **Hyperglycemia**, blood glucose levels are too high. It happens when the body doesn't make enough insulin, so glucose can't get into cells. It stays in the bloodstream instead.
  - **Hypoglycemia**, blood glucose levels are too low. If the body sends too much insulin into the blood, too much glucose will go into cells. This leaves less in the bloodstream.

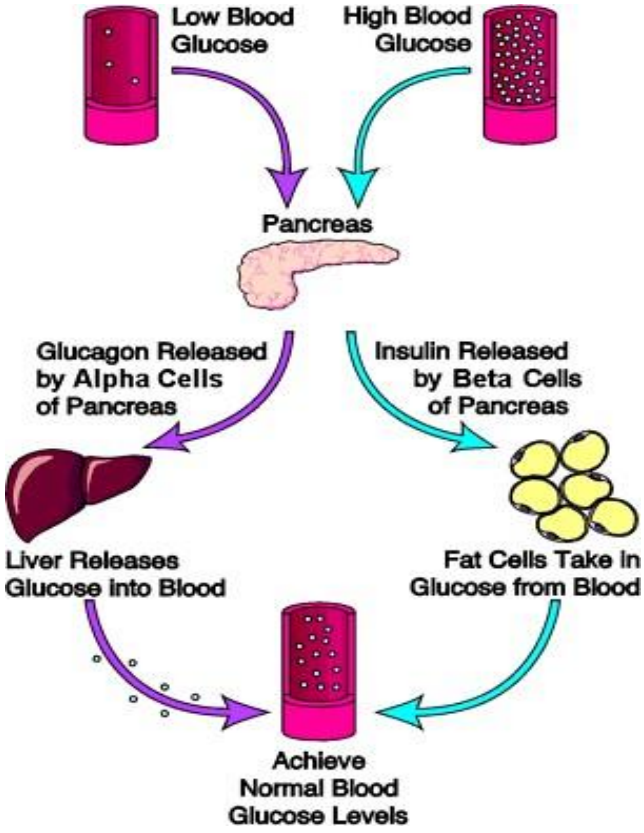






- **Diabetes** is the most common cause of abnormal glucose levels.
  - **Type 1 Diabetes.** The body makes little or no insulin at all.
  - **Type 2 Diabetes.** Body able to make insulin, but cells in the body don't respond well and can't easily take up enough glucose from blood. This is called insulin resistance.

Hormonal control



- ✓ The release of insulin is tightly regulated to balance food intake and the metabolic needs of the body. This is a complex process and other hormones found in the gut and pancreas also contribute to this regulation.
- ✓ The rise in blood glucose levels causes insulin release from the pancreas so glucose can move inside the cells and be used.
- ✓ As glucose enters the cells, the amount of glucose in the bloodstream returns to normal and insulin release slows down.
- ✓ Proteins and hormones produced by the gut stimulate insulin release.
- ✓ Hormones released in times of acute stress, **such as adrenaline**, stop the release of insulin.
- ✓ Insulin works in tandem with glucagon, another hormone produced by the pancreas. Insulin lowers blood sugar levels if needed, glucagon raises blood sugar levels if they fall too low.

Purpose of the insulin test

- ❖ Evaluate insulin production by the beta cells; in this case, a C-peptide test may also be done.
- ❖ Measures insulin from both sources while the C-peptide test reflects insulin produced by the pancreas.
- ❖ Diagnose insulin-producing tumor in the islet cells (insulinoma).
- ❖ Determine the cause of low blood glucose (hypoglycemia).
- ❖ Determine when a type 2 diabetic needs to start taking insulin to supplement oral medications.
- ❖ Determine and monitor the success of an islet cell transplant.

❖ Insulin levels are also sometimes used in conjunction with the glucose tolerance test (GTT). In this situation, blood glucose and insulin levels are measured at pre-established time intervals to evaluate insulin resistance.

Principle of the test

- Insulin ELISA test is based on simultaneous binding of human insulin by two monoclonal antibodies, one immobilized on microwell plates and the other conjugates with horseradish peroxidase (HRP).
- After incubation, the bound/free separation is performed by simple solid-phase washing.
- The enzyme HRP in the bound fraction reacts with the substrate (H_2O_2) and the TMB substrate and develops a blue color that changes into yellow when the stop solution (H_2SO_4) is added.
- The color intensity is proportional to the insulin concentration in the sample.

Preparation of the sample

- A fasting morning serum sample should be obtained.
- To obtain the serum, the blood should be collected in a venipuncture tube without additives or anticoagulants.
- Allow blood to clot; centrifuge the specimen to separate serum from the cells.
- Samples may be refrigerated at 2-8°C for a maximum period of 5 days. If the specimens cannot be assayed within this time, they may be stored at -20°C for up to 30 days. Avoid repetitive freezing and thawing.
- Patient specimens with insulin concentrations above 200 µIU/mL may be diluted (for example 1:10 or higher) with Calibrator zero and re-assayed.

Procedure

Allow all reagents to reach room temperature (22-28°C).

- Unused coated microwell strips should be released securely in the foil pouch containing desiccant and stored at 2-8°C.
- To avoid potential microbial and/or chemical contamination, unused reagents should never be transferred into the original vials.
- As it is necessary to perform the determination in duplicate in order to improve the accuracy of the test results.

Reagent	Calibrator	Sample/control	Blank
Calibrator C0-C5	100 µL	-	-
Sample/Control	-	100 µL	-
Conjugate	100 µL	100 µL	-
Incubate 1 hr. at room temperature (22-28°C). Remove the contents from each well. Wash the wells 3 times with 300 µL of diluted wash solution.			
TMB substrate	100 µL	100 µL	100 µL
Incubate 15 minutes in the dark at room temperature (22-28°C).			
Stop solution	100 µL	100 µL	100 µL
Shake the microplate gently. Read the absorbance at 450 nm within 5 min.			

Calculation of result

- Insulin concentration in the sample is calculated based on a calibration curve.

Reference values

❖ Insulin values are consistently higher in plasma than in serum; thus, the serum is preferred.

	µIU/mL
Children < 2 years	**< 10**
Adults (Normal)	**0.7-9**
Diabetic (Type II)	**0.7-25**

Abnormalities of insulin level in serum

A) Elevated insulin levels are seen with:

- Type 2 diabetes (not in the chronic phase).
- Insulin resistance syndrome.
- Hypoglycemia.
- Cushing's syndrome, a disorder of the adrenal glands. Adrenal glands make hormones that help the body break down fat and protein.
- An insulinoma (pancreatic tumor).

B) Decreased insulin levels are seen with:

- Hyperglycemia in type 2 diabetes at an early stage.
- Type 1 diabetes.
- Pancreatitis.

Self-Study Questions

I- **Complete the following.**

1- In Type 1 Diabetes your body ……..… while in Type 2 your body …….

2- Elevated insulin levels are seen ……, ……., …...……, …….

3- Insulin levels are most frequently ordered following a …….

4- High glucose level stimulates ..... hormone while low glucose level stimulates .....hormone.

II- Choose the correct answer.

1- In Type 1 Diabetes:

a) body makes little or no insulin at all.
b) body don't respond well to insulin.
c) body make enough insulin.
d) none of the above.

2- Insulin resistance usually result in ……

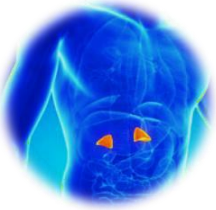
a) Extra insulin in the bloodstream.
b) Hypoglycemia.
c) Type 2 Diabetes.
d) All answers are right.

3- Insulin plays a key role in keeping glucose in the bloodstream in …….

a) high level.
b) low level.
c) normal level.
d) threshold level.

4- Insulin is a hormone mainly made by …….

a) beta cells.
b) delta cells.
c) gamma cells.
d) alpha cells.

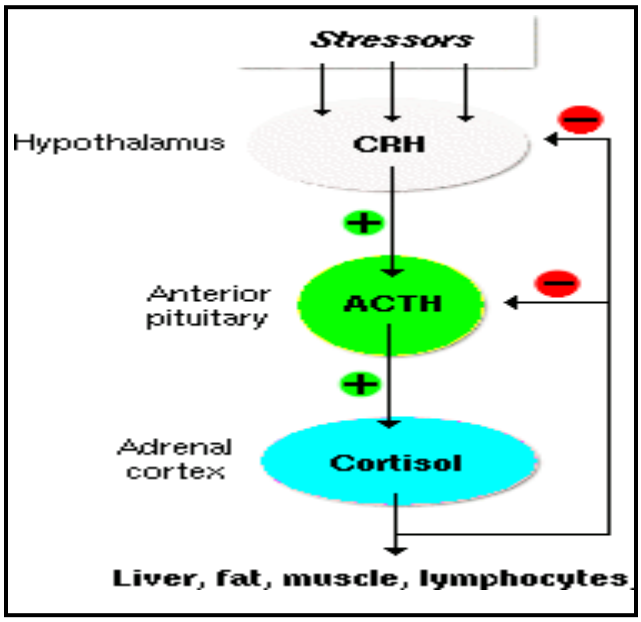


Quantitative Determination of Cortisol

- Cortisol is a steroid hormone synthesized from cholesterol in the cortex of the adrenal gland.
- Plays a role in the metabolism of carbohydrates, fat and protein, the maintenance of myocardial function and the adaptation to stress.
- Approximately 90% of cortisol in plasma or serum is protein bound to cortisol-binding-globulin (CBG).

Hormonal control

- Production of the hormone is regulated by the **hypothalamus** in the brain and by the pituitary gland. When the blood cortisol level falls, the hypothalamus releases (CRH), which directs the **pituitary gland** to produce ACTH.
- ACTH stimulates the adrenal glands to produce and release **cortisol.**



Purpose of the cortisol test

- Monitor the function of the adrenal and pituitary gland.
- Adrenal insufficiency, Addison disease and Cushing syndrome can be diagnosed based on the results of a cortisol level test.
- Measurement of unbound cortisol found in saliva is an accurate method to assess the biologically active free plasma cortisol.

Sample collection and preparation

- Blood is drawn from a vein in the arm, but sometimes **urine (24-hour) or saliva.** The timing of blood sampling is therefore very important at **9 A.M. and 9 P.M.**
- Cortisol levels in blood increase during the early morning (**highest at about 8 a.m.**) and decrease slightly in the evening and during the early phase of sleep.

- **Salivary cortisol** has some advantages over the assessment of cortisol in the **blood.**

Procedure

1- Add 20 µL standard, control or sample into their respective wells.
2- Add 200 µL cortisol-HRP conjugate to each well. Leave a blank well.
3- Incubate for 1 hour at 37°C.
4- Wash each well 4 times with 300 µL diluted washing solution. Avoid overflows.
Note: Washing is critical, insufficient washing results in poor precision and falsely elevated absorbance values.
5- Add 100 µL TMB substrate into all wells.
6- Incubate for exactly 15 minutes at room temperature in the dark.
7- Add 100 µL stop solution.
8- Measure the absorbance of the sample at 450 nm within 5 minutes.

Calculation of result

- Construct the standard curve; plot the absorbance for Cortisol standards (vertical axis) versus Cortisol standard concentrations (horizontal axis) on a linear graph paper. Draw the best curve through the points.
- Record the concentrations of each unknown sample.

Normal values

- Each laboratory should establish its own normal ranges based on a representative sampling of the local population. The following values may be used as initial guideline ranges only:

Time	Range	Unit
09:00 – 10:00 A.M.	50-230	nmol/L
04:00 P.M.	30-150	nmol/L

Abnormal values

a. Factors reducing cortisol level

- Cushing syndrome.
- Magnesium supplementation decreases serum cortisol levels after aerobic exercise.
- Omega-3 fatty acids (fish oil) have a dose-dependent effect.
- Soy-derived phosphatidylserine interacts with cortisol.
- High-dosage treatment with ascorbic acid (vitamin C).

b. Factors increasing cortisol level

- Addison disease.
- Viral infections.
- Caffeine and sleep deprivation.
- Intense (high VO_2 max) or prolonged aerobic exercise.
- Severe trauma or stressful events.
- Anorexia nervosa.

Self-Study Questions

Choose the correct answer:

1. Cortisol helps in responding to and coping with:

a. Stress b. Trauma

c. Environmental extreme d. All the above

2. Elevated cortisol levels over a prolonged period of time can produce:

a. Blood sugar imbalances such as hyperglycemia

b. Lowered immunity

c. Neither of these

d. Both of these

3. Cortisol is synthesized in the:

a. liver b. kidney c. brain d. Adrenal

4. How does a high level of cortisol make us feel?

a. Anxious b. Angry c. Agitated d. All the above

5. Reference ranges of cortisol test depend on ……

a. the sample type (blood or urine)

b. the analytical method used

c. factors such as age and sex

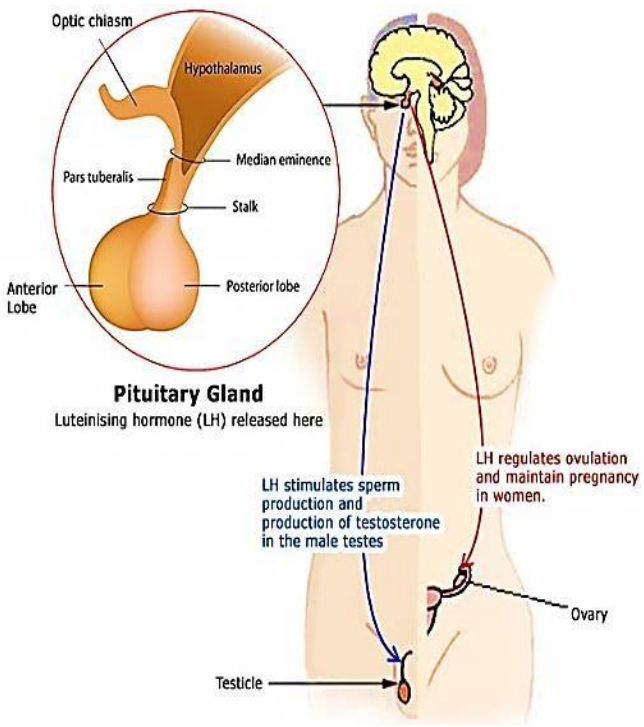
d. all the above

6. Factor reducing cortisol levels.

a. viral infection b. Cushing syndrome

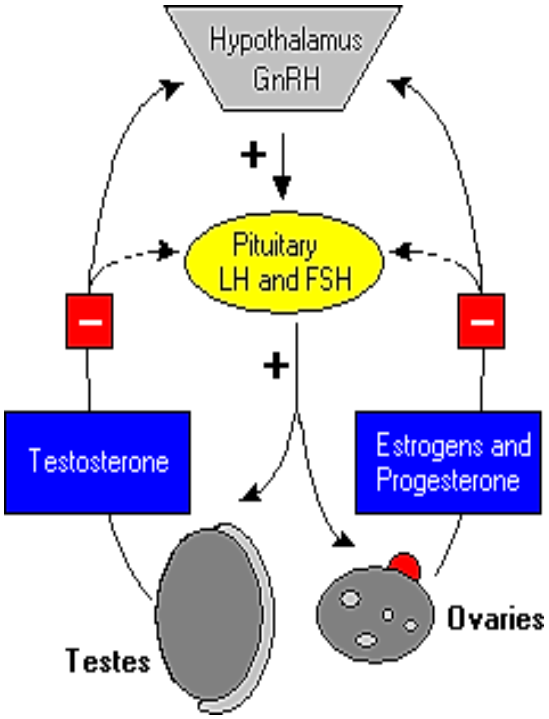
c. Anorexia nervosa d. Addison disease

Quantitative Determination of FSH and LH



- Follicle-stimulating hormone (FSH or folliculotropin) and interstitial cell-stimulating hormone (ICSH) or lutropin or the formal name, Luteinizing hormone (LH); are gonadotrophic hormones (GnRH) produced and released by cells in the anterior pituitary gland.
- They are crucial in regulating the function of the testes in men and ovaries in women.
- They play a role in sexual development and reproduction in both males and females.
- **In females**, FSH stimulates the growth of ovarian follicles in the ovary.
- Also increases estradiol production.
- **In males**, it acts on the **Sertoli cells** of the testes to stimulate sperm production
- **In men**, LH stimulates Leydig cells in the testes to produce testosterone.
- ***In women***, LH carries out different roles in the two halves of the menstrual cycle. In weeks (1-2) of the cycle, LH is required to stimulate the ovarian follicles to produce the female sex hormone, estradiol.
- Around day 14 of the cycle, a surge in LH levels causes the ovarian follicle to tear and release a mature oocyte (egg) from the ovary, a process called ovulation.
- For the remainder of the cycle (weeks three to four), the remnants of the ovarian follicle form a corpus luteum. LH stimulates the corpus luteum to produce progesterone, which is required to support the early stages of pregnancy if fertilization occurs.

Hormonal control of FSH and LH



- The secretion and release of FSH and LH from the **anterior pituitary gland** are regulated through a system called the **hypothalamic-pituitary-gonadal axis.**
- GnRH is released from the **hypothalamus** and binds to its receptors to stimulate both the synthesis and release of FSH and LH.
- The released FSH and LH are carried in the bloodstream where it binds to receptors in the testes and ovaries and controls the functions.
- The release of hormones from the gonads can suppress the secretion of GnRH and, in turn, LH and FSH from the anterior pituitary gland.
- When levels of hormones from the gonads fall, the reverse happens and GnRH rise. This is known **as *negative feedback*.**

The purpose of the FSH test

The most common reasons for an FSH test to be done include:

1) Test for Women

- Assessing infertility problems.
- Assessing women having difficulty getting pregnant or irregular or an absence of menstrual cycles.
- Diagnosing disorders of the pituitary gland or diseases involving the ovaries.

2) Test for Men

- Evaluate a low sperm count (hypogonadism or gonadal failure).
- Assess testicular dysfunction.
- Man infertility.
- When a man has low muscle mass or decreased sex drive.
- Pituitary gland disorders.

3) Test for Children

- **In children,** LH and FSH may be ordered when a boy or girl does not appear to be entering puberty at an appropriate age (too late or too soon).

Principle of the test

- The FSH or LH test is based on solid-phase enzyme immunoassay.
- This assay uses two mouse monoclonal antibodies directed against distinct antigenic determinants on the FSH molecule.
- The polystyrene wells are coated with a captured antibody against FSH or LH. Standards, controls, and specimen are added to (solid phase) and incubate.
- The FSH or LH present in the wells is bound to the anti-FSH or LH antibodies. The unbound material is removed by aspiration and washing.
- After washing, the HRP labeled anti-FSH or LH Mab is added to the wells.
- After second incubation and washing, a solution of TMB is added to each well, resulting in the development of a blue color. The intensity of the color is proportional to the amount of FSH or LH present in the sample.
- The color development is stopped by the addition of stop solution, causing the blue color to change to yellow.

Specimen collection and preparation

- ✓ The assay can be performed on serum or heparinized plasma samples.
- ✓ Keep samples at 2-8°C for 1-2 weeks; for a longer period, it is recommended to store the sample in aliquots at -20°C. Avoid repeated freezing and thawing of samples.
- ✓ Prior to assay, frozen specimens should be slowly brought to room temperature and gently mixed by hand.
- ✓ Do not vortex patient samples.

Assay procedures

1. Dispense 25 µl of LH of FSH standards, control serums, and patient samples.
2. Pipette 100 µl of HRP anti-LH or FSH conjugates to well and mix the plate for 15 seconds.
3. Incubate the strips for 1 hour at room temperature.
4. Wash each well 4 times with 300 µl of diluted wash solution.
5. Dispense 100 µl of TMB substrate into each well.

6. Incubate at room temperature in the dark for 10 minutes.
7. Add 100 µl of stop solution and mix for 10 seconds.
8. Read absorbance at 450 nm (with a reference wavelength at 630 nm).

Calculation of result

- The FSH or LH concentration in the sample is calculated based on a calibration curve.
- Read the absorbance for controls and each unknown sample from the curve. Record the value for each control or unknown sample.
- Plot the absorbance for standards (vertical axis) versus standard concentrations (horizontal axis) on a linear graph paper. Draw the best curve through the points.

Normal values

- Based on random selected outpatient clinical laboratory samples, the mean FSH values are:

Expected values for FSH

Adult	mIU/ml
Males:	1.5-12.4
Females:	
Follicular	3.5-12.5
Ovulatory Peak	4.7-21.5
Luteal	1.7-7.7

Expected values for LH

Adult	mIU/ml
Males:	1.7-8.6
Females:	
Follicular	2.4-12.6
Ovulatory Peak	14-95.6
Luteal	1.5-11.4
Postmenopausal	7.7-58.5

Abnormalities of FSH secretion

1) (Hypergonadotropic-Hypogonadism)

This is a condition in which levels of **FSH** in the bloodstream are **raised.**

- ✓ Gonads fail to create enough estrogen, testosterone and/or inhibin.
- ✓ **Klinefelter's syndrome** in men and **Turner syndrome** in women.
- ✓ Ovarian hyperstimulation syndrome.

2) (Hypogonadotropic-Hypogonadism)

This is a condition in which levels of **FSH** in the bloodstream are **low.**

In women

- ✓ Ovarian failure.
- ✓ **Kallmann's syndrome**, which is associated with a reduced sense of smell.

In men

- ✓ Lack of puberty and infertility due to lack of sperm (azoospermia).
- ✓ Partial FSH deficiency in men can cause delayed puberty and limited sperm production (oligozoospermia).

Abnormalities of LH secretion

In women

- ✓ **High levels of LH;** can be a sign of what's called "primary ovarian failure" which means that the problem is with the ovaries themselves.
- ✓ **Low levels of LH;** may be a sign of "secondary ovarian failure," which means the problem starts with the pituitary gland or hypothalamus (a part of the brain).

In men

- ✓ **High levels of LH**; in the blood are a sign of a problem with the testicles.
- ✓ **Low levels of LH**; mean the issue is with the pituitary gland or hypothalamus.

Self-Study Questions

I) Complete the following:

1- **In women**, incomplete development at puberty and poor ovarian function are due to …… and this syndrome is known as …….…….

2- The production and release of FSH hormone are regulated by the levels of a number of circulating hormones called …….

3- FSH in females stimulates ……..…. while in males stimulates ……..…

4- **In men,** azoospermia can occur due to but oligozoospermia can occur due to ……

5- Luteinizing hormone is also called ……. or ……...…….

6- LH and FSH may be ordered when a boy or girl does not ……

7- In females, LH at the midpoint of menstrual cycle triggers …….

8- A high level of LH in women is a sign of …….

II) Choose the correct answer:

1- the correct feedback control of FSH production from the pituitary gland is achieved by …….

a) estrogen b) testosterone

c) inhibin d) all of previous

2- Too high levels of FSH in women due to failure of ovaries is called ……

a) Klinefelter's syndrome b) Turner syndrome

c) Kallmann's syndrome d) None of the above

3- All of the following is true about <u>ovarian hyperstimulation syndrome</u> except;

a. It is very rare pituitary conditions
b. It can reduce the levels of FSH in the bloodstream
c. It causes enlarging of the ovaries
d. It results in pain in the pelvic area.

4- LH is secreted from …….

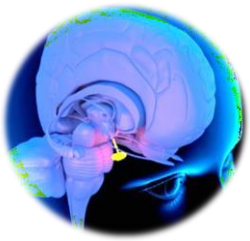
a. The anterior portion of pituitary gland
b. The anterior portion of hypothalamus
c. The posterior portion of pituitary gland
d. The posterior portion of hypothalamus

5- LH testing is required when a ……

a. man's partner cannot get pregnant
b. man has a low testosterone level
c. man has low muscle mass or decreased sex drive
d. all previous are correct

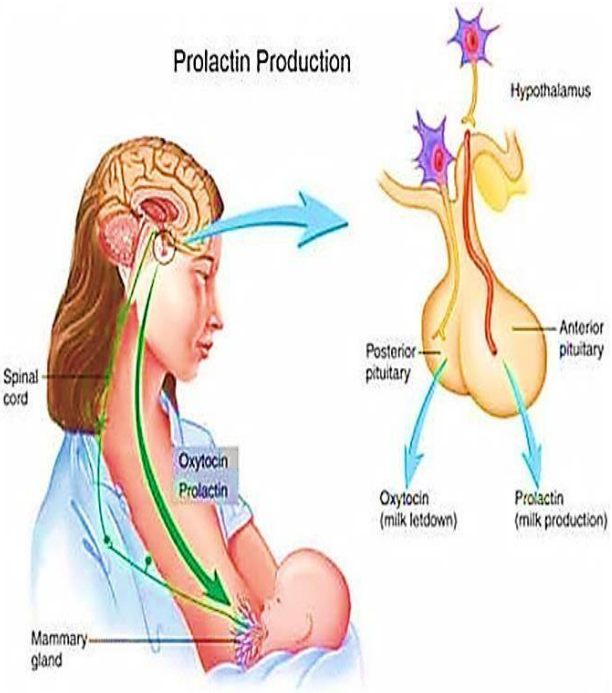
6- High levels of LH in females may be a sign of …….

a. secondary ovarian failure
b. problem with the pituitary gland or
c. problem with hypothalamus
d. primary ovarian failure



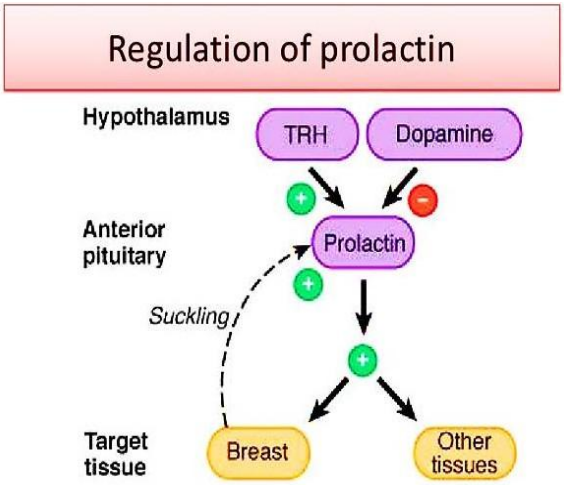
Quantitative Determination of Prolactin

- Prolactin (PRL) is a hormone originally named after its function to promote milk production (lactation).
- Plays a role in reproductive, metabolic, regulation of fluids (*osmoregulation*), regulation of the immune system (*immunoregulation)* and behavioral functions.
- In humans, prolactin is produced both in the anterior pituitary gland. Lactotroph cells in the pituitary gland produce prolactin, where it is stored and then released into the bloodstream.
- Human prolactin is also produced in the uterus, immune cells, brain, breasts, prostate, skin, and adipose tissue.



Hormonal control of prolactin

- **Dopamine** hormone produced by the hypothalamus regulates of the production of prolactin from the pituitary gland.
- **Estrogen** is another key regulator of prolactin and increases the production and secretion of prolactin from the pituitary gland.
- In addition to dopamine and estrogen, a whole range of other hormones can both increase and decrease the amount of prolactin released in the body, with some examples thyrotrophic-releasing hormone, oxytocin, and anti-diuretic hormone.



The Purpose of the prolactin level test

For women

- Irregular or no periods.
- Infertility.
- Breast milk discharge when you're not pregnant or nursing.
- Tenderness in your breast.
- Menopausal symptoms such as hot flashes and vaginal dryness.

For men

- Decreased sex drive.
- Difficulty in getting an erection.
- Breast tenderness or enlargement.
- Breast milk production (very rare).

For both

- Unexplained headaches.
- Vision problems.

Principle of the test

- The Prolactin test is based on solid-phase enzyme immunoassay (EIA).
- This assay system uses two mouse monoclonal antibodies directed against distinct antigenic determinants on the prolactin molecule.
- The polystyrene wells are coated with a captured antibody against prolactin.
- Standards, controls, and patient samples are added to the wells and incubate.
- The prolactin present in the wells is bound to the anti-prolactin antibodies.
- The unbound material is removed by aspiration and washing.
- After washing, the HRP labeled anti-prolactin Mab is added to the wells.
- After second incubation and washing, a solution of TMB is added to each well, resulting in the development of a blue color.
- The intensity of the color is proportional to the amount of prolactin present in the sample. The color stopped by addition of stop solution, causing the blue color to change to yellow.

Specimen collection and preparation

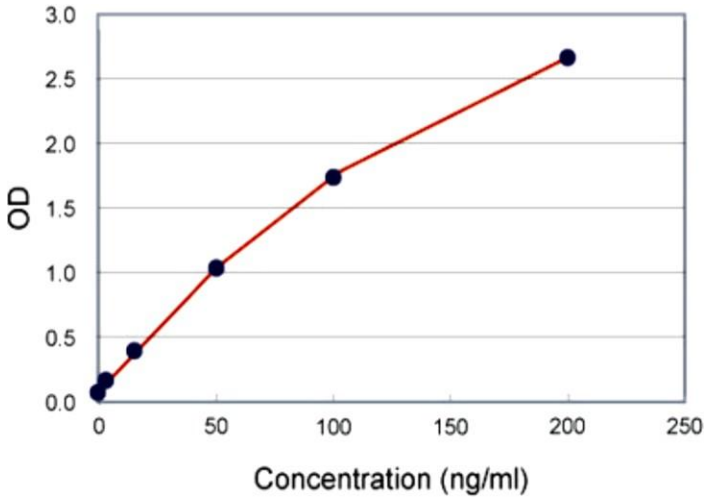
- The assay can be performed on serum or heparinized plasma samples.
- Keep samples at 2-8°C for 1 week; for longer periods it is recommended to store the sample in aliquots at -20°C.
- Avoid repeated freezing and thawing of samples.
- Prior to assay, frozen specimens should be slowly brought to room temperature and gently mixed by hand.
- Do not vortex patient samples.

Procedure

1- Dispense 50 µL of standards, control, and patient serums into well.
2- Pipette 50 µL of assay buffer to each well and mix the plate for 15 seconds.
3- Incubate the strips for 30 minutes at room temperature (without shaking).
4- Aspirate and wash each well 4 times with 300 µL of diluted wash solution.
5- Add 50 µL of HRP anti-prolactin. Then incubate for 30 minutes at RT.
6- Aspirate and wash each well 4 times with 300 µL of diluted wash solution.
7- Dispense 100 µL of TMB substrate into each well.
8- Incubate at room temperature in the dark for 10 minutes.
9- Add 100 µL of stop solution and mix for 10 seconds.
10- Read absorbance at 450 nm (with a reference wavelength at 630 nm) within 15 minutes after the addition of stop solution.

Calculation of result

- Prolactin concentration in the sample is calculated based on a calibration curve.



Normal range

- ✶ Each laboratory must establish its own normal ranges based on patient population.
- ✶ The upper limit for circulating prolactin in healthy individuals, as suggested in the literature, is up to 30 ng/ml.

Adult	Range (ng/ml)
Male:	3-14.7
Female:	3.8-23.2
Pregnancy, 3rd trimester	95-473

Abnormalities of prolactin secretion

1) **Hyperprolactinemia**. The condition of having too much (higher than the normal range) prolactin circulating in the blood.
   - *The most common causes of **hyperprolactinemia** include*;
   - ✓ Pregnancy.
   - ✓ Medications that reduce dopamine action in the body.
   - ✓ Thyroid under activity.
   - ✓ Benign pituitary tumors (known as prolactinomas).
   - ✓ Disturbances to the menstrual cycle.
   - ✓ Estrogen deficiency (in women) or testosterone deficiency (in men).

- The vast majority of patients with a prolactinoma can be treated successfully using drugs which mimic the action of **dopamine.** The most commonly used is **cabergoline**.

2) **Hypoprolactinemia.** The condition of having too little (below the normal range) prolactin circulating in the blood. This condition is very rare and may occur in people with pituitary under activity that's known as hypopituitarism.

3) **A decrease in the amount of prolactin** secreted can lead to insufficient milk being produced after giving birth. Most people with low prolactin levels do not have any specific medical problems, although preliminary evidence suggests they might have reduced immune responses to some infections.

Self-Study Questions

i. Complete the following.

1- Prolactin hormone has shown several functions in the body including ……., ……., ……., ……
2- Hypoprolactinemia is defined as …….
3- Decrease of prolactin in males may lead to ……..…., ……., ……

i) Choose the correct answer.

1-Hyperprolactinaemia is a condition that …….

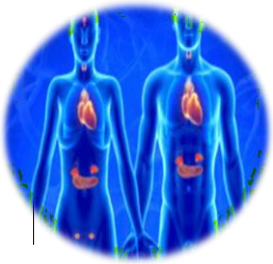
a. little prolactin circulating in the blood.
b. is very rare and may occur in people with pituitary underactivity.
c. occurs in case of hypopituitarism.
d. occurs in case of pregnancy.

2- Dopamine and estrogen hormone can affect the production of …….

a. Insulin
b. Albumin
c. Glucagon
d. Prolactin

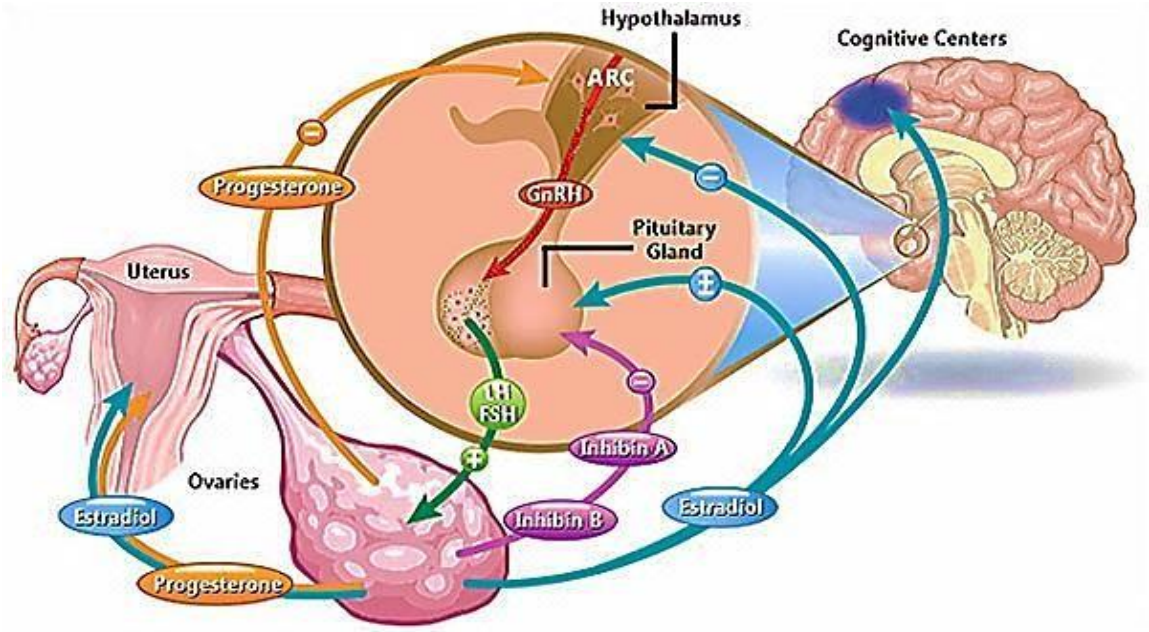
3- The vast majority of patients with a prolactinoma can be treated successfully using drugs which mimic the action of …….

a. Insulin
b. Albumin
c. Dopamine
d. Prolactin



Quantitative Determination of Gonads Hormones

1. Estrogen and progesterone



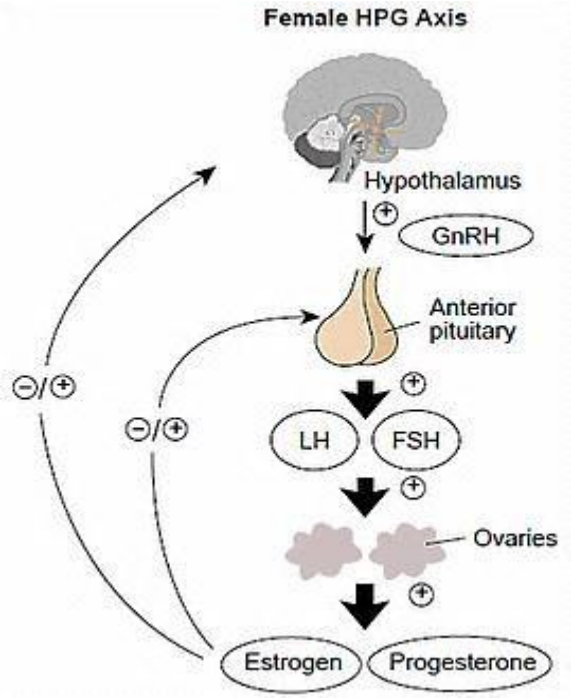
- Steroid hormones derived from enzymatic modifications of cholesterol.
- In women, production of estrogen and progesterone occurs primarily in the **ovary**, but also in the placenta during pregnancy, in smaller amounts by the adrenal cortex, and the male testes.
- Estrogen and progesterone levels are low throughout childhood but increase dramatically at the onset of puberty.

The related hormones in the estrogen family include:

- **Estrone (E1):** A weak form. The only type found in women after menopause.
- **Estradiol (E2):** This is the strongest type (the dominant type > 90% of total estrogen). Produced by the ovaries.
- **Estriol (E3):** The weakest type of the estrogens and is a waste product made after the body uses estradiol.
- **Estetrol (E4):** Produced only during pregnancy.

Hormonal control

- Gonadotropin-releasing hormone (GnRH), produced and released by the hypothalamus, stimulates the production and pulsatile release of follicle-stimulating hormone (FSH) and luteinizing hormone (LH) in the anterior pituitary.
- FSH and LH modulate ovarian sex hormone synthesis and secretion. LH in synergy with (FSH) stimulates follicular growth and ovulation. Thus, normal follicular growth is the result of the complementary action of FSH and LH.



Purpose of the test

Estradiol (E2)

- Measure or monitor estrogen levels or hormone imbalance.
- Monitor treatment for infertility or symptoms of menopause.
- Sometimes to test for fetal-placental status during early stages of pregnancy.

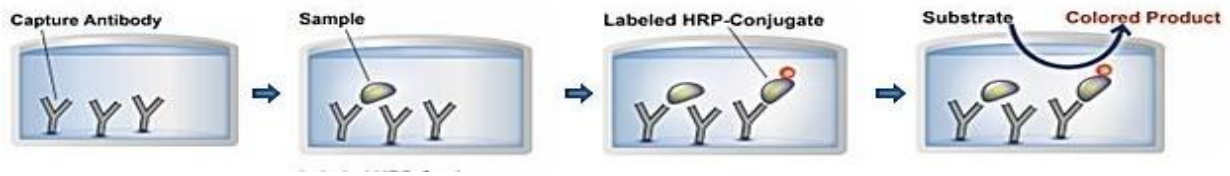
Progesterone

- To determine the cause of infertility or track ovulation.
- To diagnose an ectopic or failing pregnancy.
- Monitor the health of a pregnancy.
- Monitor progesterone replacement therapy.
- To diagnose the cause of abnormal uterine bleeding.

Principle of the test

- The Estradiol or Progesterone ELISA kit is a solid phase competitive ELISA.
- The samples and (Estradiol or Progesterone) enzyme conjugate are added to the wells coated with anti- Estradiol or Progesterone monoclonal antibody.
- Estradiol or Progesterone in the sample competes with the (Estradiol or Progesterone) enzyme conjugate for binding sites.

- Unbound (Estradiol or Progesterone) and (Estradiol or Progesterone) enzyme conjugate are washed off by washing buffer.
- Upon the addition of the substrate, the intensity of the color is inversely proportional to the concentration of (Estradiol or Progesterone) in the samples.
- A standard curve is prepared relating color intensity to the concentration of the (Estradiol or Progesterone).



Sample preparation

Samples are drawn from a vein or a **24-hour urine sample**. Menstrual and peripheral blood samples of **estrogen** collected from cycling women on day **1-3** of menstrual cycle. Blood samples of **progesterone** collected at **21** days of M. cycle.

- Collect blood specimens and separate the serum immediately.
- Specimens may be stored refrigerated at (2–8°C) for 5 days. For long term storage frozen at (-20°C) for up to one month.
- Avoid multiple freeze-thaw cycles.
- Frozen sera should be completely thawed and mixed well.

Procedure

- Prior to assay bring all reagents to room temperature. Gently mix all reagents.

1. Pipette 10 µL of standards, control, and serum samples.
2. Add 200 µL of (Estradiol or Progesterone) enzyme conjugate to all wells.
3. Incubate for 60 minutes at room temperature (18-26°C).
4. Wash wells 4 times with 300 µL of 1x wash buffer.
5. Add 100 µL of TMB substrate to all wells.
6. Incubate for 15 minutes at room temperature.
7. Add 50 µL of stop solution to all wells.
8. Read absorbance on ELISA Reader at 450 nm within 15 minutes after adding the stop solution.

Calculation of results

- Calculate the mean absorbance value (A_{450}) for each set of reference standards, controls, and samples.

- ❖ Construct a standard curve by plotting absorbance value against the concentration of each standard ng/mL on a linear-linear graph paper, with absorbance values on the vertical or Y-axis and concentrations on the horizontal or X-axis.

Normal values

** Each laboratory should establish its own normal range based on the patient population.

Estradiol reference range

- ➢ **Males:** 10–50 pg/mL
- ➢ **Females:**
  - postmenopausal phase: 0–30 pg/mL
  - ovulating: 30–400 pg/mL
  - early follicular: 30–100 pg/mL
  - late follicular: 100–400 pg/mL
  - luteal phase: 50–200 pg/mL
  - pregnant normal: up to 35,000 pg/mL
  - prepubertal children, normal: <10 pg/mL

Progesterone reference range

- ➢ **Males:** < 0.1 ng/mL
- ➢ **Females:**
  - Follicular phase: 0.1 – 1.4 ng/mL
  - Mid-luteal phase: 4.0 – 25.0 ng/mL
  - Menopause: < 1.0 ng/mL
  - Pregnancy: *increase according to weak of pregnancy*

Weak	ng/mL
✓ 18 – 21	53 – 76
✓ 22 – 25	60 – 86
✓ 26 – 29	71 – 133
✓ 30 – 33	86 – 142
✓ 34 – 37	104 – 175
✓ 38 – 41	117 – 187

Abnormal values

A) *Causes of low level:*

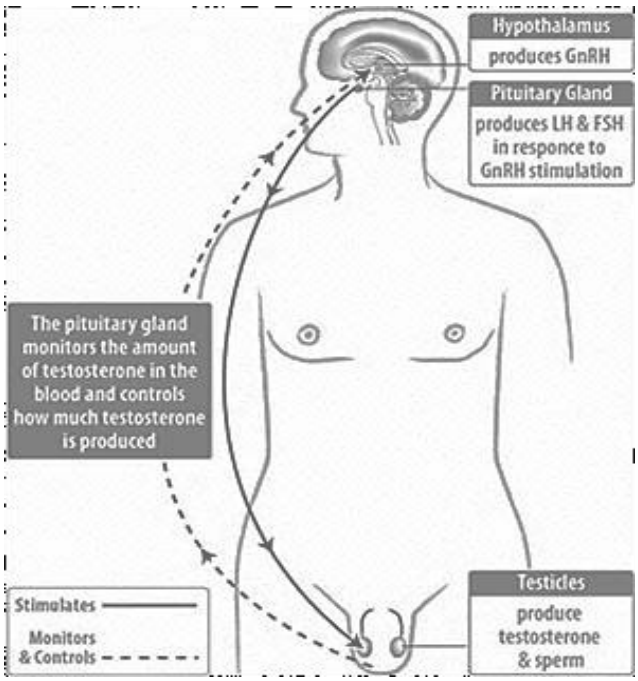
Progesterone	Estrogen
➢ Fetal death / Miscarriage. ➢ Ectopic pregnancy. ➢ Pre-eclampsia. ➢ Toxemia - a condition that can happen late in your pregnancy that could be serious if not treated ➢ Decreased function of ovaries or not working normally. ➢ Not menstruating or lack of menstruation (amenorrhea).	➢ Turner syndrome: an inherited condition in women caused by a missing or abnormal X chromosome. ➢ Low level of pituitary hormones (hypopituitarism). ➢ Dysfunction of the ovaries (female hypogonadism). ➢ Failing pregnancy (estriol). ➢ Eating disorders such as anorexia nervosa. ➢ After menopause (estradiol). ➢ Extreme endurance exercise.

B) *Causes of high level:*

Progesterone	Estrogen
Girls and Women: ➢ Pregnancy. ➢ The second half of the M. cycle. ➢ Ovarian cysts. ➢ The rare form of ovarian cancer. ➢ Non-viable pregnancies known as molar pregnancies. **Both Women and Men:** ➢ Adrenal cancer. ➢ Overproduction of progesterone by the adrenal glands. ➢ Congenital adrenal hyperplasia.	**Girls and Women:** ➢ Early (precocious) puberty. ➢ Tumors of the ovary or adrenal glands. **Boys and Men:** ➢ Enlarged breasts (gynecomastia). ➢ Tumors of the testicles (testicular cancer) or adrenal glands. ➢ Delayed puberty. **Both Women and Men:** ➢ Hyperthyroidism. ➢ Cirrhosis.

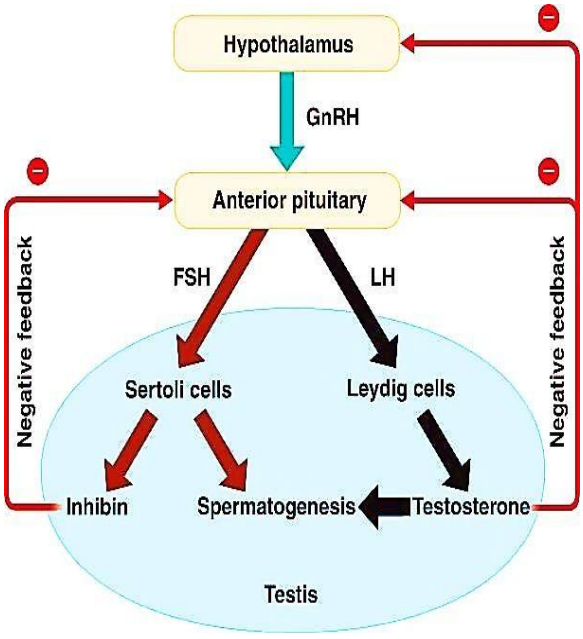
2. Testosterone

- Steroid hormone biosynthesized in several steps from cholesterol.
- Secreted primarily by the **testes** of males (> 95%) and, to a lesser extent, the ovaries of females and during pregnancy by the placenta. Small amounts are also secreted by the adrenal glands.
- In adult males, levels are about **7 to 8 times** as great as in adult females.
- In men, low production causes infertility but in women, overproduction may cause fertility problem.



Hormonal control of testosterone

- Hypothalamic GnRH stimulates the production of LH and FSH by the pituitary.
- LH is transported in the bloodstream to the testes, where it stimulates Leydig cells to produce testosterone.
- The testes, in turn, in negative feedback on the hypothalamus and the pituitary via testosterone and inhibin secretion to limit GnRH and gonadotropin production.



Purpose of the test

- In males, to help diagnose the cause of erectile dysfunction or infertility.
- In females, to help diagnose the cause of masculine physical features (virilization), infertility, or polycystic ovary syndrome (PCOS).

- In children, to help determine the cause of genitals those aren't clearly male or female (ambiguous genitalia) or delayed or early puberty.

Principle of the test

- The testosterone ELISA kit is used for the quantitative measurement in human serum or plasma.
- It is based on the principle of competitive binding between testosterone in the test specimen and Testosterone HRP conjugate for a constant amount of mouse anti-testosterone antibody for binding sites.
- Unbound testosterone and testosterone enzyme conjugate are washed off by washing buffer.
- Upon the addition of the substrate, the intensity of the color is inversely proportional to the concentration of testosterone in the samples.
- A standard curve is prepared relating color intensity to the concentration of the testosterone.

Sample preparation

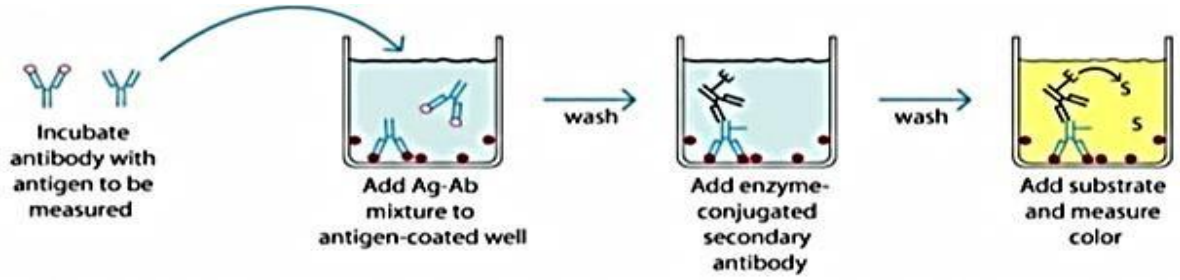
A morning blood sample is preferred.

- Collect blood specimens and separate the serum immediately.
- Specimens may be stored refrigerated at (2–8°C) for 5 days. If storage time exceeds 5 days, store frozen at (-20°C) for up to one month.
- Avoid multiple freeze-thaw cycles.
- Frozen sera should be completely thawed and mixed.

Procedure

- Before proceeding, bring all reagents to room temperature (18–26°C).

1. Pipette 25 µL of the standards, control, or specimen into the assigned well.
2. Add 100 µL of working testosterone-enzyme conjugate reagent to all wells.
3. Swirl the microplate gently for 20–30 seconds to mix the reagents.
4. Cover the plate and incubate for 60 minutes at room temperature.
5. Wash wells 4 times with 300 µL of 1x wash buffer.
6. Add 100 µL of TMB substrate reagent to all wells.
7. Cover the plate and incubate at room temperature for 15 minutes.
8. Add 50 µL of stop solution to each well and gently mix for 15–20 seconds.
9. Read the absorbance on ELISA Reader at 450 nm within 15 minutes after adding the stop solution.



Calculation of results

- Calculate the mean absorbance value (A_{450}) for each set of reference standards, controls, and samples.
- Construct a standard curve by plotting absorbance value against the concentration of each standard ng/mL on a linear-linear graph paper, with absorbance values on the vertical or Y-axis and concentrations on the horizontal or X-axis.

Normal values

- It is recommended that each laboratory establish its own normal ranges based on a representative sampling of the local population. The following testosterone ranges values may be used as an initial guideline only:

For Total Testosterone:

- **Males:**
  - Prepubertal (late): 0.1 – 0.2 ng/ml
  - Adult: 3.0 – 10.0 ng/ml

- **Females:**
  - Prepubertal (late): 0.1 – 0.2 ng/ml
  - Follicular phase: 0.2 – 0.8 ng/ml
  - Luteal phase: 0.2 – 0.8 ng/ml
  - Post-menopausal: 0.08 – 0.35 ng/ml

For Free Testosterone

- **Adult Males:** 5 – 30 pg/mL
- **Adult Females:** 0 – 3 pg/mL
- **Children (**1–10 year): 0.1 – 1.251 pg/mL

Abnormal values

A) Causes of low level of testosterone (hypogonadism):

In Males: Decreased testosterone levels indicate partial or complete hypogonadism. Serum testosterone levels are usually below the reference range. The cause is either primary or secondary/tertiary (pituitary/hypothalamic) testicular failure.

- Hypothalamic or pituitary disease.
- Genetic diseases that can cause decreased testosterone production in young men (Klinefelter, Kallmann and Prader-Willi syndromes) or testicular failure and infertility (as in myotonic dystrophy, a form of muscular dystrophy).
- Impaired testosterone production because of acquired damage to the testes, such as from alcoholism, physical injury, or viral diseases like mumps.
- Chronic disease, such as diabetes.

In Females:

- In women, testosterone levels are normally low but decreased testosterone levels may be observed in primary or secondary ovarian failure.
- Most women with oophorectomy have a significant decrease in testosterone.

B) Causes of high levels of testosterone (hypergonadism):

In Males:

- Testicular tumors.
- Adrenal tumors that are producing testosterone.
- Use of androgens (also called anabolic steroids).
- Early puberty of unknown cause in boys.
- Congenital adrenal hyperplasia in babies and children.

In Females:

- Polycystic ovary syndrome (PCOS).
- Ovarian or adrenal gland tumor.
- Congenital adrenal hyperplasia.

Self-Study Questions

Choose the correct answer.

1) Stimulates sex hormone secretion, reproductive organ growth, and reproductive processes.
   a. Parathyroid hormone (PTH) b. Calcitonin
   c. Gonadotropic hormones (FSH & LH d. Oxytocin
2) Corpus luteum secrets …….
   a) LH b) Progesterone
   c) Progesterone and LH d) Progesterone and estrogen

3) Which of these hormones is produced by females?
   a) Estrogen b) oxytocin
   c) Testosterone d) all of these

4) Which of the following stimulates the production of progesterone by the corpus luteum?
   a) LH b) FSH c) TSH d) GH

5) Which one of the following hormones binds to the pituitary and stimulates the release of LH and FSH?
   a) Gonadotrophin releasing hormone (GnRH)
   b) Corticotropin-releasing hormone (CRH)
   c) Adrenocorticotropic hormone (ACTH)

6) The secretions from which of these glands differs between males and females?
   a) Adrenal b) Parathyroid c) Gonadal d) Pancreas

7) Most estrone and estriol are formed from estradiol in this organ.
   a) Ovary b) Liver c) Adrenal d) Kidney

8) What are gonads?
   a) Reproductive glands only b) Reproductive organs only
   c) Reproductive organs and glands d) Neither reproductive organs nor glands

9) What hormones are secreted by the testis?
   a) Testosterone only b) Estrogen and progesterone
   c) Estrogen and testosterone d) Progesterone and testosterone

Study Cases

Choose the most correct answer:

1- Man with increased heart rate and weight loss, is diagnosed as:

a. Dwarfism b. D. Mellitus c. Myxedema d. Hyperthyroidism

2- Girl (20 yrs.) with a missed menstrual cycle:

a. Diagnosed as secondary amenorrhea
b. Recommended for FSH, LH, PRL and testosterone
c. Recommended for semen analysis for her husband
d. Diagnosis as Virilism

3- Polyuria (8 L/day) - colorless urine with a low specific gravity in child

a. Diagnosed as diabetes mellitus
b. Diagnosed as acute kidney disease
c. Recommended for: ADH, if normal; OGTT
d. Recommended for an insulin test

4- Repeated abortion in women:

a. Recommended for progesterone level
b. Recommended for TORCH analysis
c. Recommended for E_2 & Oxytocin
d. Diagnosed as menopause

Case history 1

A patient has been diagnosed with acromegaly and referred to an endocrinologist. MRI demonstrates a large pituitary mass extending to and compressing the optic chiasm. Serum PRL was 57 ng/mL, TSH was undetectable, fT4 was 0.4 ng/dL and an ACTH stimulation test gave a serum cortisol value at 30 min. of 10.9 µg/dL. **What do these biochemistry results indicate?**

Case history 2

A 40-year-old woman had attended her family doctor for a cervical smear. She saw a new doctor; her previous doctor having known her since childhood. The new doctor was concerned by the patient's coarse facial appearance and asked some questions. The woman was surprised to be asked about her shoe size but confirmed that most of her shoes were now a size larger than 10 years ago.

What diagnosis is being considered?

What other questions should be asked?

What specific features of the examination should be sought?

What tests would confirm the doctor's suspicion?

Full Exam

Choose the correct answer. (Kindly, answer only on the provided bubble sheet):

1. Which of the following statements concerning hormonal regulation is correct?

a. A hormone does not inhibit its own release.

b. The substrate a hormone regulates does not affect that hormone's release.

c. Negative feedback regulation occurs only at the level of the anterior pituitary.

d. Feedback inhibition may be exerted by nutrients and hormones.

2. RIA can be replaced by:

a. PCR b. ELISA c. Latex agglutination d. Radial immunodiffusion

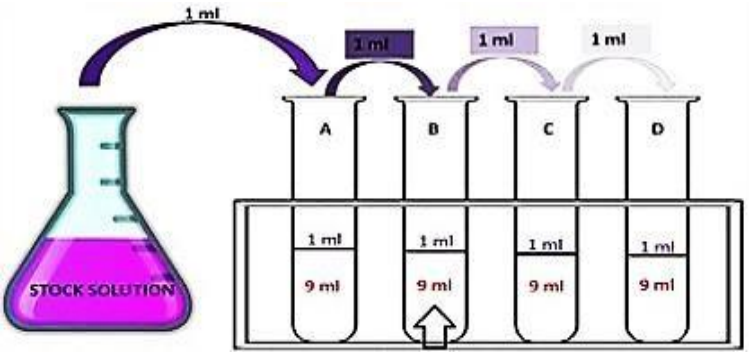
3. Which of the following ELISA uses two different antibodies?

I. Direct **II. Sandwich** **III. Competitive**

a. I & II only b. II and III only c. II only d. III only

4. What would be the 2nd tube dilution, in the given figure?

a. 1/10 b. 2/10 c. 1/100 d. 1/1000

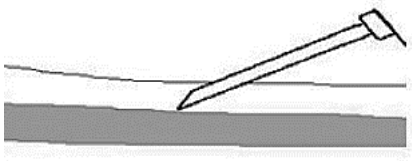


5.....is among the many factors that influence hormone levels.

a. circadian rhythm b. sleep pattern c. gender

d. all of them e. none of them

6. Regarding venipuncture, this trouble is ..... and the suggested solution is ……



a. Hematoma; withdraw the needle.

b. Incomplete collection; move the needle forward.

c. Hematoma; move the needle backward.

d. Incomplete collection; adjust the angle of the needle.

7. If an antigen has only one epitope, which type of ELISA should you use to detect that antigen?

a. Direct b. Competitive c. Sandwich d. RIA

8. Reference ranges of hormones vary from lab to lab.

a. True b. False

9. In enzyme-linked immunosorbent assay:

a. Antibody or antigen is bound to an enzyme, which catalyzes the reaction.

b. Substrates in the reaction are converted to a colored end-product.

c. Antigen or antibody can be detected quantitatively with extreme sensitivity.

d. all of them. e. none of them.

10. Free T3 is .....form circulates unattached in circulation.

a. less abundant b. high abundant

c. very high abundant d. not from the above

11. The ……. test is the most common type of T3 blood test, measures both the bound and free forms of T3 in your child's blood.

a. total T4 test b.T3 total test

c. free T4 test d. free T3 test

12. Cortisol levels in the blood are.....during the early morning.

a. highest b. lowest

c. not changed d. not of the above

13. One causes of a high level of TSH is …….

a. primary hyperthyroidism b. Hashimoto thyroiditis

c. secondary hypothyroidism d. tertiary hypothyroidism

14. Factors reducing cortisol levels …….

a. viral infection b. Cushing syndrome

c. Anorexia nervosa d. Addison disease

15. A low TSH result may mean that .....

a. presence of Graves' disease b. the pituitary tumor

c. presences of Hashimoto thyroiditis

d. An underactive thyroid gland (hypothyroidism)

16. Cortisol is a steroid produced from …….

a. thyroid gland b. adrenal cortex

c. adrenal medulla d. none of the above

17. Normal …… hormone levels typically fall between 0.4 and 4.0 mU/L, according to the American Thyroid Association (ATA).

a. LH b. TSH c. FSH d. T4

18. The T4 EIA kit is measured by …….

a. Immunometric assays (Sandwich ELISA) b. flow cytometry technique

c. a solid phase competitive binding enzyme immunoassay d. none of the above

19. What test is used to diagnose growth hormone deficiency?

a. growth hormone stimulation test b. pituitary test

c. lumbar puncture d. a simple blood test

20. Which of the following is Growth hormone inhibiting hormone?

a. FSH b. Insulin c. GHRH d. Somatostatin

21. The main role of GH on hepatocytes is to induce the formation of …….

a. FSH b. TRH c. IGF-1 d. Somatostatin

22. Sex hormone secretion and release stimulated by ……

a. Parathyroid hormone (PTH) b. Calcitonin

c. Gonadotropic hormone d. Oxytocin

23. Corpus luteum secrets ……

a. LH b. Progesterone

c. Progesterone and LH d. Progesterone and estrogen

24. Which of these hormones is mainly produced by males?

a. Estrogen b. Oxytocin c. Testosterone d. All of these

25. Which of the following inhibit the production of progesterone?

a. LH b. FSH c. Inhibin d. GH

26. Which one of the following hormones binds to the pituitary and stimulates the release of LH and FSH?

a. Gonadotrophin releasing hormone (GnRH)

b. Corticotropin-releasing hormone (CRH)

c. Adrenocorticotropic hormone (ACTH)

27. The secretions from which of these glands differs between males and females?

a. Adrenal b. Parathyroid c. Gonadal d. Pancreas

28. The most one formed from estrogen is …….

a. Estrone b. Estradiol c. Estetrol d. estriol

29. An inherited condition in women caused by a missing X chromosome ……

a. Klinefelter syndrome b. Turner syndrome

c. Kallmann syndrome d. Prader-Willi syndrome

30. One of these causes a low level of testosterone …….

a. Testicular tumors b. Congenital adrenal hyperplasia

c. Polycystic ovary syndrome d. Mumps

31. Production of FSH is regulated by ……

a. Testosterone b. Inhibin

c. a and b d. No answer is correct

32. Dopamine and estrogen hormone can affect the production of ……

a. Insulin b. Albumin

c. Glucagon d. Prolactin

33. High levels of LH in females may be a sign of ……

a. Secondary ovarian failure.

b. Problem with the pituitary gland.

c. Problem with hypothalamus.

d. Primary ovarian failure.

34. Elevated insulin levels are seen in …….

a. Hyperglycemia b. Type 1 diabetes

c. Pancreatitis d. Hypoglycemia

35. FSH level in case of oligozoospermia is …….

a. Totally absent b. Partially decreased

c. Not effected c. Increased

36. Insulin works in tandem with ……

a. Glucagon b. Prolactin

c. Dopamine d. Adrenalin

37. LH is secreted from …….

a. Anterior portion of pituitary gland.

b. Anterior portion of hypothalamus.

c. Posterior portion of pituitary gland.

d. Posterior portion of hypothalamus.

38. Insulin allows..... to enter cells to be used as energy

a. Lipids b. Proteins

c. Glucose d. Amino acids

39. Too high levels of FSH in women due to failure of ovaries are called …….

a. Klinefelter's syndrome b. Turner syndrome

c. Kallmann’s syndrome c. None of the above

Appendix 1: Normal values of metabolic parameters and tests of endocrine function

Test name	Sample	Normal for 25 years old only	Unit
Cortisol (Free), Urine	5ml from 24 hrs. urine	3.5-45	µg/24hr
Cortisol (Extracted), Urine	5ml from 24 hrs. urine	55.5-286	µg/24hr
Cortisol (9 A.M.)	Serum	4.3-22.4	µg/dl
Cortisol (9 P.M.)	Serum	3.09-16.66	µg/dl
Estradiol (E2)	Serum	Males: 10-52 Females: Early Follicular: 20-150 Late Follicular: 40-350 Midcycle: 150-170 Luteal Phase: 30-450 Post-Menopausal: < 21	pg/ml
Estriol unconj. (uE3)	Serum	Males: Up to 2.0 Females: Non Pregnant Early: < 2.0 16W: 0.3-1.05 18W: 0.63-2.3 34w: 5.3-18.3 35W: 5.2-26.4 36W: 8.2-28.1 37W: 8.0-30.0 38 W: 8.6-38.0 39W: 7.2-34.3 40W: 9.6-28.9	ng/mL

FSH	Serum	Males: 1.4-15.4 Females: Early Follicular: 1.4-9.9 Midcycle: 6.17-17.2 Luteal Phase: 1.1-9.2 Post-Menopausal: 19.3-100.6	mIU/ml
Growth Hormone (After Stimulation)	Serum	Insulin Test > 10 Arginine Test > 7.5 L-Dopa > 7.5	ng/mL
Growth Hormone (Basal)	Serum	Up to 13.0	ng/mL
Insulin Level (Fasting)	Serum	2-25	mIU/ml
Insulin Level (P.P.)	Serum	16-166	mIU/ml
LH	Serum	Males: 1.5-9.3 Females: Follicular: 1.7-15 Midcycle: 21.9-56.6 Luteal Phase: 0.5-16.9 Post-Menopausal: 14.2-52.3	mIU/ml
Progesterone	Serum	Males: 0.28-1.22 Females: Follicular: 0.15-1.40 Luteal Phase: 3.34-25.56 Mid-Luteal Phase: 4.44-28.03 Post-Menopausal: 14.2-52.3	mIU/ml
Prolactin	Serum	Males: 4.04-15.2 Females: 4.79-23.3	ng/mL
TSH	Serum	0.4-5	µIU/mL

T3-Free	Serum	2-4.4	pg/mL
T3-Total	Serum	Male & Female: 70-204 Female: Preg. 1st Trimester: 81-190 2nd & 3rd Trimester: 100-260	ng/dL
T3 uptake	Serum	Male: 27-37 Female: 20-37	%
T4-Free	Serum	0.93-1.7	ng/dL
T4-Total	Serum	5.1-14.1	ng/dL
Testosterone-Total	Serum	Male ≥ 18 yrs.: 2.5-11 Female ≥ 18 yrs.: 0.15-0.7	ng/mL
Testosterone-Free	Serum	Male: 50-210 Female: 0.1-6.4	pg/mL

Appendix 2: The endocrine organs and their hormones*

Gland	Hormone	Molecular characteristics
Hypothalamus/ median eminence	Releasing and inhibiting hormones:	
	Thyrotrophin-releasing hormone (TRH)	Peptide
	Somatostatin (SS; inhibits GH))	Peptide
	Gonadotrophin-releasing hormone (GnRH)	Peptide
	Corticotrophin-releasing hormone (CRH)	Peptide
	Growth hormone-releasing hormone (GHRH)	Peptide
	Dopamine (inhibits prolactin)	Tyrosine derivative
Anterior pituitary	Thyrotrophin or thyroid-stimulating hormone (TSH)	Glycoprotein
	Luteinizing hormone (LH)	Glycoprotein
	Follicle-stimulating hormone (FSH)	Glycoprotein
	Growth hormone (GH) (also called somatotrophin)	Protein
	Prolactin (PRL)	Protein
	Adrenocorticotrophic hormone (ACTH)	Peptide
Posterior pituitary	Vasopressin [also called antidiuretic hormone (ADH)]	Peptide
	Oxytocin	Peptide
Thyroid	Thyroxine (T4) and tri-iodothyronine (T3)	Tyrosine derivatives
	Calcitonin	Peptide
Parathyroid	Parathyroid hormone (PTH)	Peptide
Adrenal cortex	Aldosterone	Steroid
	Cortisol	Steroid
	Androstenedione	Steroid
	Dehydroepiandrosterone (DHEA)	Steroid
Adrenal medulla	Epinephrine (also called adrenaline)	Tyrosine derivative
	Norepinephrine (also called noradrenaline)	Tyrosine derivative
Stomach†	Gastrin	Peptide
Pancreas (islets of Langerhans)†	Insulin	Protein
	Glucagon	Protein
	Somatostatin (SS)	Protein
Duodenum and jejunum†	Secretin	Protein
	Cholecystokinin	Protein
Liver	Insulin-like growth factor I (IGF-I)	Protein
Ovary	Oestrogens	Steroid
	Progesterone	Steroid
Testis	Testosterone	Steroid

* The distinction between peptide and protein is somewhat arbitrary. Shorter than 50 amino acids is termed a peptide in this table.

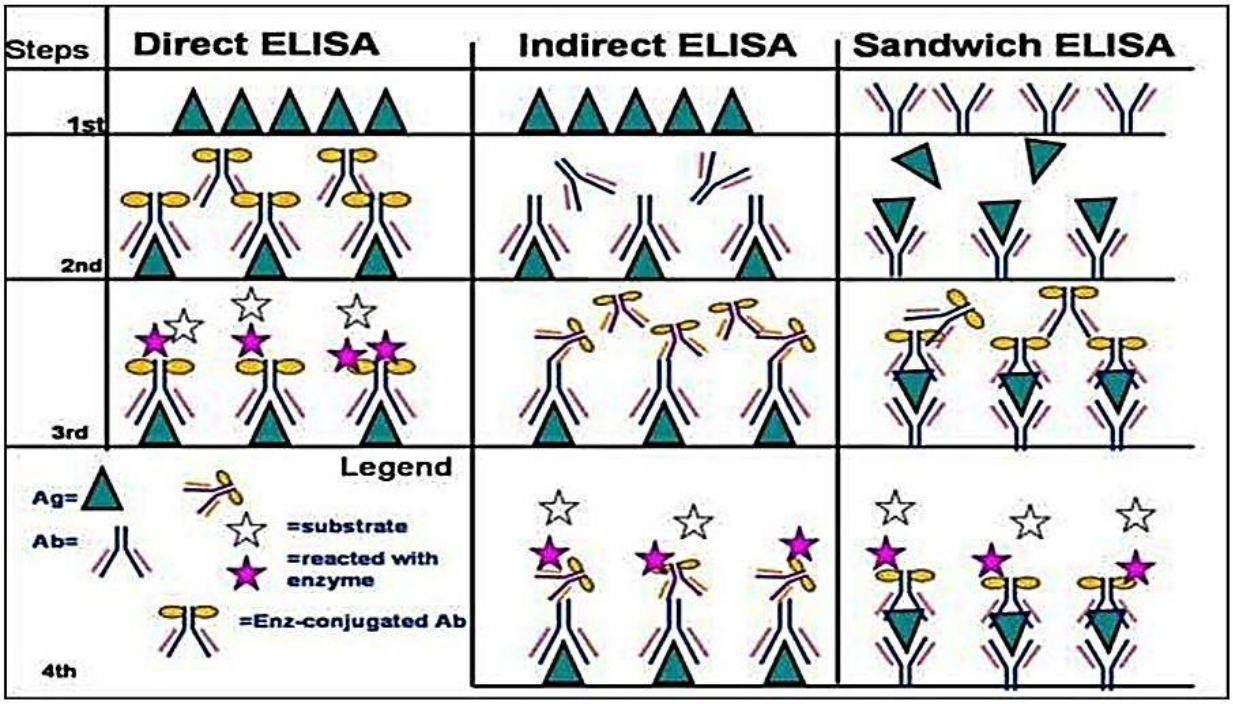
Appendix 3

Random sampling vs. dynamic testing

Hormone	Random or dynamic sampling?
GH	Glucose tolerance test Insulin stress test or alternative if suspect low
IGF-1	Random
LH, FSH	Random in males, post-menopausal females Timed with menstrual cycle in pre-menopausal females Random or stimulated in pre-pubertal children
Testosterone	Random
Oestrogen (*oestradiol*)	Random in males, post-menopausal females Timed with menstrual cycle in pre-menopausal females
ACTH	Random
Cortisol	Dexamethasone suppression test for excess Synacthen stimulation test if suspect low
TSH	Random
T4 & T3	Random
Prolactin	Random
ADH/vasopressin	Don't normally measure directly
Osmolality	Water deprivation test if suspect diabetes insipidus
Parathyroid hormone	Random, but need simultaneous calcium value
Insulin	Fasting, plus simultaneous glucose value
Calcitonin	Random
Renin/aldosterone	Upright usually, off medication
Catecholamines	Measure in urine, 24h sample
5HIAAs	Measure in urine, 24h sample

Appendix 4

Types of Non Competitive ELISA



Appendix 5: Advantages and disadvantages of each ELISA type

	Advantages	Disadvantages
Direct ELISA	1. Simple protocol, time-saving, and reagents-saving. 2. No cross-reactivity from secondary antibody.	1. High background. 2. No signal amplification, since only a primary antibody is used and a secondary antibody is not needed. 3. Low flexibility, since the primary antibody must be labeled.
Indirect ELISA	1. Signal amplification, since one or more secondary antibodies can be used to bind to the primary antibody. 2. High flexibility, since the same secondary antibody can be used for various primary antibodies.	1. Complex protocol compared with direct ELISA. 2. Cross-reactivity from secondary antibody.
Sandwich ELISA	1. High flexibility. 2. High sensitivity. 3. High specificity, since different antibodies bind to the same antigen for detection.	1. The antigen of interest must be large enough so that two different antibodies can bind to it at different epitopes. 2. It's sometimes difficult to find two different antibodies that recognize different epitopes on the antigen of interest and cooperate well in a sandwich format.
Competitive ELISA	1. High flexibility. 2. High sensitivity. 3. Best for the detection of small antigens, even when they are present in low concentrations.	1. Relatively complex protocol. 2. Needs the use of inhibitor antigen.

https://www.cusabio.com/c-20659.html

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Lab. Worksheet

Name: …….. **ID: ……**

Date: ……..……

I- From your practical activity in the lab, complete the following:

TSH = …….

T4 = …….

LH = ……

Estradiol = …….

Interpretation:

……

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……

……

Signature: ……