Chapter one
Introduction

1-1 Anatomy

The Thyroid glands are located in the neck just below your Adam's apple,in close approximation to the first part of the trachea. In humans, the thyroid gland has a "butterfly" shape, with two lateral lobes that are connected by a narrow section called the isthmus. Most animals, however, have two separate glands on either side of the trachea. Thyroid glands are brownish-red in color.

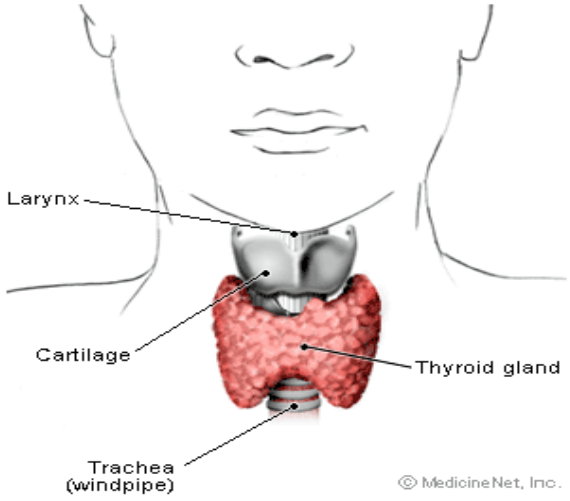


Figure 1-1 location of thyroid gland in the body

The microscopic structure of the thyroid is quite distinctive. Thyroid epithelial cells - the cells responsible for synthesis of thyroid hormones - are arranged in spheres

called thyroid follicles. Follicles are filled with colloid, a proteinaceous depot of thyroid hormone precursor. In the low (left) and high-magnification (right) images of a cat thyroid below, follicles are cut in cross section at different levels, appearing as roughly circular forms of varying size. In standard histologic preparations such as these, colloid stains pink.

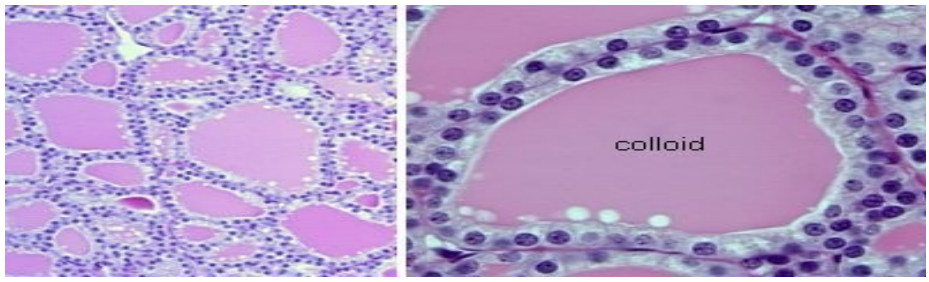


Figure 1-2 thyroid tissue

In addition to thyroid epithelial cells, the thyroid gland houses one other important endocrine cell. Nestled in spaces between thyroid follicles are Para follicular or C cells, which secrete the hormone calcitonin. The structure of a parathyroid gland is distinctly different from a thyroid gland. The cells that synthesize and secrete parathyroid hormone are arranged in rather dense cords or nests around abundant capillaries. The image below shows a section of a feline parathyroid gland on the left, associated with thyroid gland (note the follicles) on the right

1-2 Physiology

Thyroid hormones are synthesized by mechanisms fundamentally different from what is seen in other endocrine systems. Thyroid follicles Serve as both factory and

warehouse for production of thyroid hormones (T4 and T3 hormones) that affect your body's metabolism and energy level. the production of this hormone is not possible without stimulation from the pituitary gland (TSH) which in turn is also regulated by the hypothalamus's TSH Releasing Hormone

1-3Thyroid problems

The thyroid gland is prone to several very distinct problems, some of which are extremely common. These problems can be broken down into:

- Those concerning the production of hormone (too much, or too little).
- Those due to increased growth of the thyroid causing compression of important neck structures or simply appearing as a mass in the neck.
- The formation of nodules or lumps within the thyroid which are worrisome for the presence of thyroid cancer. And
- Those which are cancerous.

1-4 Thyroid function tests

1-4-1 Definition

Thyroid function tests are blood tests used to evaluate how effectively the thyroid gland is working . The thyroid gland function depend on the presence of iodine content Is the most popular test .Because thyroid is the only organ in the body that takes up and uses iodine These tests include the thyroid-stimulating hormone test (TSH), the thyroxine test (T4), the triiodothyronine test (T3), the thyroxine-binding globulin test (TBG), the triiodothyronine resin uptake test (T3RU), and the long-acting thyroid stimulator test (LATS).

1-4-2 Purpose

Thyroid function tests are used to:

- help diagnose an underactive thyroid(hypothyroidism) and an overactive thyroid(hyperthyrodism)
- evaluate thyroid gland activity
- monitor response to thyroid therapy

1-4-3 Precautions

- Thyroid treatment must be stopped one month before blood is drawn for a thyroxine (T4) test.

- Steroids, propranolol (Inderal), cholestyramine (Questran), and other medications that may influence thyroid activity are usually stopped before a triiodothyronine (T3) test.

- Estrogens, anabolic steroids, phenytoin, and thyroid medications may be discontinued prior to a thyroxine-binding globulin (TBG) test. The laboratory analyzing the blood sample must be told if the patient cannot stop taking any of these medications. Some patients will be told to take these medications as usual so that the doctor can determine how they affect thyroxine-binding globulin.

- Patients are asked not to take estrogens, androgens, phenytoin (Dilantin), salicylates, and thyroid medications before having a triiodothyronine resin uptake (T3RU) test.

- Prior to taking a long-acting thyroid stimulant (LATS) test, the patient will probably be told to stop taking all drugs that could affect test results.

1-5 Other test used to diagnose thyroid gland:

Measurement of Serum Thyroid Hormones T4 by RIA: T4 by RIA (radioimmunoassay) is the most used thyroid test of all. It is frequently referred to as

a T7 which means that a resin T3 uptake (RT3u) has been done to correct for certain medications such as birth control pills, other hormones, seizure medication, cardiac drugs, or even aspirin that may alter the routine T4 test. The T4 reflects the amount of thyroxin in the blood. If the patient does not take any type of thyroid medication, this test is usually a good measure of thyroid function.

Measurement of Serum Thyroid Hormones T3 by RIA: As stated on our thyroid hormone production page, thyroxin (T4) represents 80% of the thyroid hormone produced by the normal gland and generally represents the overall function of the gland. The other 20% is triiodothyronine measured as T3 by RIA. Sometimes the diseased thyroid gland will start producing very high levels of T3 but still produce normal levels of T4. Therefore measurement of both hormones provides an even more accurate evaluation of thyroid function

Thyroid Binding Globulin: Most of the thyroid hormones in the blood are attached to a protein called thyroid binding globulin (TBG). If there is an excess or deficiency of this protein it alters the T4 or T3 measurement but does not affect the action of the hormone. If a patient appears to have normal thyroid function, but an unexplained high or low T4, or T3, it may be due to an increase or decrease of TBG. Direct measurement of TBG can be done and will explain the abnormal value. Excess TBG

or low levels of TBG are found in some families as in hereditary trait. It causes no problem except falsely elevating or lowering the T4 level. These people are frequently misdiagnosed as being hyperthyroid or hypothyroid, but they have no thyroid problem and need no treatment.

Measurement of Pituitary Production of TSH: Pituitary production of TSH is measured by a method referred to as IRMA (immunoradiometric assay). Normally, low levels (less than 5 units) of TSH are sufficient to keep the normal thyroid gland functioning properly. when the thyroid gland becomes inefficient such as in early hypothyroidism, the TSH becomes elevated even though the T4 and T3 may still be within the "normal" range. This rise in TSH represents the pituitary gland's response to a drop in circulating thyroid hormone; it is usually the first indication of thyroid gland failure. Since TSH is normally low when the thyroid gland is functioning properly, the failure of TSH to rise when circulating thyroid hormones are low is an indication of impaired pituitary function. The new "sensitive" TSH test will show very low levels of TSH when the thyroid is overactive (as a normal response of the pituitary to try to decrease thyroid stimulation). interpretations of the TSH level depends upon the level of thyroid hormone; therefore, the TSH is usually used in combination with other thyroid tests such as the T4 RIA and T3 RIA.

TRH Test: In normal people TSH secretion from the pituitary can be increased by giving a shot containing TSH Releasing Hormone (TRH...the hormone released by the hypothalamus which tells the pituitary to produce TSH). A baseline TSH of 5 or less usually goes up to 10-20 after giving an injection of TRH. Patients with too much thyroid hormone (thyroxin or triiodothyronine) will not show a rise in TSH when given TRH. This "TRH test" is presently the most sensitive test in detecting early hyperthyroidism. Patients who show too much response to TRH (TSH rises greater than 40) may be hypothyroid. This test is also used in cancer patients who are taking thyroid replacement to see if they are on sufficient medication. It is sometimes used to measure if the pituitary gland is functioning. The new "sensitive" TSH test (above) has eliminated the necessity of performing a TRH test in most clinical situations.

Table 1-1 shows the normal value of thyroid lab investigation.

Test	Abbreviation	Typical Ranges
Serum thyroxin	T4	4.6-12 ug/dl
Free thyroxin fraction	FT4F	0.03-0.005%
Free Thyroxin	FT4	0.7-1.9 ng/dl
Thyroid hormone binding ratio	THBR	0.9-1.1

Free Thyroxin index	FT4I	4-11
Serum Triiodothyronine	T3	80-180 ng/dl
Free Triiodothyronine l	FT3	230-619 pg/d
Free T3 Index	FT3I	80-180
Radioactive iodine uptake	RAIU	10-30%
Serum thyrotropin	TSH	0.5-6 uU/ml
Thyroxin-binding globulin	TBG	12-20 ug/dl T4 +1.8 ugm
TRH stimulation test Peak	TSH	9-30 uIU/ml at 20-30 min
Serum thyroglobulin l	Tg	0-30 ng/m
Thyroid microsomal antibody titer	TMAb	Varies with method
Thyroglobulin antibody titer	TgAb	Varies with method

Thyroid Uptake Test: A means of measuring thyroid function is to measure how much iodine is taken up by the thyroid gland (RAI uptake). Remember, cells of the thyroid normally absorb iodine from our blood stream (obtained from foods we eat) and use it to make thyroid hormone (described on our thyroid function page). Hypothyroid patients usually take up too little iodine and hyperthyroid patients take up too much iodine. The test is performed by giving a dose of radioactive iodine on an empty stomach. The iodine is concentrated in the thyroid gland or excreted in the urine over

the next few hours. The amount of iodine that goes into the thyroid gland can be measured by a "Thyroid Uptake". Of course, patients who are taking thyroid medication will not take up as much iodine in their thyroid gland because their own thyroid gland is turned off and is not functioning. At other times the gland will concentrate iodine normally but will be unable to convert the iodine into thyroid hormone; therefore, interpretation of the iodine uptake is usually done in conjunction with blood tests.

Thyroid Scan: Taking a "picture" of how well the thyroid gland is functioning requires giving a radioisotope to the patient and letting the thyroid gland concentrate the isotope (just like the iodine uptake scan above). Therefore, it is usually done at the same time that the iodine uptake test is performed. Although other isotopes, such as technetium, will be concentrated by the thyroid gland; these isotopes will not measure iodine uptake which is what we really want to know because the production of thyroid hormone is dependent upon absorbing iodine. It has also been found that thyroid nodules that concentrate iodine are rarely cancerous; this is not true if the scan is done with technetium. Therefore, all scans are now done with radioactive iodine. Both of the scans above show normal sized thyroid glands, but the one on the left has a "HOT" nodule in the lower aspect of the right lobe, while the scan on the right has a

"COLD" nodule in the lower aspect of the left lobe (outlined in red and yellow). Pregnant women should not have thyroid scans performed because the iodine can cause development troubles within the baby's thyroid gland .two types of thyroid scans are available. A camera scan is performed most commonly which uses a gamma camera operating in a fixed position viewing the entire thyroid gland at once. This type of scan takes only five to ten minutes. In the 1990's, a new scanner called a Computerized Rectilinear Thyroid (CRT) scanner was introduced. The CRT scanner utilizes computer technology to improve the clarity of thyroid scans and enhance thyroid nodules. It measures both thyroid function and thyroid size. A life-sized 1:1 color scan of the thyroid is obtained giving the size in square centimeters and the weight in grams. The precise size and activity of nodules in relation to the rest of the gland is also measured. CTS of the normal thyroid gland In addition to making thyroid diagnosis more accurate, the CRT scanner improves the results of thyroid biopsy. The accurate sizing of the thyroid gland aids in the follow-up of nodules to see if they are growing or getting smaller in size. Knowing the weight of the thyroid gland allows more accurate radioactive treatment in patients who have Graves' disease. Specifically thyroid Scans are used for the following reasons:

- Identifying Nodules and Determining If They Are "Hot" Or "Cold".

- Measuring the size of the goiter prior to treatment.

- Follow-up of thyroid cancer patients after surgery.

- Locating thyroid tissue outside the neck, i.e. base of the tongue or in the chest.

*Thyroid Ultrasound scans:*Thyroid ultrasound refers to the use of high frequency sound waves to obtain an image of the thyroid gland and identify nodules. It tells if a nodule is "solid" or a fluid-filled cyst, but it will not tell if a nodule is benign or malignant. Ultrasound allows accurate measurement of a nodule's size and can determine if a nodule is getting smaller or is growing larger during treatment. Ultrasound aids in performing thyroid needle biopsy by improving accuracy if the nodule cannot be felt easily on examination.

*Thyroid Antibodies:*The body normally produces antibodies to foreign substances such as bacteria; however, some people are found to have antibodies against their own thyroid tissue. A condition known as Hashimoto's Thyroiditis is associated with a high level of these thyroid antibodies in the blood. Whether the antibodies cause the disease or whether the disease causes the antibodies is not known; however, the finding of a high level of thyroid antibodies is strong evidence of this disease. Occasionally, low levels of thyroid antibodies are found with other types of thyroid

disease. When Hashimoto's thyroiditis presents as a thyroid nodule rather than a diffuse goiter, the thyroid antibodies may not be present.

Thyroid Needle Biopsy: This has become the most reliable test to differentiate the "cold" nodule that is cancer from the "cold" nodule that is benign ("hot" nodules are rarely cancerous). It provides information that no other thyroid test will provide. While not perfect, it will provide definitive information in 75% of the nodules biopsied. Thyroid nodules increase with age and are present in almost ten percent of the adult population. Autopsy studies reveal the presence of thyroid nodules in 50 percent of the population, so they are fairly common. Ninety-five percent of solitary thyroid nodules are benign, and therefore, only five percent of thyroid nodules are malignant. Common types of the benign thyroid nodules are adenomas (overgrowths of "normal" thyroid tissue), thyroid cysts, and Hashimoto's thyroiditis. Uncommon types of benign thyroid nodules are due to sub acute thyroiditis, painless thyroiditis, unilateral lobe agenesis, or Riedel's struma. As noted on previous pages, those few nodules which are cancerous are usually due to the most common types of thyroid cancers which are the differentiated" thyroid cancers. Papillary carcinoma accounts for 60 percent, follicular carcinoma accounts for 12 percent, and the follicular variant of papillary carcinoma accounting for six percent. These well differentiated thyroid

cancers are usually curable, but they must be found first. Fine needle biopsy is a safe, effective, and easy way to determine if a nodule is cancerous.

Thyroid cancers typically present as a dominant solitary thyroid nodule which can be felt by the patient or even seen as a lump in the neck by his/her family and friends. This is illustrated in the picture above. As pointed out on our page introducing thyroid nodules, we must differentiate benign nodules from cancerous solitary thyroid nodules. While history, examination by a physician, laboratory tests, ultrasound, and thyroid scans (shown in the picture below) can all provide information regarding a solitary thyroid nodule, the only test which can differentiate benign from cancerous thyroid nodules is a biopsy (the term biopsy means to obtain a sample of the tissue and examine it under the microscope to see if the cells have taken on the characteristics of cancer cells). Thyroid cancer is no different in this situation from all other tissues of the body...the only way to see if something is cancerous is to biopsy it. However, thyroid tissues are easily accessible to needles, so rather than operating to remove a chunk of tissue with a knife, we can stick a very small needle into it and remove cells for microscopic examination. This method of biopsy is called a fine needle aspiration biopsy, or "FNA". Cold nodule: Thyroid cells absorb iodine so they can make thyroid hormone out of it. When radioactive iodine is given, a butterfly

image will be obtained on x-ray film showing the outline of the thyroid. If a nodule is composed of cells which do not make thyroid hormone (don't absorb iodine) then it will appear "cold" on the x-ray film. A nodule which is producing too much hormone will show up darker and is called "hot". The evaluation of a solitary thyroid nodule should always include history and examination by a physician. Certain aspects of the history and physical exam will suggest a benign or malignant condition. Remember, a biopsy of some sort is the only way to tell for sure

Thyroid fine needle aspiration (FNA) biopsy: is the only non-surgical method which can differentiate malignant and benign nodules in most, but not all, cases. The needle is placed into the nodule several times and cells are aspirated into a syringe. The cells are placed on a microscope slide, stained, and examined by a pathologist. The nodule is then classified as no diagnostic, benign, suspicious or malignant.

No diagnostic indicates that there are an insufficient number of thyroid cells in the aspirate and no diagnosis is possible. A no diagnostic aspirate should be repeated, as a diagnostic aspirate will be obtained approximately 50 percent of the time when the aspirate is repeated. Overall, five to 10 percent of biopsies are no diagnostic, and the patient should then undergo either an ultrasound or a thyroid scan for further evaluation.

Benign thyroid aspirations are the most common (as we would suspect since most nodules are benign) and consist of benign follicular epithelium with a variable amount of thyroid hormone protein (colloid).

Malignant thyroid aspirations can diagnose the following thyroid cancer types: papillary, follicular variant of papillary, medullary, anaplastic, thyroid lymphoma, and metastases to the thyroid. Follicular carcinoma and Hurthle cell carcinoma cannot be diagnosed by FNA biopsy. This is an important point. Since benign follicular adenomas cannot be differentiated from follicular cancer (~12% of all thyroid cancers) these patients often end up needing a formal surgical biopsy, which usually entails removal of the thyroid lobe which harbors the nodule.

Suspicious cytologies make up approximately 10 percent of FNA's. The thyroid cells on these aspirates are neither clearly benign nor malignant. Twenty five percent of suspicious lesions are found to be malignant when these patients undergo thyroid surgery. These are usually follicular or Hurthle cell cancers. Therefore, surgery is recommended for the treatment of thyroid nodules from which a suspicious aspiration has been obtained.

FNA is the first, and in the vast majority of cases, the only test required for the evaluation of a solitary thyroid nodule. (A TSH value should also be obtained to

evaluate thyroid function.) Thyroid ultrasound and thyroid scans are usually not required for evaluation of a solitary thyroid nodule. FNA has reduced the cost for evaluation and treatment of thyroid nodules, and has improved yield of cancer found at thyroid surgery. Although a solitary thyroid nodule can enlarge or shrink over time, the natural history of solitary nodules reveals that most nodules change little with it.

1-6 Problem of the study

As mention before There Were two method to diagnose the function of thyroid gland by blood one by using ELISA and other by using RIA, However those methods done by the same request ordered by the doctor and are the same in using serum of the blood after centrifuge it but Unfortunately, the result which determinate the function (amount of T3, T4, TSH) isn't similar to either method, in spite of differences these tests are common used because of their diagnostic usefulness. To make sure about this problem I took 5 sample and the two methods were applied on the same samples and the difference have been noticed between them, so we will continue by gathering data and do test by each method to confirm the differences, to try to find a solution to make it disappear or at least lessen from it because this differ will cause the big problem and lead to misdiagnoses.

1-7 Objectives

The general objective of this study was to assess human thyroid function using radioimmunoassay (RIA) and enzyme-linked-immuno-sorbent-assay (ELISA) to validate the reliability of ELISA relative to RIA method.

1:7:1Specific objectives

- To cross-correlate between the ELISA and RIA results
- To evaluate the result according to the patient age, weight, height.
- To correlate between the RIA and ELISA result and the patient body characteristics
- To find a correction factor that might minimize the difference between the two method
- To investigate the cause of the discrepancy between the two methods

1-8 Significant of the study

This study can play more active role in highlighting the sensitivity and accuracy of diagnosing of thyroid function test also it give better result when we focus to the difference and cause of it and try to find the solution in addition it may help other researcher to discover new way for investigation of thyroid function with minimizing the false percent.

1-9 Overview of the study:

This study falls into five chapters with chapter one is an introduction which include the tools used for investigation of thyroid disorder, problem of the study, purpose of the study, objective of the study and the overview of the study. Chapter two include theoretical background and literature review, while chapter three demonstrate the method and material used in this study, chapter four include presentation of the result and finally chapter five include the discussion, recommendation and references list.

CHAPTER TWO

Section one

Theoretical background

2-1 Immunoassay

For nearly half a century, immunoassays have been the primary source for detection of analytes of interest in biological samples for both life science research and clinical diagnostics. This began with the quest to measure insulin levels and culminated in the development of the radioimmunoassy (RIA) by Yallow and Berson (1960). The desire to use less hazardous detection methods than radioisotopes lead to the development of

the enzyme-linked immunosorbent assay (ELISA) by Engvall and Perlmann (1971). An immunoassay is essentially the measurement of a substance using the reaction of an antibody considered as the immunoreagent. The word measurement is applied to the both quality and quantity measurement .it depend on interactions between antigens (Ag) and antibodies (Ab) The nature of this reaction is Lock and Key Concept – The combining site of an antibody is located in the Fab portion of the molecule and is constructed from the hyper variable regions of the heavy and light chains. X-Ray crystallography studies of antigens and an antibody interacting shows that the antigenic determinant nestles in a cleft formed by the combining site of the antibody. Thus, our concept of Ag-Ab reactions is one of a key (*i.e.* the Ag) which fits into a lock (*i.e.* the Ab).

e.g.:

Ab + Ag ⟶ **Ab . Ag**

- where k1= association constant
- and k2 = dissociation constant
- Applying the law of mass action
- [Ab.Ag]/ [Ab][Ag]=k1/k2=K
- where K = affinity constant

- [Ab] = antibody concentration
- [Ag] = antigen concentration
- [Ab.Ag]= antibody-antigen complex concentration
- At the beginning of the reaction , the antibody (Ab) and the antigen (Ag) to form a complex (Ab: Ag) .
- Ab + Ag ----→ Ab:Ag

This reaction is reversible and dissociated

- Ab : Ag------→ Ab + Ag

This reaction is more complex and increasing amount of dissociation .

- After a certain time the association will be balanced by the same amount of dissociation and the reaction will be at equilibrium ,i.e:

  Ab+Ag---→Ab:Ag .

2-1 Factor that affect the reaction:

- *Reaction pH*

Optimal (working)assay pH usually-7.0

Binding inhibited at extremes of pH(acid or basic)

Masking of epitopes may occur at extremes of pH

- *Ionic strength*

High ionic strength(molarity)may inhabit binding

At low ionic strength,pH may not be controlled

- *Temperature*

Affects the association & dissociation constants($k_1$and k_2)

Some immunological reactions are thermolabile evaporation may influence Ab-Ag binding

- *Reaction matrix*

Matrices containing high concentration of proteins may inhibit Ab-Ag binding

Matrices containing low concentration may favour Ab-Ag binding

Other substances may inhibit Ab-Ag binding

2-2 Radioimmunoassay (RIA)

One of the earlier type of immunoassay which is the mother of all other immunoassays is an in vitro nuclear medicine test. Is simple, reliable and reproducible laboratory techniques used extensively in clinical laboratories for the last three decades worldwide because of its great sensitivity. Using antibodies of high affinity (K0 = 108–1011 M−1), it is possible to detect a few 22iluents22d (10−12 g) of antigen in the tube. This Nobel prize winning technique is based on the antigen-antibody reaction, imparts the highest degree of specificity, and utility of radioisotopes offers astonishing degree of sensitivity .They are primarily utilized in diagnosis and management of thyroid disorders and infertility. As the name suggests there is an antigen – antibody reaction between the antigen (Hormone) of interest and its specific antibodies raised from laboratory animals. The quantum of antigen-antibody binding is monitored using a radiolabelled antigen or

antibody. In brief the RIA kit contains test tubes which have been coated with the antibody. To these tubes, standards or samples are added (usually 25 to 200 ul volume) along with the tracer, The contents are incubated for 1 – 2 hours and the tubes are emptied by decanting the contents or aspirating. The tubes are washed with the wash solution provided and are counted in a gamma counter. The counts obtained for various known standards used in the assay are utilized for generating a dose – response curve and the unknown concentration in serum samples are extrapolated from the data. Basically there are classified into two types and they are: a limited antibody assay (RIA-original) and an excess antibody assay (IRMA – also called RIA). For e.g. T3 and T4 are done using the conventional RIA while the estimation of TSH, FSH, LH and PRL are using IRMA. All the pituitary hormones are done with IRMA which has got a wider working range, improved sensitivity and shorter incubation time.

2:2:1Competitive radioimmunoassay (RIA)

The Competitive assay method used Limited antibody concentration and Requires use of 23iluent analyte as tracer analyte

- Analyte: Antigen (substance to be analyzed)
- 125 I- 23iluent antigen= radioactive tracer
- Description of other assay components
- B= bound fraction (Ab-Ag complex formed)
- F= free fraction (Ag not bound to Ab)
- T= total radioactivity (quantity of tracer used)

analyte Labelled analyte Ab Ab- bound fraction Free fraction

Separate from free fraction

Measure label in Ab – bound fraction

figure 2:1 competitive RIA

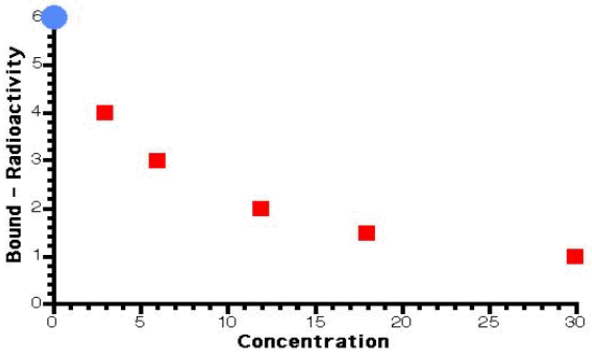


Figure 2:2 Curve of competitive method

2:2:2 Non competitive assay method :

- also called as Immunoradiometric assay (IRMA) or sandwich
- it Need for large quantities of pure antibody (monoclonal antibodies usually employed)and required 2 antibody binding sites (limit range of analysis)
- High dose “hook” effect

- Need for multiple washing steps
- Non specific interference due to heterophyllic antibodies

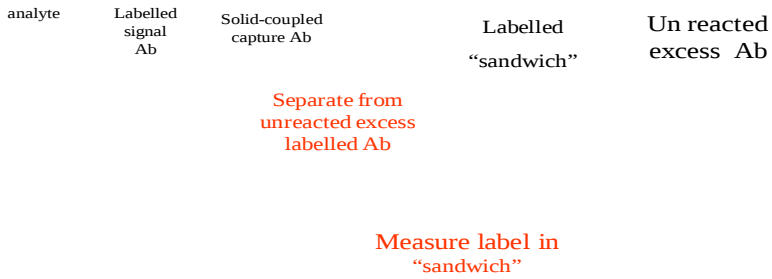


figure 2:3 non competitive method

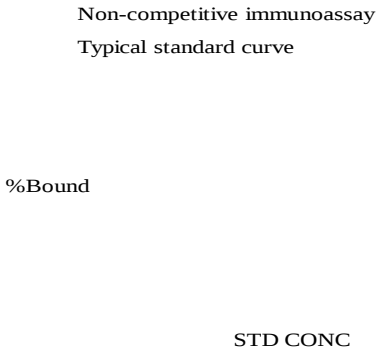


figure 2:4 curve of non-competitive method

2:2:3 Radioimmunoassay is widely-used because it have a lot of advantage:

- **sensitivity** (Using antibodies of high affinity (K0 = 108–1011 M−1), it is possible to detect a few 26iluents26d (10−12 g) of antigen in the tube).

- A number of substances of biological significant found in blood, put in position showing their approximate concentration using 26iluen-chemical procedures.

- Our plant earth from space is magnified we can see more details about the earth this means that we make more sensitive to see these details which can't be seen unless we magnify .
- Another example for sensitivity if we take 1.4 mm of epidermis a cross and magnified by a factor to show more details than what can see by neck eyes so this is the advantages of RIA in terms of sensitivity when related to the other analytical techniques .
- **specific**(specific antibody to specific antigen), it requires specialized equipment(gamma counter), If we look at steroids hormones for example oestradiol and oilstone and look at the molecular structures of these two steroid , a small changes of molecular structure , the RIA is specified and easy detected it
- **Simplicity:** In RIA once reagent were prepared it's possible to investigate small volume of biological fluid such as blood , it does not need extraction techniques or purification or at a certain concentration for detecting , so these advantages of simplicity in an analytical techniques were increase the speed of investigation using these methods
- **Universal application:** The word universal means here in this topic is a wide use of immunoassay where the works in 1950's show that antisera could produce using chemical substance even it were not organic in themselves, by production of

specific antisera when coupled to larger immunogens like protein albumin but not unique

Disadvantage of RIA:

- it is not considered to be as environmentally friendly because:
- Required specialized manipulation and store
- Requires specialized disposal of the residual radioisotopes,
- requires special precautions and licensing

2-3 ELISA

Was first described in 1971, and since then has become a more and more important technique in diagnostic virology. ELISA has replaced a number of more cumbersome and time consuming “classical” serological techniques, and has also widened the scope of the detection methods for viruses and their related markers of infection. When a foreign particle enters the body, the body tries to synthesize antibodies (defense particles) , which combine with the antigen making it susceptible to destruction or phagocytosis. The same interaction of antigen and antibody outside the body–in the laboratory–can be used to determine whether a patient has an infectious or an autoimmune disease. The test measures whether a specific antibody associated with an illness can be found in a patient’s blood. A positive result indicates that the antibody is there and implies that the person has encountered a particular disease.

A portion of serum possibly containing the antibody is allowed to react with the target antigen. A correct match causes the antigen and antibody to bind together. Detection becomes possible when a second antibody is added. This antibody is prepared from the

serum of an animal injected previously with human antibody; the human antibody in this case serves as an antigen and the animal thus produces an antibody against the human antibody. Once isolated, the second antibody can be chemically linked to a system that can produce a detectable signal. In ELISA the label that is added to produce a detectable signal is an enzyme and therefore it is called enzyme linked immunosorbent assay.

2:3:1 The basic principles of the ELISA test are as follows:

- Antigens solubilised in an appropriate buffer can be coated on a plastic surface, like polystyrene. This may be directly or via an antibody. When serum is added, antibodies can attach to the antigen on the solid phase.(Antigen-antibody binding).

- The presence or absence of these antibodies can be demonstrated with the help of anti-human immunoglobulin conjugate (indirect method) or with conjugate specific against the appropriate antigen (direct method) respectively. The antibodies are conjugated to an enzyme, for example peroxidase.(conjugate binding).

- Adding a substrate, like HRPO, will detect the amount of bound conjugate by a degree of colour produced, which can be quantified. ELISA can also be used for detection of antigens by using specific antibody on the solid phase. Adding an enzyme-linked antibody and a substrate leads to colour production in proportion to the amount of antigen present. Commercially produced ELISA kits are now available for a wide range of viral antigens and antibodies, including IgM

antibodies. The advantages of ELISA are its ease-of-use, flexibility, the ability of any lab to build assays, and low cost as evidenced by nearly 10,000 studies published per year utilizing the technique (Lequin, 2005).

2-3-2 Types of ELISA

Direct ELISA Indirect ELISA Dot ELISA Sandwich ELISA Competitive ELISA Reverse ELISA Multiplex ELISA

Direct ELISA :

Direct ELISA The direct detection method originated in the 1940s when Coons and colleagues labeled antibodies with a fluorescent tag to mark tissue antigens. The direct ELISA uses the method of directly labeling the antibody itself. Microwell plates are coated with a sample containing the target antigen, and the binding of labeled antibody is quantitated by a colorimetric, chemiluminescent, or fluorescent end-point. Since the secondary antibody step is omitted, the direct ELISA is relatively quick, and avoids potential problems of cross-reactivity of the secondary antibody with components in the antigen sample.

Advantages of Direct ELISA: Advantages of Direct ELISA Quick methodology since only one antibody is used. Cross-reactivity of secondary antibody is eliminated.

Disadvantages of Direct ELISA: Disadvantages of Direct ELISA Immuno reactivity of the primary antibody may be reduced as a result of labeling. Labeling of every primary antibody is time-consuming and expensive. No flexibility in choice of primary antibody label from one experiment to another. Little signal amplification

Indirect ELISA

It is two-step method uses a labeled secondary antibody for detection. First, a primary antibody is incubated with the antigen. This is followed by incubation with a labeled secondary antibody that recognizes the primary antibody.

Then secondary antibody will bind any antibody produced by a member of the donor's species (for example, an antibody produced in a mouse that will bind any rabbit antibody). This secondary antibody often has an enzyme attached to it, which has a negligible effect on the binding properties of the antibody.

A substrate for this enzyme is then added. Often, this substrate changes color upon reaction with the enzyme. The color change shows that secondary antibody has bound to primary antibody, which strongly implies that the donor has had an immune reaction to the test antigen. This can be helpful in a clinical testing, and in R&D. The higher the concentration of the primary antibody that was present in the serum, the stronger the color change. Often a spectrometer is used to give quantitative values for color strength

Advantages of Indirect ELISA: Advantages of Indirect ELISA A wide variety of labeled secondary antibodies are available commercially. Versatile, since many primary antibodies can be made in one species and the same labeled secondary antibody can be used for detection. Immuno-reactivity of the primary antibody is not affected by labeling. Sensitivity is increased because each primary antibody contains several epitopes that can be bound by the labeled secondary antibody, allowing for signal amplification. Different visualization markers can be used with the same primary antibody.

Disadvantages of Indirect Detection: Disadvantages of Indirect Detection Cross-reactivity may occur with the secondary antibody, resulting in nonspecific signal. An extra incubation step is required in the procedure.

DOT ELISA

DOT ELISA In this type ,we use nitrocellulose membrane instead of polysterene plate. The Substrate used is Bromocreso indoylpyrophosphate. This gives blue colour. This is useful in immunoblot technique. Disadvantage: Quantification is not possible.

Sandwich ELISA

Sandwich ELISA Sandwich ELISA is used to determine the antigen concentration in unknown samples. The sandwich ELISA measures the amount of antigen between two layers of antibodies. The antigens to be measured must contain at least two antigenic sites, capable of binding to antibody, since at least two antibodies act in the sandwich.

So sandwich assays are restricted to the quantification of multivalent antigens such as proteins or polysaccharides. Sandwich ELISAs for quantification of antigens are especially valuable when the concentration of antigens is low and/or they are contained in high concentrations of contaminating protein. Sensitivity of the sandwich ELISA is dependent on four factors :

- The number of molecules of the first antibody that are bound to the solid phase.
- The avidity of the first antibody for the antigen.
- The avidity of the second antibody for the antigen.

- The specific activity of the second antibody.

Applications of sandwich ELISA: to detect and quantitate large molecules with multiple antigenic sites, usually a protein, in a sample. To test recognition or binding between antigen and antibody, where polyclonals are usually used, to make an ELISA when a pair of monoclonal antibody is not immediately available.

Competitive ELISA: Competitive ELISA Unlabeled antibody is incubated in the presence of its antigen (Sample) These bound antibody/antigen complexes are then added to an antigen coated well. The plate is washed, so that unbound antibody is removed. (The more antigen in the sample, the less antibody will be able to bind to the antigen in the well, hence “competition”). The secondary antibody, specific to the primary antibody is added. This second antibody is coupled to the enzyme. A substrate is added, and remaining enzymes elicit a chromogenic or fluorescent signal.

Reverse ELISA:

Reverse ELISA A new technique uses a solid phase made up of an immunosorbent polystyrene rod with 4-12 protruding ogives. An ogive (pronounced oh-JYVE) is the roundly tapered end of a two-dimensional or three-dimensional object. The entire device is immersed in a test tube containing the collected sample and the following steps (washing, incubation in conjugate and incubation in chromogenous) are carried out by dipping the ogives in microwells of standard microplates pre-filled with reagents.

Advantages: The ogives can each be sensitized to a different reagent, allowing the simultaneous detection of different antibodies and different antigens for multi-target assays; The sample volume can be increased to improve the test sensitivity in clinical (saliva, urine), food (bulk milk, pooled eggs) and environmental (water) samples; One ogive is left unsensitized to measure the non-specific reactions of the sample; The use of laboratory supplies for dispensing sample aliquots, washing solution and reagents in microwells is not required, facilitating ready-to-use lab-kits and on-site kits.

Multiplex ELISA: A logical progression of the widely used microtiter plate ELISA is toward a protein array format that allows simultaneous detection of multiple analytes at multiple array addresses within a single well. There are different types of multiplex ELISA have been developed and in practice. One of the examples is to measure antigens by coating or printing capture antibodies in an array format within a single well to allow for the construction of “sandwich” ELISA quantification assays. Generally, multiplex ELISA can also be achieved through antibody array, where different primary antibodies can printed on glass plate to capture corresponding antigens in a biological sample such as plasma, cell lysate, or tissue extract. Detection method can be direct or indirect, sandwich or competitive, labeling or non-labeling, depending upon antibody array technologies.

Advantages of ELISA: Advantages of ELISA tests are generally highly sensitive and specific and compare to radioimmune assay (RIA) tests. They have the added advantages of not needing radioisotopes (radioactive substances) or a costly radiation counter (a radiation-counting apparatus). Reagents are relatively cheap & have a long shelf life

ELISA is highly specific and sensitive No radiation hazards occur during 34iluents or disposal of waste. Easy to perform and quick procedures Equipment can be inexpensive and widely available. ELISA can be used to a variety of infections.

Disadvantage of ELISA: Disadvantage of ELISA It requires skilled laboratory technicians and specialized laboratory equipment. Measurement of enzyme activity can be more complex than measurement of activity of some type of radioisotopes. Enzyme activity may be affected by plasma constituents. Kits are commercially available, but not cheap Very specific to a particular antigen. Won't recognize any other antigen False positives/negatives possible, especially with mutated/altered antigen. ELISA with weak signal: Wash buffer not adequately drained after every wash step. Inadequate incubation times. Detection reagents too dilute. Perform checkerboard titrations. Enzyme conjugate defective or inhibited by contaminant. Substrate defective or contaminated. Microwell plates poorly coated. Loss of capture antibody during blocking/washing. Decrease or eliminate use of Tween-20.

Section two
Literature review

Hamza et al. (2003) find out the effect of pregnancy on thyroid hormones by Measurement of thyroid hormones during all trimesters of pregnancy. To detects the correlation between thyroid gland activities with pregnancy. In these study One hundred and twenty Sudanese pregnant women at different stages of pregnancy were chosen for this study and classified according to gestational age into three subgroups: first trimester 5–12 weeks, second trimester 13–27 weeks and third trimester 28–40weeks. The Study

was conducted during the period 2001 to 2003 at Omdurman Maternity, Khartoum and Khartoum North Teaching Hospitals. Forty healthy non-pregnant women of the same age were used as control. Thyroid hormones were measured by using a highly sensitive specific radioimmunoassay (RIA) technique. Represent study show that There was an increase in the concentration of total thyroid hormones (T4 and T3), but the increase did not proceed at steady level during the different trimesters, both hormones increased significantly in all trimesters compared with control group and continued thereafter, but at slower rate. Thyroid stimulating hormone (TSH) level remained within the normal range throughout the pregnancy period, although (12.5%) pregnant women with TSH values below normal range in the first trimester.

Abdelgadir et al. (2009) determined antibodies to thyroglobulin (TgAb) and thyroid peroxidase (TPOAb) to assess its diagnostic value, especially in autoimmune thyroid disease. Using an ELISA technique for measured the anti-thyroid (anti-Tg, and anti-TPO) Antibodies concentrations, in 208 patients with various thyroid pathologies attending Fedail clinic, and Khartoum teaching hospital in Khartoum, Sudan. Patients were grouped into five categories according to presentation, family history and laboratory investigation. Blood specimens also collected from normal Sudanese individuals with no family history of thyroid disease and matching ethnicity and sex. The patients were categorized according to the stock of Sudanese tribes into 11 groups, which represents all the ethnic groups of the Sudanese. The result show that TPOAb and TgAb were found in 5% and 10%, respectively, of healthy adult controls, in 66.7 % and 27.8% of patients with GD, in 100% and 66.7% of patients with Hashimoto, and in non autoimmune thyroid diseases of this study, hyperthyroidism, hypothyroidism, and goiter, anti-TPO antibody was positive

in 17.5% (10/57), 19.4% (13/67)and 9.5% (6/63), respectively, while in control group positive in 5% (3/60). Anti-Tg antibody positive in 14% (8/57), 7.5% (5/67), and 15.9% (10/63) respectively, while in control group positive in 10% (6/60).

Nishimura et al. (2000) evaluated the RIA of myelin basic protein (MBP) in cerebrospinal fluid (CSF) which used as a biochemical marker of demyelination in patients with multiple sclerosis (MS) and develop a sufficiently sensitive ELISA for Myelin basic protein and evaluate it clinically in patients with multiple sclerosis. Samples were taken by lumbar puncture from 84 Japanese MS patients 14–68 years of age, 55 patients 18–64 years of age with other neurological diseases (12 with chronic cerebrovascular disease, 14 with amyotrophic lateral sclerosis, 12 with Parkinson disease, 5 with polyneuropathy, 4 with Behcet disease, 5 with meningitis, and 3 with Guillain-Barré syndrome), and 45 patients 19–67 years of age with nonneurological diseases. The RIA & ELISA methods used for measured MBP in CSF from patients with MS and other neurological diseases and MBP concentrations obtained by this new ELISA were compared with those obtained by the previously developed RIA. The study showed that The respective within- and between-assay CVs were 4.7% and 7.2% at 200 ng/L, and 6.3% and 8.8% at 2000 ng/L. The detection limit was 30 ng/L. Most of the MS patients with acute exacerbations had markedly increased MBP in the CSF. Longitudinal studies of six MS patients with recurrent exacerbation confirmed this observation. MBP concentrations from 78 MS patients, as tested with our ELISA, correlated well with those obtained by RIA ($r = 0.9$; $P < 0.01$), but the detection limit of the ELISA was much lower than that of the RIA. But it has higher sensitivity than the existing assays is a suitable

routine assay that provides a diagnostic indicator of myelin breakdown in the central nervous system; moreover, it is an excellent indicator of MS disease activity.

Shoukat et al. (2001) studied the correlates between radioactive iodine uptakes (RAIU) by thyroid gland with the Tri-iodothyronine (T3), Tetra-iodothyronine (T4) and thyroid stimulating hormone (TSH) radioimmunoassay (RIA) levels. 134 adult patients in the age range of20 to 60 years, that included 76 females and 58 males were chosen and referred to the department of Nuclear Medicine, for evaluate the thyroid radioactive iodine uptakes to study the correlation between them. Thyroid RAIU was performed on each patient at 2, 24 and 48 hours using thyroid uptake probe also the Radioimmunoassay on blood samples was carried out in the department of Immunology, Using kits procured from Bhabha Atomic Research Centre Correlation coefficients© were calculated and tested for statistical significance. Regression lines for various variables were computed using the Microsoft excel. Two hour thyroid RAIU values had a statistically significant positive correlation (r=0.645 and p<O.OO I) and (r = 0.56 and p = <0.00 I) with T3 and T4 values respectively assessed by radioimmunoassay (RIA). The 2 hours thyroid RAIU values showed an expected negative correlation (r= - 0.07) with the TSH values, 24 hours thyroid RAIU values had a positive correlation (r=0.65) with T3 values also had statistically significant (p=<O.OO I) correlation with T4 values The expected negative correlation (r=-0.18) with TSH values The 48 hours thyroid RAIU values statistically insignificant (p>O.1 00) correlation with T3, T4 and TSH values . It was observed that there was a significant positive correlation between 2 and 24 hours thyroid RAIU values with T3 & T4, RIA values.

Koutsky et al. (2005-2007) evaluated the ELISA-method for p16INK4a as more accurate cervical cancer screening strategies because of the low sensitivity of cytology and low specificity of human papillomavirus testing. 1,781 women attending to Planned Parenthood clinics for routine Papanicolaou (Pap) screening in Washington state were invited to participate in this study to undergoing routine screening provided cervical specimens for p16INK4a ELISA (original and enhanced versions of a prototype), liquid-based cytology, and Hybrid Capture II (hc2) testing. All women with a positive result and a random sample of those with negative results on all tests were referred for histological diagnosis. Cervical intraepithelial neoplasia grade ≥3 (≥CIN3) was the main outcome. The original analysis included all ≥CIN3 outcomes (n = 28). The a posteriori analysis was used to represent clinically relevant results with ≥CIN3 as outcomes only when detected after a positive screening test (n = 27). Participants had a median age of 23 years. The prevalence of high-risk human papillomavirus DNA was 30.6%. In a posteriori analyses, the sensitivity and specificity for p16INK4a ELISA (≥8 pg/mL cut-point), cytology, and hc2 were 50.9%, 58.1%, and 100.0%, respectively, and 90.4%, 89.3%, and 69.2%, respectively. Referral to colposcopy of women with positive results for hc2 and p16INK4a (enhanced ELISA, ≥6 pg/mL cut-point) had a sensitivity of 91.8% (95% confidence interval, 79.1-100.0%) and specificity of 86.0% (95% confidence interval, 82.0-89.0%). Results of the original analyses had similar specificity but substantially lower sensitivity due to the strong influence of the single CIN3 case with completely negative screening results. An enhanced version of this prototypic p16INK4a ELISA showed promise in screening.

Robbins et al. (1988) determined the Levels of antibodies against Haemophilus influenza type b capsular polysaccharide in acute-phase and convalescent-phase and compared the three method of determining level of antibody against H.influenza type b CPS. Our study included 65 serum samples obtained from children the age between (3month_4 years) 21 with invasive H. influenza type b infections and from 44 children vaccinated with two H. influenza type b vaccines. Amounts of immunoglobulin G (IgG), IgM, and IgA antibodies were measured by direct and indirect enzyme-linked immunosorbent assay (ELISA), and the total amount of antibodies was measured by radioimmunoassay (RIA). Results obtained by ELISA were calculated by multiple-point parallel-line comparison and by endpoint analysis. A very good correlation was obtained between direct and indirect ELISA values. In the lower range of antibody concentrations, the correlation between ELISA values obtained by endpoint analysis and those obtained by multiple-point parallel-line comparison was poor, since the latter method of calculation yielded values of up to 1 microgram/ml in sera that were negative according to endpoint analysis. These sera with negative endpoint titers also had undetectable or very low antibody concentrations as measured by RIA. Consistent with this finding, in acute-phase and prevaccination sera with undetectable or low antibody concentrations as measured by RIA, ELISA values calculated by multiple-point parallel-line comparison were much higher. In sera with higher antibody concentrations, however, parallel-line comparisons showed good correlation between RIA and ELISA values. Although no reference method for measuring true antibody concentrations is available, ELISA values as calculated by multiple-point parallel-line comparison appear to overestimate antibody concentrations in

sera containing low antibody concentrations, whereas ELISA values obtained by endpoint analysis are less well correlated with RIA values at higher concentrations

Reinhardt et al. (2004). Demonstrate that there are narrow variations of serum T4 and T3 within individuals with and without thyroid disorders. Although the diagnosis of abnormalities of thyroid function is generally based on the measurement of thyroid hormones and TSH in blood. The recommended reference ranges for serum T4 and T3 as well as TSH are quite wide as the result of large differences in thyroid function tests in healthy persons. It has been proven that the individual variation within an individual is small, compared with the variation between individuals. The studied investigated long term variations of these parameters in patients with and without benign thyroid diseases. And performed long term follow-up serum determinations of T3, T4, and TSH in a total of 150 patients for a time period of 3 to 13 years. The majority of patients had been put on L-thyroxin. Values of total T3, total T4, and free T4 were measured with an almost unmodified test (RIA) over the years. The result show that the lowest relative coefficient of variation (<10%) was observed in the group of patients who had been treated with L-thyroxin only. Even for TSH, relatively low coefficients of variation were observed in this group. In the group of patients who had not received any medication, T3 and T4 showed also a variation of 10%. FT4 and TSH revealed a wider range of variation. Even after radioiodine therapy, T3 and T4 showed only a quite small variation, while TSH demonstrated a wide range with a variation of >30%.

Leovegildo et al. (1996) determined the prevalence of Hepatitis B infection in pregnant women and evaluates the risk factors for infection, and compare the effectiveness of

immunopathology as a screening tool in our setting. In this study, the enzyme-linked immunoassay (ELISA) used for screening and diagnosis of hepatitis infection. It belongs to the third generation of techniques like the radioimmunoassay (RIA). However, the latter is still the gold standard of test because of a sensitivity and specificity close to 99%. The study was performed on 929 pregnant patients from the out-patient clinic and admitting section between July 25 – September 1, 1996. Information regarding risk factors was gathered using questionnaires and evaluated. The existing ELISA method on the same set of patients was compared with the test result from RIA. The serum collected was screened for HbsAg using radioimmunoassay (RIA) with the test kits (IMK – 413 CIAE HbsAg SPRIA KIT, Beijing, China). Likewise, the results of hepatitis screening regularly done in the institution (using the ELISA – HEPANOSTIKA HbsAg UNI-FORM II, Organon Teknika) were copied from the logbook for comparison with the result of this study. The Differences were analyzed by Fischer's exact test for small numbers and the Chi square test. Differences in proportions were calculated using the 95% confidence intervals. Thirty-six pregnant patients had positive results for Hepatitis B surface antigen (prevalence of 3.9%). Of the risk factors defined by CDC, age ($p = 0.011$) and history of previous hepatitis in 8/16 ($p = 0.00$) cases was statistically significant. The rest of the risk factors studied were not significant (age, obstetric history, history of STD, drug abuse, blood transfusion, hepatitis exposure, occupational exposure, diagnosis, education, status, promiscuity, tattoo, dental/surgical procedures, and hospitalization). Using RIA as the gold standard, ELISA has a specificity of 98.8% and sensitivity of 33%. False positivity was 1.23% while false negativity was 66.67%. Hepatitis screening with radioimmunoassay allows for more accurate identification of Hepatitis B surface

Omrani et al. (1999) correlates the Dehydroepiandrosterone (DHEA) or its sulfate derivative (DHEA-S) is the major C19 steroid hormone secreted by the adrenal cortex with atherogenesis through its antiproliferative effect. And examine the effect of DHEA-S on coronary artery disease (CAD). In a prospective randomized study 202 patients with possible coronary artery disease who underwent coronary angiography between January 1999 and June 1999 were studied. They were classified into two groups, group 1 (n=142, female: 39, male: 103) included patients who had more than 75 percent cross sectional area narrowing of at least one coronary artery, and group 2 (n=60, female: 28, male: 32) included patients who had no coronary artery disease. The age range for two groups was 18-75 years. The Level of DHEA-S measured by two different methods; first by RIA (RA-1000 automatic analyzer, Technicon Co, and Kontron gamma counter) and then by the ELISA method. Various blood parameters such as fasting blood sugar, TG, total cholesterol, LDL-C, HDL-C were measured and compared between the two groups. The average age was 54.9 years in group one and 53.2 in group two without statistically significant differences (t=-1.69, p=0.27). The result showed that, coronary disease cases had significantly higher mean values of LDL-C, total cholesterol, FBS, TG, and lower levels of HDL. BMI was not significantly different between CAD cases and controls. Rechecking the DHEA-S values by the RIA method and statistical analysis again showed no statistically significant difference between DHEA-S in CAD cases and controls. The patients with angiographically proven CAD, there was no significant difference in the mean DHEA-S level between high (FBS = 126) and normal (FBS < 126) fasting blood sugar groups. Also there was no significant relationship between smoking, history of

hypertension and serum DHEA-S level, in different age groups in either males or females Results of analysis were almost the same for both ELISA and RIA methods

HIROSHI YAMASAKI et al. (2005) evaluate the prevalence of toxocariasis in children in Jaboatão northeastern Brazil, the study included 215 children serum sample (age range = 1–17 years) the group was composed of 104 males and 94 females this sample were examined by an enzyme-linked immunosorbent assay (ELISA) using a recombinant Toxocara canis *antigen*. 53 serum samples were selected based on the results of the Serologic tests on an ELISA; 26 (12.1%) of 215 were ELISA-positive samples and 27 were ELISA-negative samples from individuals infected with helminthes and those not infected, the diagnostic results correlated with those obtained by the ELISA. Moreover, it has been confirmed that the recombinant *T. canis* antigen was highly specific for toxocariasis by ELISA using serum samples positive for antibody to *Ascaris* lumbricoides. Considering the specificity of the recombinant antigen to toxocariasis, the ELISA or dot-blot assay using the recombinant *T. canis* antigen is recommended in tropical and sub-tropical regions where various parasitic infections are commonly endemic.

Elmansury (2007) determined serum thyroid hormones (Thyroxin T4, Triiodothyronine T3) and the anterior pituitary secretion Thyroid Stimulating Hormone (TSH) in normal healthy donkeys in Sudan. Detection of such hormone was done using Radio-immune assay (RIA) and 43iluen-radiometric assay (IRMA) techniques. This study included 13 male and 13 female donkeys; 4-10 years. The animals were housed in Central Veterinary Research Laboratories (CVRL) Soba, Sudan. Blood samples of 5ml were collected from

the jugular vein and Thyroxin (T4) and triiodothyronine (T 3) were measured in donkey's serum using RIA method. Thyroid Stimulating Hormone (TSH) was measured using the IRMA technique. The obtained values (mean ± standard deviation, SD) of male and female animals were comparable and Male donkeys showed higher concentration of serum Thyroxin (T4) than female donkeys also The result show that Both methods used (RIA and IRMA) are able to detect the thyroid hormones with sensitivity of 0.08 ng/ml, 0.13 ng/ml, and 0.24 mIUlL for T3, T4 and TSH respectively. And There were significant differences in the thyroid hormones concentration related to time of blood collection during the day and also reported the mean values for serum thyroxin (T4) were 8.46 ± 7.19 (l .40 -25.73) nglml, the serum triiodothyronine (T3) 0.52 ± 0.21 (0.20 -1.03) ng/rol, and thyroid stimulating hormone (TSH) were 0.86 ± 0.1 3 (0.64 -1.15) mTIJ/L. also

Baraka (1997) studied the relationship between the administration of Ivermectin and the serum progesterone profile in the female camel Blood samples were collected for 35 days at 5 days intervals from four female camels aging 8-12 years and weighing between 390 and 450 kg. The progesterone level of the sera was assessed to be as control group. Then, treatment with ivermectin was injected subcutaneously at two intervals. Two different doses of Ivermectin were administered subcutaneously at 150Jlg/kg and 200 Jlg/kg body weight. Progesterone level was measured before and after injection of the drug using radioimmunoassay (RIA). At least three out of the four animals showed an observable increase in progesterone when Ivermectin was administered within the breeding season, while those injected outside the season showed decreased levels of progesterone.

Yoshitaka et al (2003) measured GnRH release activities from different GnRH systems because there are multiple GnRH systems in the vertebrate brains also show that GnRH functions not only as a hypophysiotropic hormone that controls reproductive functions but also as a neuromodulator that controls motivational or arousal states of the animal. First GnRH release from the brain-pituitary slices of the dwarf gourami was measured and separated into functionally different two parts (POA- and TN-TEG-GnRH slices), by a static incubation system and RIA using a single antibody that almost equally recognizes various molecular species of GnRH also the similarities and differences of GnRH release from the two functionally different systems were compared and the result show that the GnRH release when the POA-GnRH slices and TN-TEG-GnRH slices from male and female fish were stimulated with high [K+]o Ringer solutions [(K+)o = 20, 40, 60, and 100 mm]. The high [K+]o Ringer solutions significantly increased the GnRH release from POA- and TN-TEG-GnRH slices in a dose-dependent manner

Rollin F. (2007) developed and validated a radioimmunoassay (RIA) for bovine TSH (bTSH) Under physiologic and pathologic conditions because Hypothyroidism is the most common type of thyroid disorder in cattle in this study the Thyrotropin-releasing hormone challenge and 24-hr sampling were first performed to obtain a physiologic validation of the bTSH assay. Thereafter, reference interval was defined on a large population of clinically healthy cows. Last, the bTSH RIA was tested under pathologic conditions on goitrous calves. The RIA was performed in duplicate in polystyrene tubes. For the standard curve determination and Laboratory validation included research of minimal detection limit, accuracy, and reproducibility the result show that The minimum detection limit (MDL) for bTSH assay was 1.3 μU/ml. The recovery was 101% to 106%.

The intra- and interassay coefficients of variation (CVs) ranged from 5% to 11% and 11% to 15%, respectively. The RIA covered the whole range of physiologic bTSH values, as shown by bTSH values induced by TRH-challenge. A pulsatile secretion of bTSH was observed, accompanied by a diurnal variation with lower night values than day values. Reference intervals of bTSH ranged from 1.3 to 13.0 μU/ml for beef and dairy breeds. Finally, bTSH easily discriminated goitrous newborn calves from healthy ones, leading to the definition of a cutoff value of 35 μU/ml. The bTSH assay positively reacted to physiologic and pathologic conditions. The accuracy and precision of the RIA were satisfying.

Rivera-Fillat (1983) developed and evaluated a radioimmunoassay for arginine-vasopressin in human plasma with use of a commercially available antibody, 35 Blood was collected and centrifuged and the plasma was stored one or two weeks before it was extracted and assayed it as extracted by two technique (a) acetone-ether, and (b) ethanol extraction. The concentration of the AVP in the plasma is then measured by RIA and that is by mixing 100 L of either standard or sample with 100 L of anti-AVP serum from the result the Recovery of unlabeled AVP added to plasma averaged 50.8 ± 6.0% (n = 30) when acetone-ether was used as a method of extraction and 65.3 ± 3.7% when the ethanol method was used ($p < .00\ 1$) Was observed .Thus we chose the ethanol method for the extraction of AVP from plasma and the results for samples for extraction losses, using 65% as the mean percentage of recovery

Salamonsen et al. (1972) examined the radioimmunoassay for ovine follicle stimulating hormone in sheep plasma and investigated the behavior of antiserum to ovine follicle stimulating hormone. This examination done by using RIA technique to represent the

proportion of total radioactivity specifically bound to anti-OFSH or anti-albumin antiserum at any point and that is by labeled 30 serum with I125 and radioactivity were measured and the sensitivity and precision were calculated by using computer program and were found (97.5%) & (4.8%) respectively this study show that a homologous RIA system will not be satisfactory for measurement of the hormone in plasma. Investigation are being carried out into heterogonous RIA methods

Prabakaran et al. (2011) developed a novel peptide ELISA for H5N1 antibody detection by incorporating the peptide comprising the amino acid sequence ‘‘CNTKCQTPMGAINSS’’. Because Peptide based enzyme immunoassay (ELISA) has been widely used for the sero-diagnosis of bacterial and viral infections. Three serum panels from human (n= 50) were used. First panel comprised human sera from 15 individuals with confirmed H5N1 infection. Second panel comprises 15 human sera from patients recovered from seasonal influenza The third panel consisted of 20 serum samples from healthy volunteers with no influenza vaccination or any illness related to influenza, the antibodies to H5N1 in immunized animals or convalescent human sera were detected using peptide ELISA. The peptide ELISA results were compared with hemagglutinin inhibition (HI), and immunofluorescence assay and immunodot blot that utilize recombinant HA1 as the capture antigen. The result show that The peptide-ELISA based on the highly conserved and antigenic H5 epitope (CNTKCQTP) provides sensitive and highly specific detection of antibodies to H5N1 influenza viruses. This study highlighted the use of synthetic peptide as a capture antigen in rapid detection of antibodies to H5N1 in human and animal sera that is robust, simple and cost effective and is particularly beneficial for developing countries and rural areas.

Robles et al. (2011) evaluated an indirect enzyme-linked immunosorbent assay (I-ELISA) kit In order to improve the serological diagnosis of bovine brucellosis in Patagonia, Argentina. This study contained 3group of serum Group 1. Sera from 286 cows older than 24 mo from 13 herds free from bovine brucellosis Group 2. Sera were used from 459 cows originated from 11 farms that were older than 24 mo, vaccinated against bovine brucellosis Group 3. Sera were used from 156 cows older than 24 mo originated from 10 herds with at least 2% of animals positive to the rose 48iluen test (RB) and 2-mercaptoethanol test (2ME) . The I-ELISA technique was performed using an I-ELISA brucellosis kit. The result show that The threshold for the ELISA technique was 29%. Therefore, for further calculations, sera with threshold higher than 29% was considered to be positive, while sera with threshold below this value were considered to be negative. The diagnostic specificity of all the tests used for non vaccinated Only 3 out of the 286 sera tested were positive by The diagnostic specificity of all the tests used for the negative but vaccinated herds is depicted in Only 13 of the sera gave a positive reaction in the I-ELISA. That is to say The specificity of the I-ELISA (98.9% and 97.2%) was similar to that of the BPA, RB, 2ME and complement fixation (CF) tests when used to test sera from negative non vaccinated and negative but vaccinated animals, respectively The sensitivity of all tests estimated with sera positive in the RB and 2ME tests Only 2 of the 156 sera gave threshold values below the threshold of 29% also to say The sensitivity of the I-ELISA (98.7%) was higher than the BPA test (96.1%) and the CF test (95.2%) The I-ELISA kit evaluated in this study was thought to be a valuable tool for the diagnosis of bovine brucellosis in the Patagonia region where little epidemiological

information is available about this disease, and where large numbers of sera should be tested to obtain such information

Wattanakrai et al. (2008) evaluated the sensitivity and specificity of ELISA for detecting antidesmoglein 1 and 3 in Thai patients with pemphigus. 48 serum samples were collected from 39 pemphigus patients 27 Pemphigus vulgaris & 7 pemphigus Foliaceus and 14 patients with other dermatologic disorders. The ELISAs were performed to the serum using MESACUP desmoglein test kits. The result show that The sensitivity of Dsg1 and Dsg3 ELISA for all patients with PV was 64% and 77.8% respectively. When subgrouped into only PV patients with new diagnosis, the sensitivity of Dsg 1 and Dsg 3 ELISA increased to 85.7% and 100%. In all PF patients, the sensitivity of anti-Dsg 1 ELISA was 71.4% and 100% for newly diagnosed PF cases. Anti-Dsg 3 was not detected in the PF group. The specificity of ELISA for anti-Dsg 1 and anti-Dsg 3 in both types of pemphigus was 85.7% and 92.3% respectively. From the study Dsg 1 and Dsg 3 ELISA is a simple, highly sensitive and specific test in Thai pemphigus patients with 100% sensitivity in the diagnosis of both new pemphigus vulgaris and foliaceus patients.

2Rajpal et al. (2007) described a prospective evaluation for demonstrating Antigen (Ag) 85 complex in the sera from TB patients. In the present study serum specimens from patients with TB were examined for the presence of *M. tuberculosis* Ag 85 complex was done by sensitive and specific indirect ELISA method and that is by employing monoclonal antibodies (mAb) against the purified Ag 85 complex. Serum samples were obtained from 197 different groups of patients : confirmed TB {n = 24}, clinically diagnosed TB {n = 104}, disease controls {n = 49} and healthy controls {n = 20}and

comparison between TB and non-TB groups were done by the chi-square test, the result show that 82% sensitivity and 86% specificity for the diagnosis of TB. The serum positivities for Ag 85 complex in cases of confirmed and clinically diagnosed TB patients were 96% (23/24) and 79% (82/104) respectively, while the positivity for patients in the non-tuberculosis group was 14% (10/69). From this study the detection of Ag 85 complex in sera from TB patients by indirect ELISA using mAb against purified Ag 85 complex gives a reliable diagnosis and does not give false results with other non-tuberculosis diseases. It could be used to develop an immunodiagnostic assay with increased sensitivity and specificity.

Joakim (2004) evaluated the Heparin-coated microtitre plates for capturing conformationally correct virus-like particle (VLPs) and improving the type specificity of human papillomavirus (HPV) serology. In the present study, the heparin-coated plates could be used to improve performance of VLP serology were evaluated. HPV16/11 hybrid VLPs that had significant reactivity with children's sera and a batch of HPV18 VLPs that had failed the quality control because of significant reactivity with sera from virginal women were tested in parallel with heparin-based ELISA, direct ELISA and type-specific mAb capture ELISA using positive- and negative-control validation panels of serum samples. The result shows that heparin-coated plates will select a subset of VLPs with intact heparin-binding activity. At least for some HPVs, intact heparin-binding ability was found to be correlated with serological specificity, allowing for a straightforward and simple improvement of the performance of HPV serology

Choudhury et al. (2011) suggested a protocol for re-entry of the blood donors who are confirmed hepatitis C virus (HCV) negative by nucleic acid test (NAT) and recombinant immunoblot assay (RIBA). A group of repeat voluntary donors were followed retrospectively who became reactive on a cross sectional study and showed HCV reactivity while donating blood regularly. A total of 51,023 voluntary non remunerated blood donors were screened for anti-HCV ELISA routinely. If anybody showed positivity, they were tested by two ELISA kits (screening and confirmatory) and then confirmed infection status by NAT and or RIBA. The previous HCV test results of repeat donors reactive by anti-HCV ELISA were looked back from the records Data of donors who were repeat reactive with single ELISA kit were analyzed separately from those reactive with two ELISA kits the result show that 140 (0.27%) donors who were reactive by anti HCV ELISA were included. Out of them, 35 were repeat voluntary donors and 16 (11.43%) were reactive with single ELISA kit. All 16 donors were reactive by single ELISA kit occasionally in previous donations. Their present ELISA positive donations were negative for HCV NAT and RIBA. A total of 19 (13.57%) donors were reactive with two ELISA kits. In their previous donations, the donors who were reactive even once with two ELISA kits were consistently reactive by the same two ELISA kits in their next donations also. Donor sample reactive by only single ELISA kit may not be considered as infectious for disposal as they were negative by NAT and or RIBA. One time ELISA positivity was found probably due to ELISA kit specificity and sensitivity. Donors reactive with two ELISA kit should be discarded as there is a high positivity with NAT/ RIBA. However, donors reactive by two ELISA kits and negative by NAT and RIBA should be followed up and may not be deferred permanently

Huang et al. (2004) Developed Envelope and membrane (E/M) and nonstructural protein NS1 serotype-specific capture Immunoglobulin M (IgM) enzyme-linked immunosorbent assays (ELISAs) to differentiate four dengue virus serotypes. This study included 115 samples 93 anti-dengue virus IgM-positive serum samples collected between days 5 and 45 of illness from 59 confirmed dengue patients were analyzed and 72 serum samples were from primary dengue virus infection and 21 serum samples were from secondary dengue virus infection and the ELISA method were done to them .The results showed that positive serotype specificity could be identified for 86.1 and 47.6% of serum samples tested for E/M-specific IgM antibodies versus 83.3 and 42.9% of serum samples tested for NS1-specific IgM antibodies from patients with primary and secondary dengue virus infections, respectively. Dual analyses with both E/M and NS1 serotype-specific capture IgM ELISAs showed that positive serotype specificity could be correctly identified for 98.6 and 61.9% of all of the primary and secondary serum samples tested, respectively. These findings suggested that E/M and NS1 serotype-specific capture IgM ELISAs have the potential to be of use in dengue virus serotyping.

Kemppainen et al. (2002) evaluated an in-house ELISA for measurement of total serum thyroxin in dogs and cats and compared the serum total thyroxin (T4) concentrations obtained with an in-house ELISA and a validated radioimmunoassay (RIA). 50 canine and 50 feline serum samples submitted for measurement of total T4 concentration with the RIA technique and the same sample measured by ELISA technique the Results of the ELISA and RIA were compared by calculating correlation coefficients the result show that Correlation coefficients for results of the 2 methods were 0.84 for the canine

samples and 0.59 for the feline samples. Examination of bias plots revealed large variations in ELISA results, compared with RIA results. For the feline samples, the ELISA consistently overestimated total T4 concentration obtained with the RIA. When results of the 2 methods were categorized (low, borderline low, normal, borderline high, or high), results were discordant for 24 (48%) and 29 (58%) of the canine samples and for 18 (36%) and 28 (56%) of the feline samples (depending on whether borderline high ELISA results were considered normal or high). Reliance on results of the ELISA would have led to inappropriate clinical decisions for 31 (62%) canine samples and 25 (50%) feline samples. The ELISA coefficients of variation for the pooled canine and feline samples were 18 and 28%, respectively. From the result Substantial discrepancies between ELISA and RIA results for T4 concentrations were detected. Thus, we concluded that the in-house ELISA kit was not accurate for determining serum total T4 concentrations in dogs and cats.

Robbins et al. (1985) determined the Levels of antibodies against Haemophilus 53iluents53 type b capsular polysaccharide in acute-phase and convalescent-phase this study were include 65 serum samples obtained from 21 children with invasive H. 53iluents53 type b infections and from 44 children vaccinated with two H. 53iluents53 type b vaccines. The Amounts of immunoglobulin G (IgG), IgM, and IgA antibodies were measured by using direct and indirect enzyme-linked immunosorbent assay (ELISA), and the total amount of antibodies was measured by radioimmunoassay (RIA). Results obtained by ELISA were calculated by multiple-point parallel-line comparison and by endpoint analysis. A very good correlation was obtained between direct and indirect ELISA values. In the lower range of antibody concentrations, , since the latter

method of calculation yielded values of up to 1 microgram/ml in sera that were negative according to endpoint analysis. These sera with negative endpoint titers also had undetectable or very low antibody concentrations as measured by RIA. Consistent with this finding, in acute-phase and prevaccination sera with undetectable or low antibody concentrations as measured by RIA, ELISA values calculated by multiple-point parallel-line comparison were much higher. In sera with higher antibody concentrations, however, parallel-line comparisons showed good correlation between RIA and ELISA values. Although no reference method for measuring true antibody concentrations is available, ELISA values as calculated by multiple-point parallel-line comparison appear to overestimate antibody concentrations in sera containing low antibody concentrations, whereas ELISA values obtained by endpoint analysis are less well correlated with RIA values at higher concentrations.

Wadah (2010) studied the relationship between thyroid function test and thyroid uptake using 99mTc. The study was also designed to help in determining the normal range of the thyroid uptake in Sudanese people as well as the possibility of using thyroid uptake with accuracy similar to the TFT. This study includes 77 patients in different age, sex, center of origin and type of food and drink intake. The study was conducted at RICK; nuclear medicine department (gamma camera and RIA) for five months From MAY to SEP 2009. The levels of thyroid hormones T4, T3, TSH and thyroid uptake are (5.6±3.6, 79.8±6.5, 6.7±0.8 and 6.3±2.4 respectively), in the subject's blood were measured using sensitive RIA method against the thyroid uptake value in the gamma camera (mediso).the result of this study showed that, there was a direct relationship between thyroid uptake and the level of the thyroid related hormones (for individual). There is strong and significant

correlation at p = 0.05 between the thyroid uptake versus T3 and T4.The percentage of thyroid uptake for the subjects included in the study was ranging between 5.78 and 6.12. Also the study indicated that there were possibilities of using thyroid uptake only as a diagnostic tool for thyroid activity without TFT due to the ability of thyroid uptake to giving sufficient information concerning thyroid status.

Osama (2004) studied 711 Sudanese female have been analyzed for thyroid function. Thyroid related hormones were measured thyroxin (T4), triiodothyronine (T3) and thyroid stimulating hormone (TSH). The study had been held during one complete year. The female subjects were refered to Sudan Atomic Energy Commission Radioimmunoassay laboratory from different hospitals in Khartoum State. The age of females was varied from less than one year up to 70 years. The age was divided in to 10 years interval in order to study the dominst thyroid disorder in each interval. Statistical package for social science (SPSS) program was used in the study as data analysis tool. The clear observation from this study was the high incidence of disorders among the age between 20 up to 40 years

Abdulrahman (2005) studied the effects of age, sex and residence on the incidence of thyroid disorders, and to know the influence of iodine status of a subject on thyroid function by measuring urinary iodine concentration. A total of 100 Sudanese patients, with thyroid disorders visiting the Radiation and Isotope Centre (RICK) at Khartoum, during the period of February 2005 to July 2005, were selected randomly to contribute in this study. A total of 30 healthy subjects from the co-patients were volunteered to participate in this study as a control group. Specimens of sera and urine samples were

collected from all patients and controls to estimate thyroid hormones, T3 & T4 by (RIA) method and TSH by (IRMA) method. Urine samples were used to measure urinary iodine concentration by Sandle Koltholt Reaction (using ammonium per sulfate as a catalyst).The patients were categorized as hyperthyroidism and hypothyroidism. There is an increased incidence of hyperthyroidism in the middle aged patients, while hypothyroidism is more common in the elderly, the results were found to be as Females were more susceptible to thyroid disorders than males for both hyperthyroid and hypothyroidism: In hyperthyroidism females were 65 out of 80 (81,3%) . In hypothyroidism females were 14 out of 20 (70%). The disease was found to be distributed in all regions of Sudan, with increased incidence of hyperthyroidism in Central-Sudan (Khartoum State & Gazera area) (47.5%), while for hypothyroidism there was increased incidence in the West (45%), urinary iodine concentration was found to be highly significantly raised in patients with hyperthyroidism compared to the control ($P<0.01$), while highly significantly reduced in patients with hypothyroidism ($P<0.01$). From this study it is recommended that urinary iodine could be used as a screening and diagnostic test for both hyper and hypothyroidism.

Nishi et al. (1996) reported the intra-individual and seasonal variations of thyroid function tests in healthy subjects. Blood samples were obtained from thirteen healthy males and seven healthy females every two weeks over a period of one year, and totally 25 samplings of each were made. Serum thyrotrophic (TSH), free thyroxin (FT4) and free triiodothyronine (FT3) were measured after the completion of the sampling. The 25 samples from each subject were always assayed with the same assay run. Variations of FT4 and FT3 in each subject were narrow and approximately one-third of normal

reference ranges. The magnitude of individual variation of TSH values was proportional to the average of TSH in each individual. Serum TSH and FT3 values during winter were significantly higher than those during summer, but such change was not observed on serum FT4.

Shoukat Hussain et al . (2004) control subject which were not suffering from any disease, especially hypothyroidism, with random distribution of age and sex during the period May – December 2004. Also Measurements were determined in (40) subjects with hypothyroidism. Thyroid hormones, triiodothyronine (T3), tetraiodothyronine (T4) and thyroid stimulating hormone (TSH) were measured by using RIA method, also age and sex was determined then the catalytic activity of creatine kinase were measured for control and hypothyroidism subject. Measurements obtained in this study revealed that creatine kinase enzyme of the hypothyroidism subjects (40) were significantly elevated than the levels of the control subjects (20). This was indicated that hypothyroidism causes elevation to the total catalytic activity of creatine kinase (CK). This study appeared that depending on the degree of hormones deficiency (T3, T4 & TSH), skeletal muscle involvement occurs on hypothyroidism. Further studies may explain the abnormalities in the skeletal muscles function in hypothyroidism subject and time caused of recovery in these abnormalities after treatment by thyroid hormone administration.

Abeer (2009) determined percentage and level of anti- GLURP IgG and IgG1 and IgG3 antibodies, and their relation to the level of parasitemia and Hb concentrations among subjects. A number of 114 malarial patients with Plasmodium falciparum were enrolled

in the study. Enzyme linked immunosorbent assay (ELISA) was performed to detect anti-GLURP IgG antibodies. 29 of malaria patients have normal hemoglobin level, 16 with mild anemia. 50 have moderate anemia and 19 with severe anemia. Patients under five years of age comprised approximately half (49.1%) of study subject. The overall prevalence of seropositive samples among study group was high ranging from (59.6%) of IgG against R2 GLURP and to (79.8%) IgG1 R0, and (78.9%) IgG3 R0.. The acquisition of antibody is age dependent. The severity of anemia correlated to IgG3 against R0-GLURP, IgG1 R1 GLURP; as well as with parasitemia. Natural acquired humoral responses against R0-R2 GLURP are prevalent among falciparum malaria patient. Call for Encouraging approaches to vaccination against P.falciparum. Further studies should be conducted to clarify whether the presence of antibody responses against GLURP is associated with reduction of malaria incidence.

Sidig (2007) determined the prevalence of Hepatitis C Virus (HCV) antibodies among blood donors. Two hundred and forty (240) voluntary blood donors attending Juba Teaching Hospital during the study period were recruited, 221 of them were males and 19 females. The mean age was 35 years. Out of the 240 samples tested for anti-HCV antibodies, 10 were found to be positive by a third generation indirect Enzyme Linked Immunosorbent Assay (ELISA), giving a prevalence rate of (4.16%). When certain risk factors were investigated, previous blood transfusion and vaccination were both found to be significant risk factors predisposing to infection with hepatitis C virus (relative risk 2.85 & 2.6.2 respectively). The study also showed, that most HCV positive donors were within the age groups 26 to 45 years (6%), followed by the age group over 46 years

(5.5%). Females, on the other hand, were more affected than males. Married donors were found to have higher infection rate than unmarried donors (5.8% vs 2%) respectively. In conclusion the prevalence rate of HCV infection among blood donors in Juba Teaching Hospital (4.16%) was relatively, high, compared to results obtained in similar regions and countries of the world.

Wilkin et al. (1990) compared a recently develop competitive enzyme-linked immunosorbent assay (ELISA) with a conventional competitive radioimmunoassay (RIA) for the measurement of rat insulin in culture medium. Fifty-six samples were analyzed by both assays. There was a correlation coefficient of r = 0.783 between results obtained using the two assay systems. The binding curves of the two assays were differently shaped, so that the ELISA gave good reproducibility over the concentration range 5–50 μU/ml insulin with inter and intra-assay coefficients of variation < 14%, but poor reproducibility at higher concentrations. Conversely, the RIA showed excellent reproducibility at concentrations greater than 50 μU/ml insulin, but poor sensitivity and high coefficients of variation below this level. The ELISA procedure offers practical advantages over the RIA, and performs well when measuring physiological concentrations of insulin

Hjertsson (1980) developed four-layer antispecies radioimmunoassay (RIA) for the detection of adenovirus in stool specimens. Polystyrene beads were used as the solid phase, anti-adenovirus guinea pig immunoglobulin (1 microgram per bead) was used as the primary antibody, anti-adenovirus rabbit immunoglobulin (16 micrograms/ml) was

used as the secondary antibody, and 125I-labeled sheep anti-rabbit immunoglobulin was used as the indicator antibody. A highly purified, crystallized adenovirus type 2 hexon antigen was used as the immunizing antigen for the production of hyperimmune sera. The sensitivity of the test was 1 ng of hexon protein per ml. Each of the 13 stool specimens positive for adenovirus by electron microscopy was positive for adenovirus by the RIA. Of 200 nonconcentrated stool specimens negative by electron microscopy, 14 additional specimens were positive by the RIA, increasing the detection rate from 6% by electron microscopy to 13% by the RIA. A confirmatory test was done on the RIA-positive, electron microscopy-negative specimens, and the test indicated a true specific result with each specimen. A confirmatory test was also done on each specimen with a low positive counts per minute value. The specificity of the RIA was further demonstrated by the fact that a positive result was found with only 3 of 295 specimens positive by the rotavirus RIA. In two of these three specimens, adenovirus and rotavirus were also detected simultaneously by electron microscopy, and the third specimen was from a patient with serological evidence for a dual infection. The adenovirus and rotavirus RIAs are now in a routine diagnostic laboratory, and in the 307 stool specimens tested during the first 5 months, the positive rate was 32% for rotavirus and 9.5% for adenovirus.

Rosenfeld RG et al (1993) identified 56 patients with GH receptor deficiency (Laron syndrome) from two provinces in southern Ecuador, one group of 26 (Loja province) with a 4:1 female predominance and 30 patients from neighboring El Oro province with a normal sex ratio. There were no significant differences between the Loja and El Oro populations in stature (-5.3 to -11.5 standard deviation score), other auxologic measures,

or in biochemical measures. GH binding protein, the circulating extracellular domain of the GH receptor, was measured by ligand immunofunction assay and found to be comparably low in children and adults. Levels of insulin-like growth factor (IGF)-I and –II and the GH-dependent IGF binding protein-3 (measured by RIA) were significantly greater, and GH and IGF binding protein-2 levels significantly lower in adults than children. Levels of IGF-I (adults) and IGF binding protein-3 (children and adults) correlated inversely with statural deviation from normal ($P < 0.01$). School performance was at an exceptionally high level, 41 out of 47 who had attended school being in the top 3 in classes of 15-50 persons.

Chapter three
Methodology

3-1 instrumentation:

The instruments used to collect the data were categorized into two groups , RIA instrument which is SOURCERER RIA gamma counter, manufactured by OAKFIED Company, England at 1992 and ELISA (Elecsys) instrument (manufactured by HITACHE-ROCHE company at 2010)

The data was collected it randomly. This study was consist of 114 patient sample (93 female – 21 male) The sample includes different tribes, age, gender and problem. This take with me about (2_3) month. I collected the sample (3-5ml) of blood. This blood centrifuge (3000-5000)unit over 3 minute at military hospital which are work by (ELISA) method after investigate it the sample as a serum form was referred to radiation and isotope center (RICK) at temperature (18-25 C)which are work by (RIA) method.the investigation was done at the period july2011-october2011

3-2 The main Equipment and reagents used in RIA were including:

Adjustable micropipettes (10-200µl), Polystyrene test tubes (disposable), Vortex mixer (single and multi-tubes), Multidose micropipette. (Eppendorff).25µl and 250 µl, magnetic base, Incubator, Centrifuge and Gamma counter (connected with computer; contains IAEA software.

3:2:1 material for measurement thyroxin(T4) by RIA:

Material:

distilled water, disposal polystyrene tube, micropipettes with disposal, tips(50,200 and 500micro L), vortex type mixer, magnetic separator, water bath, and gamma counter suitable for measuring iodine125

kit

bottle(148kBq , 4 micro CI)(I125) T4 solution (55ml, red) in barbitone buffer with bovine serum albumin and ANS(8-ANILLINO-Inaphtalene sulfonic acid),1 bottle T4 antibody suspension(55ml) with antimicrobial agent,6 vials T4: pipette 1ml of distilled water into each vial of T4standard for reconstitution and stand for 5 minutes. The concentration are 0 , 20 , 40 , 80 , 160, 240ng/ml and pack insert

3:2:2 Materials for measurement tri-iodothyronine (T3) by RIA

Material:

Distilled water, disposal polystyrene tube, micropipettes with disposal tips(50,200 and 500micro L), vortex type mixer, magnetic separator, water bath and gamma counter suitable for measuring iodine125

kit

1bottle(148kBq，4 micro CI)(I125) T3 solution (55ml, red) in barbitone buffer with bovine serum albumin and ANS(8-ANILLINO-Inaphtalene sulfonic acid), 1 bottle T3 antibody suspension(55ml) with antimicrobial agent, 6 vials T3 : standard in human serum with antimicrobial agent nominally 0 , 0.5 , 1.0 , 2.0 , 4.0 , 8.0 ngT3 /ml, 1bottle NSB reagent (5ml) for the determination of nonspecific binding , with antimicrobial agent and pack insert

3:2:3Material for measurement thyroid stimulating hormone(TSH) by **RIA:**

Equipment:

disposal polystyrene tube size12*75mm, micropipettes with disposal tips(50,200 and 500micro L)and 1ml, vortex type mixer, magnetic separator, absorbent plotting paper, water bath or oven set at37 and gamma counter suitable for measuring iodine125

kit

vial I125-anti-TSH(monoclonal) solution red, 1vial magnetic TSH antibody (polyclonal) suspension, 7 vials TSH standard A B C D E F G lyophilized, 3 vial containing quality control and 1 vial concentrated wash buffer

3:3 The RIA method:

The radioimmunoassay used in this study are typical for RIA department in radiation and isotopes center of Khartoum , which include incubation of 5mL of patient blood and then follow the below procedure :

3-3-1: T4

The principle of the method depends on competition between iodine-125 labeled T4 and T4 contained in standard or in specimen to be assayed. For 65iluent and limited number of T4 antibody binding sites. After the incubation the amount ofiodine-125 labeled T4bound to antibody is inversely related to the amount of T 4 present in the sample. In the antibody suspension of the kit the antibody is covalently bound to magentisable particles. Separation of antibody bound fraction is achieved by magnetic separator and decanting the supernatant. By measuring the proportion of iodine-125 labeled T4 bound in the presence of reference standards containing various

known amount of T4. The concentration of T4 present in unknown sample can be interpolated.

One hundred and fourteen (polystyrene) test tubes were labeled in duplicates and arranged in assay rack, and then 25 µl was 66iluent into each tube of the standards, quality control sample and patient's sample. And 250 µl anti T4 antibody added to each tube, and mixed well, to the STD and QC and samples250 µl of tracer(radioactive antigen) was added Because of the ease with which iodine atoms can be introduced into tyrosine residues in a protein, the radioactive 125I are often used.

After mixing well incubated at 37 Co for 45 minutes, because 37Co is the internal temperature of the human body which the reaction was occur at this inside the body then the rack was placed in the magnetic base for 10 minutes, to separate the bound fraction free from the free fractions by decant the supernatant. Lastly each tube was counted in the gamma counter to evaluate the gamma emission per minutes, and binding percent was plotted vs. the concentration, to get standard calibration curve, and from the curve obtained the concentration of Thyroxin in the patient's samples was evaluated. This method is bioassay method, (Radioimmunoassay), Using radioactive isotope of iodine (I-125) which is gamma emitter. The standards tube and

quality control tube were measured with any group of sample patient to exposed all sample to the same climate to give more accurate result

3-3-2 T3:

The principle of the method depends on competition between iodine-125 labeled T3 and T3 contained in standard or in specimen to be assayed. For affixed and limited number of T3 antibody binding sites. After the incubation the amount ofiodine-125 labeled T3bound to antibody is inversely related to the amount of T3 present in the sample. In the antibody suspension of the kit the antibody is covalently bound to magentisable particles. Separation of antibody bound fraction is achieved by magnetic separator and decanting the supernatant. By measuring the proportion of iodine-125 labeled T3 bound in the presence of reference standards containing various known amount of T3. The concentration of T3 present in unknown sample can be interpolated. 114 numbers of test tubes were labeled and in duplicate arranged in assay rack. 25 µl of standard solutions, QC samples and patients sample were added to each target tube. 100 µl of T3- I125 tracer and 100 µl anti-T3 antibody were added to each tube and mixed the tubes and incubated at 37Co for one and half hour and then place test tube rack on magnetic separator and allow to stand for 10 minutes. Decant supernatant and keeping the separator inverted, place the tube on a pad of

absorbent paper and allowed to drain for 5 minutes and then each tube was placed in gamma counter; the principle of the assay is the same as that for T4.

3-3-3 TSH

The principle of method depend on IRMA kit utilizes a two-site sandwich immunoradiometric assay for measurement of TSH in human serum. This involve the reaction of TSH present in serum with monoclonal and polyclonal antibody . the monoclonal antibody is labeled with I125 as tracer (I-125McAb) and polyclonal antibody is coupled to magnetic iron oxide particle(PcAb<M>).The formed I125McAb-TSH-PcAb<M> complex (sandwich) is separated from unbound tracer by placing the assay tubes in the magnetic separator and decanting supernatant . the radioactivity of tracer in the tube is directly proportion to the concentration of TSH in the specimens

114 test tubes were labeled and arranged in assay rack in duplicates,100 µl of STD and QC and sample was 68iluent in target tube and 25 µl tracer (anti TSH labeled by I125)was added to each tube and vortexed then incubated at 37Co in the incubator for one hour 250 µl of anti TSH (antibody coupled to magnetic particles) was added to each tube and mixed well and incubated at 25Co for one hour then the racks were

placed in the magnetic bases for 10 minutes and the supernatant was separated by decantation.

Wash step:

In this step first the concentrated wash buffer was diluted by adding distilled water (1:9), and then 500 µl of the 69iluents was added to each tube and then vortexed well and then placed again in the magnetic base, the wash step was repeated again and all the tubes were counted in the gamma counter, to evaluate the concentration of TSH in the patient sample. The quantitative analysis of TSH is achieved by the above method, which is immunoradiometric method and it is non competitive method in which the radioactive compound (tracer) is TSH antibody, we have two antibodies react with the TSH in the analyte to get a sandwich complex.

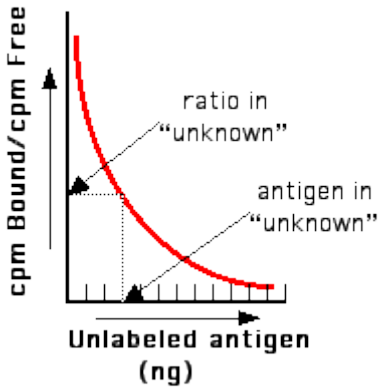


Figure 3-1 standard calibration curve

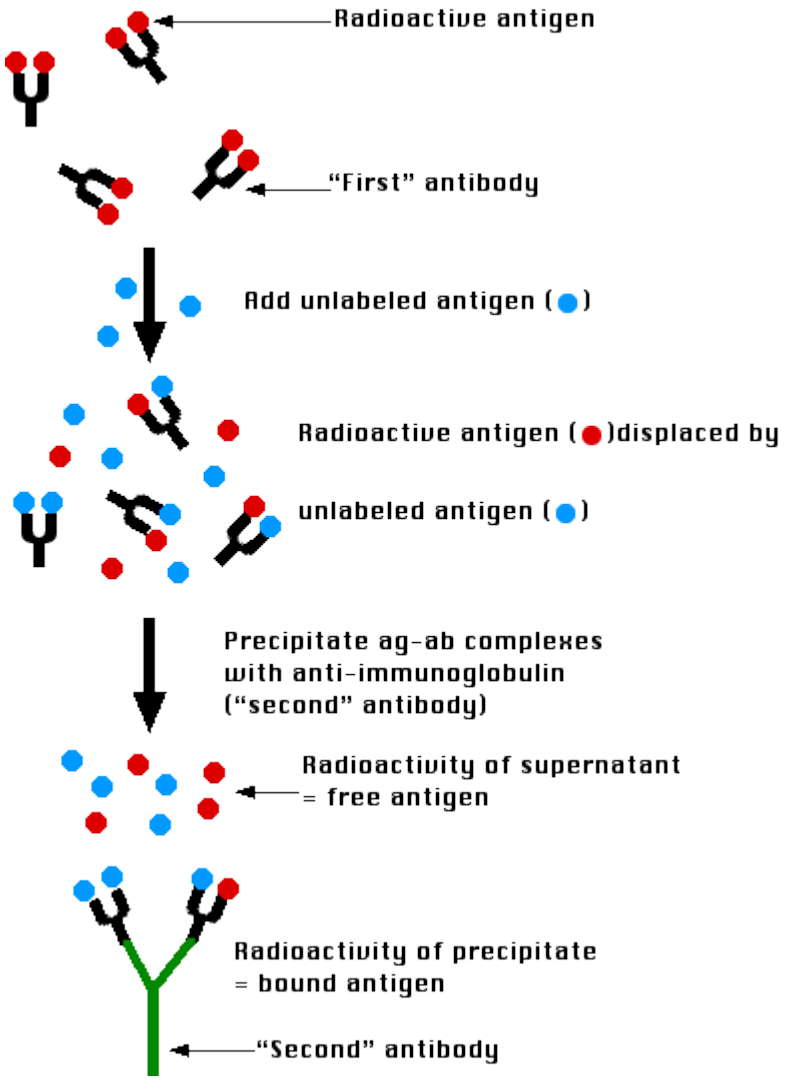


Figure 3-2 General principle of RIA

3:4 ELISA

3:4:1 For EISA test we used:

(1)Roche Diagnostics Elecsys 2010 has the power and efficiency to meet the needs of medium and large volume testing facilities. Roche Elecsys 2010, which is available in the form of sample disk or a rack handling system, can be operated in both continuous and random access modes. The throughput rate of this analyzer is 86 tests per hour and on-board capacity is of 15 tests. Elecsys 2010 disk and Elecsys 2010 rack holds 30 and 100 positions for samples respectively. With Roche Elecsys 2010 system at your workstation, you can attain benefits such as easy and trouble free operation, workflow flexibility, faster turnaround times, broad measuring ranges, and more Features

- Touch screen
- Easy to use software
- Two-dimensional barcode technology for reagents
- Calibrators and controls
- Ready-to-use reagents

- Recertified with 90 day parts warranty

- Reagents, controls, and consumables may be available

Service contracts may be available

System Fully automated, sample selective analyzer for heterogeneous immunoassays, continuous loading, self contained

Test Throughput Max. throughput 88 results/hr (typically 80-85 results/hr)

Sample Types Serum, Plasma

Sample Input/Output Load/unload capacity: 30 samples (Disk version) or 75 samples on 15 racks on one tray

Rack: 5 positions, RD standard

Tray: 15 racks/75 samples, RD standard

STAT port: STAT port (Rack version), or from the sample disk

(Disk version), samples are processed with priority

Sample Container Types Primary tubes: 5 to 10ml; 16x100, 16x75, 13x100, 13x75mm

Sample cup: 2.5ml Cup on tube:

Cup on tube: 16x75/100mm

Sample Volume 5 to 50µl

Sample Barcode Types Code 128, Codabar (NW 7), Interleaved 2 of 5, Code 39

Reagents Ready to use RackPacks with 2-D barcode, temperature controlled reagent compartment (20°C), onboard capacity max. 15 tests

Reaction Vessels 180 disposable cups (AssayCup)

Liquid Handling 360 disposable tips (AssayTip), liquid level and clot detection, sample

and test specifi c dilution

Control Unit Coloured touch-screen monitor, 73iluents73d keyboard and computer

System Interfaces RS 232 serial interface, bi-directional, query and batch mode

Electrical Requirements 115-240V / 50-60Hz / max. 1000VA

Physical Dimensions Width: 120cm (Disk version)

170cm (Rack version)

Depth: 56cm

Height: 73cm

Weight approx. 210kg (Rack version),

approx. 170kg (Disk version)

Certifi cation ISO 9001, UL, GS, DVE, CE

(2) Coating buffer: 0.2 M sodium carbonate/bicarbonate, pH 9.4

(3)capture antibody: Diluted in Coating Buffer (see Appendix for appropriate concentration range).

(4)ash buffer: 0.1 M phosphate, 0.15 M sodium chloride, pH 7.2 containing 0.05% Tween 20

(5)blocking buffer: 2% (w/v) Bovine Serum Albumin (BSA) in Wash Buffer

(6)standard diluents: 2% (w/v) BSA in Wash Buffer. Note: Ideally the standard 74iluents composition would be as close as possible to the sample matrix. For example if measuring the concentration of an antigen in culture supernatant, culture medium should be used as the standard 74iluents. However biological sample matrices such as serum are impossible to replicate, therefore BSA is commonly used in these instances. Often the blocking buffer is also used as the standard diluents

(7) Samples/standards

(8) detection antibody: Diluted in 1/5 strength standard diluents

(9) enzyme conjugate: Streptavidin-HRP diluted in 1/5 strength standard diluents

(10)substrate: TMB substrate

(11) stop solution: 2M sulfuric acid

(12) Plate reader or luminometer equipped to detect the substrate

(13) reagent

3:4:2 Basic principle of ELISA test:

- Antigens solubilised in an appropriate buffer can be coated on a plastic surface, like polystyrene. This may be directly or via an antibody. When serum is added, antibodies can attach to the antigen on the solid phase.(Antigen-antibody binding)
- The presence or absence of these antibodies can be demonstrated with the help of anti-human immunoglobulin conjugate (indirect method) or with conjugate specific against the appropriate antigen (direct method) respectively. The antibodies are conjugated to an enzyme, for example peroxidase. (conjugate binding)
- Adding a substrate, like HRPO, will detect the amount of bound conjugate by a degree of colour produced, which can be quantified. ELISA can also be used for detection of antigens by using specific antibody on the solid phase. Adding an enzyme-linked antibody and a substrate leads to colour production in proportion to the amount of antigen present. Commercially produced ELISA

kits are now available for a wide range of viral antigens and antibodies, including IgM antibodies.

General Principle of ELISA (T3 test)

The ELISA (Enzyme Linked Immunosorbent Assay) technique is based on the antibody sandwich principle First, a capture antibody specific to the analyte of interest (e.g goat anti-mouse igG)is bound to a microtiter plate to create the solid phase . a measured amount of patient serum and a certain amount of mouse monoclonal anti-T3 antibody and a constant amount of T3 conjugated with horseradish peroxidase are added to the microtitre well. During incubation period(60 min at room temperature) the mouse anti T3-antibody is bound to the second antibody on the well. And T3 and conjugated T3 compete for the limited binding sites on the anti T3 antibody . after incubation period the wells are washed 5 times by water to remove unbound T3 conjugate .then TMB(tetramethylbenzidine) solution is added and incubated for 20 minutes resulting in development of blue color .the color development is stopped with addition of stopped solution (1NHCL) and the absorbance is measured spectrophotometrically at 450 nm . The intensity of color formed is proportional to the amount of enzyme present and is inversely related to the

amount of unlabelled. T3 standards assayed in the same way, the concentration of T3 in the unknown sample is then calculated.

General Principle of ELISA (T4 test):

The ELISA (Enzyme Linked Immunosorbent Assay) technique is based on the antibody sandwich principle First, a capture antibody specific to the analyte of interest (e.g goat anti-mouse igG) is bound to a microtiter plate to create the solid phase . a measured amount of patient serum and a certain amount of mouse monoclonal anti-T3 antibody and a constant amount of T3 conjugated with horseradish peroxidase are added to the microtitre well. During incubation period(60 min at room temperature) the mouse anti T3-antibody is bound to the second antibody on the well. And T3 and conjugated T3 compete for the limited binding sites on the anti T3 antibody . after incubation period the wells are washed 5 times by water to remove unbound T3 conjugate .then TMB(tetramethylbenzidine) solution is added and incubated for 20 minutes resulting in development of blue color .the color development is stopped with addition of stopped solution (1NHCL) and the absorbance is measured spectrophotometrically at 450 nm . The intensity of color formed is proportional to the amount of enzyme present and is inversely related to the

amount of unlabelled. T3 standards assayed in the same way, the concentration of T3 in the unknown sample is then calculated.

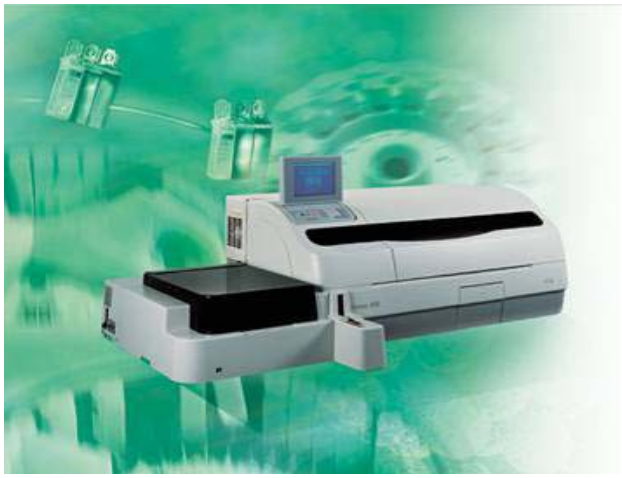


Figure 3-3 Elecsys 2010

Chapter four

Results

Introduction: the result in this chapter will be presented by using frequency tables and figures

Crosstabs

T3 (RIA)	T3 (ELISA)			Total
	Normal	Hypo	Hyper	
Normal	98	0	0	98
Hypo	0	4	0	4
Hyper	1	0	11	12
Total	99	4	11	114

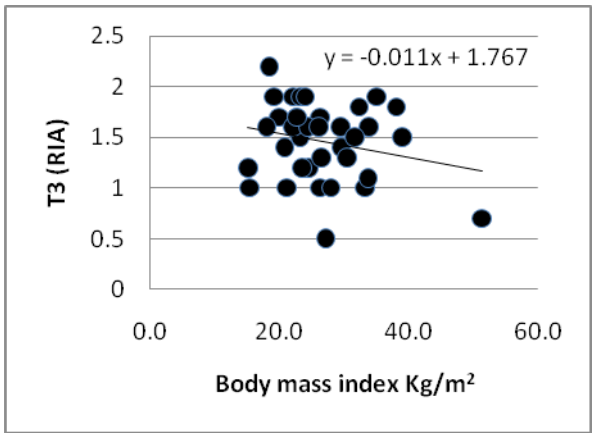
Table (1) cross tabulation table between RIA and ELISA assay result of T3

T3 (RIA)	T4 (ELISA)			Total
	Normal	Hypo	Hyper	
Normal	76	6	3	85
Hypo	2	4	0	6
Hyper	3	1	19	23
Total	81	11	22	114

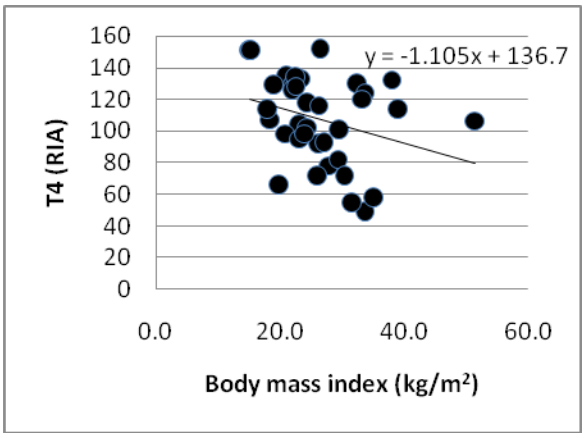
Table (2) cross tabulation table between RIA and ELISA assay result of T4

T3 (RIA)	TSH (ELISA)			Total
	Normal	Hypo	Hyper	
Normal	77	4	4	85
Hypo	4	14	0	18
Hyper	4	0	7	11
Total	85	18	11	114

Table (3) cross tabulation table between RIA and ELISA assay result of TSH



(A)



(B)

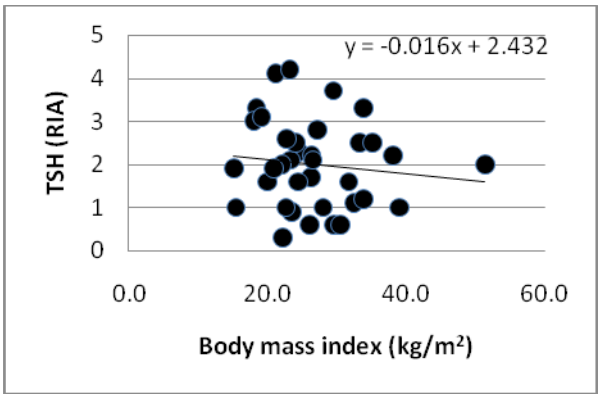


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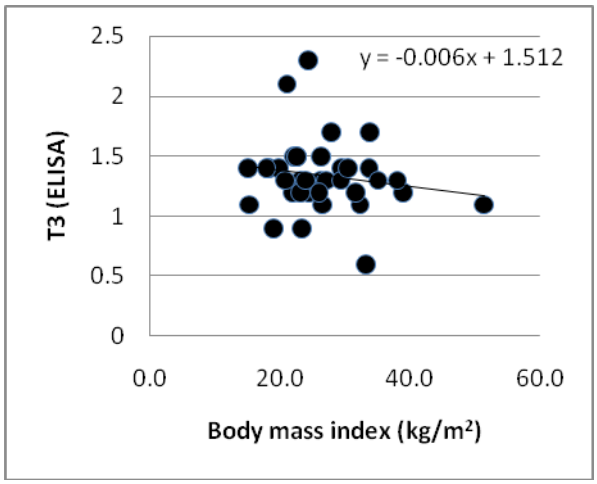
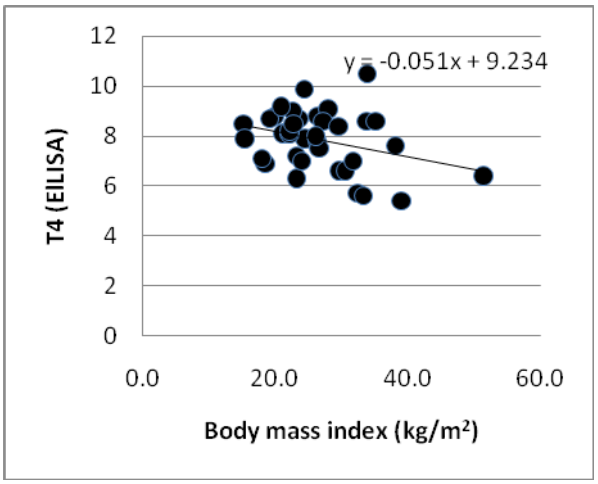
Figure (1) scatter plot show an inverse linear relationship of BMI and thyroid hormones assay result using RIA method; (A) T3, (B) T4 and (C) TSH





(A) (B)

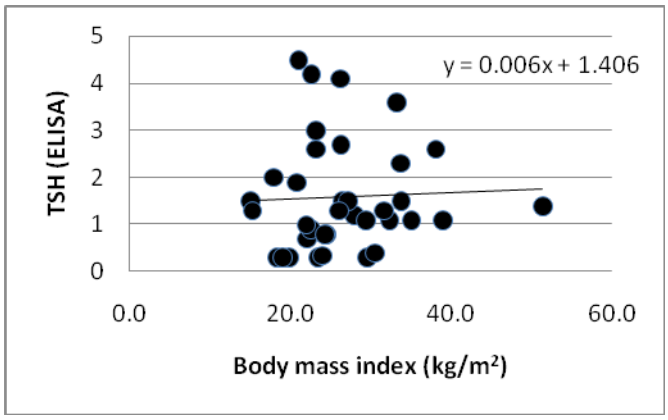


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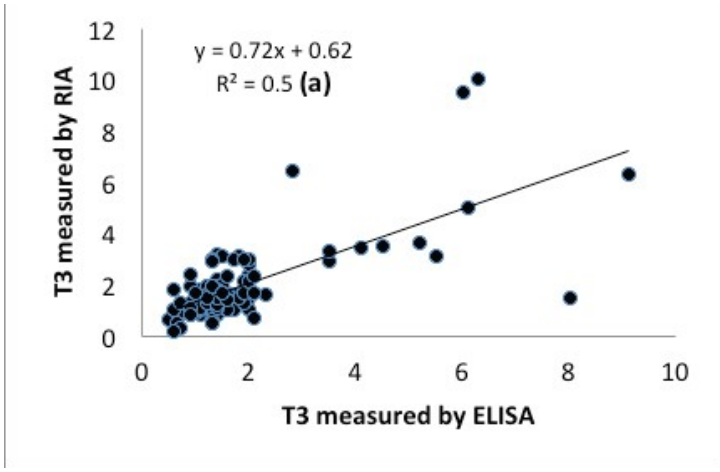
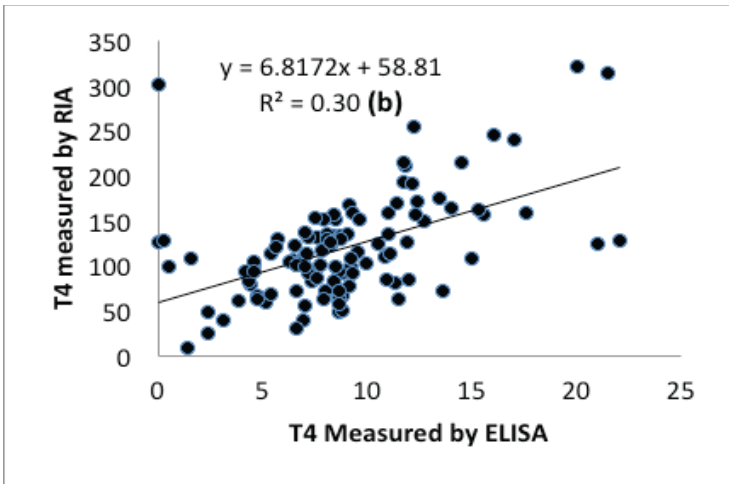
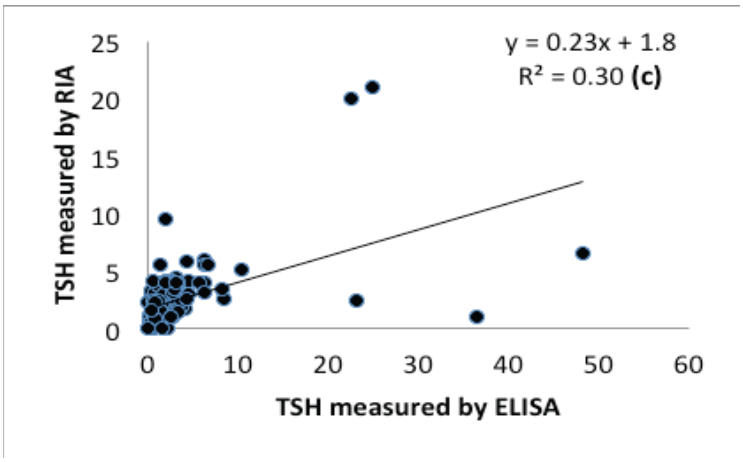
Figure (2) scatter plot show an inverse linear relationship of BMI and thyroid hormones assay result using ELISA method; (A) T3, (B) T4 and (C) TSH







***Scatter plot show a linear relationship between thyroid hormones assay results using RIA method & ELISA method** (A) T3, (B) T4 and (C) TSH*

Chapter five
Discussion Conclusion and Recommendation

5-1 Discussion

The result of RIA has been considered as reference; therefore, T3-RIA results showed that 98 samples as normal and 16 as abnormal thyroid function (Table 1). Accordingly ELISA showed sensitivity, specificity and accuracy of 93.8%,100% AND 99.1%

respectively. This result dictates that ELISA system got the same accuracy in respect to T3 assay. concerning T4-RIA results AS IN Table 2, 85 samples were normal and 29 had abnormal and accordingly ELISA showed sensitivity , specificity and accuracy of 79.3%,89.4% and 86.8% respectively. This result indicate that ELISA has lower sensitivity and relatively fair specificity and accuracy. TSH-RIA results showed that 85 samples were normal and 29 were abnormal function similar to T4 result(Table 3) This result reveal sensitivity, specificity and accuracy of 72.4%, 90.6% and 86% also this result dictate same essence as T4, where the specificity and accuracy showed relative fair result but the sensitivity showed a controversial result result, such results are in agreement with Stya et al, (1984) and Gary and James,(1999). Also the result showed a good correlation between RIA and ELISA with a direct linear relationship where ELISA result can be converted to RIA using the following coefficient 0.72, 6.8 and 0.23 for T3, T4 and TSH respectively see Figure 1. Figure2 depict an inverse linear relationship between the BMI and T3,T4 as well as TSH using RIA whereas the amount of T3, T4 and TSH decrease by 0.011nmol\kg\m2 and 1.105nmol\kg\m2 respectively. This means the increase of BMI mostly lead to decrease level of T3,T4 and TSH, this result dictate that hypothyroidism is mostly associated with obesity, which goes with general trend as has been mentioned by Resta et al,(2004);in which they found that the prevalence of hypothyroidism was higher than that was commonly reported for patient who suffered from sleep disordered breathing and were either obese or overweight. Also Brunilda et al, (2008)have examined the effect of age and BMI on TSH levels by using a multiple linear regression analysis, they found that there is statistically significant relationship at (R2=0.055), which suggests that as age and BMI increasesd, TSH levels also increased.

Similarly figure 3 portrayed inverse linear relationship between the BMI and T3 and T4 using ELISA whereas the amount of T3 and T4 decrease by 0.006 nmol\kg\m2 and 0.051nmol\kg\m2 respectively .But TSH shows a direct linear relationship with BMI where TSH slightly increased by 0.007nmol\kg\m2 .Also this result resembles the fashion of RIA except for TSH where the increase were significantly low.

5-2 Conclusion

The sensitivity of ELISA system over three tests relative to RIA showed unsatisfactory results, which mostly attributed to quality control inadequacy, where small shift in concentration can lead to a wrong reading, while in case of specificity and accuracy a few adjustment can lead to an identical result by using a correction factor in addition to, it requires skilled laboratory technologists and specialized laboratory equipment moreover the measurement of enzyme activity is more complex than measurement of activity of

some type of radioisotopes, the enzyme activity could be affected by plasma constituents and the Kits are expensive, and the antigen won't recognize any other antigen i.e. false positives/negatives ratio, especially with mutated/altered antigen (Viswanth, 2013), therefore RIA could be considered as standard and more convenient than ELISA.

5-3 Recommendation

- The application of ELISER system should be done after extensive QC test on regular bases; due to the instability output.
- ELISER should be used for minor scale, i.e. in the remote areas where there is difficulties in taken a protective measures using RIA
- Further study should be done to optimize the ELISER to have more accurate result similar to RIA method

- Comparison between different ELISER types and RIA result is important to highlight the reliable ELISER type

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