

CHEMILUMINESCENCE IMMUNOASSAY OF THYROID HORMONE IN HUMAN SERUM BY USING MINIVIDAS - A HORMONAL ANALYZER SYSTEM

Yuvaraj sampathkumar^{1*}, M.Dinakaran¹, N. Silambarashi² and S.Elumalai³.

¹ = Department of clinical laboratory, 12A, Jaffer Syrang Street, Parry's, Chennai - 600001

¹ = Department of Cardiology, 12A, Jaffer Syrang Street, Parry's, Chennai - 600001

² = Department of Microbiology, Vinayaka Mission's Kirupananda Variyar Medical College & Hospitals, Salem

³ = Department of Biotechnology, University of Madras, Guindy Campus, Chennai - 600 025

Corresponding author Email: yuvaasampath@rediffmail.com

ABSTRACT

The biomerieux minividas chemiluminescence immunoassay is a prompt and unbeatable method for determination of TSH, TT3 and TT4 in human serum sample. The hormonal tests are crucial for the reliable diagnosis and monitoring of thyroid dysfunction. The use of thyroid function test covers the elevation of TSH, TT3 and TT4 in the serum sample. TSH is widely used as a screening test in suspicion with thyroid disorders, many times TSH alone may be fallacious, in this situation TSH along with T3 and T4 should be performed which will rectify the problems. For most of the patients symptoms are non specific, so biochemical immunoassay is compulsory to recognize the thyroid disorders. The regular uses of these tests are considered based on the medical record and clinical implementation.

Keywords: Thyroid function test, TSH, T3, T4, Chemiluminescence and Immunoassay.

INTRODUCTION

In the 1950s, only one thyroid test was available, an indirect determination of the serum total (free + protein-bound) thyroxine (T4) concentration, using the protein bound iodine (PBI) technique. Since 1970, specialized advances in radioimmunoassay (RIA), immunometric assay (IMA) and more recently liquid chromatography mass spectrometry (LC-MS/MS) methodologies have progressively upgrade the specificity, reproducibility and sensitivity of thyroid testing procedures. Currently, serum-based immunoassays and LC-MS/MS techniques are accessible for measuring the total and free thyroid hormones, FT4 and FT3 concentrations, as well as the pituitary thyroid stimulator, Thyrotropin (Thyroid Releasing Hormone). The Research work are required for the proper identification of thyroid condition because the indication and expression of thyroid illness are subtle or distracted in most patients, potential biochemical works obligatory to recognize disease. The thyroid releasing hormone assessment is the effective original assay for distinguishing primary hypothyroidism and hyperthyroidism, Pituitary TSH secretion regulates TT4 and TT3 release, which apply log-linear negative feedback on pituitary thyrotropes. Due to this correlation, little variation in the application of free thyroid hormone (TH) effect in broad changes in the serum collection of Thyroid releasing hormone; therefore, TSH is the first signal of subtle changes in thyroid production.

First-generation assays

The first-generation TSH radio immunoassays, introduced in the 1960s, were depends on the basis of competitive binding using one polyclonal antibody and a radioactive isotope label. The functional sensitivity level was between 1 and 2 ml U/L.

Second-generation assays

The second-generation TSH immunoassays, introduced in the 1970s, are based on noncompetitive or two-site immunometric methods. These so-called sandwich immunoassays were initially constructed with two different polyclonal antibodies to bind the antigen at two epitopes. A radioisotope or an enzyme, such as alkaline phosphatase or horseradish peroxidase, was the label.

Third-generation assays.

The development of the third generation assays for the measurement of serum thyroid releasing hormone and the log-linear relationship with free thyroxine (T4), established TSH as the hallmark of thyroid function testing. Third generation assays have good reactivity in the weak TSH range correlated to the second-generation test and around hundred time's high sensitive than the first-generation TSH test.

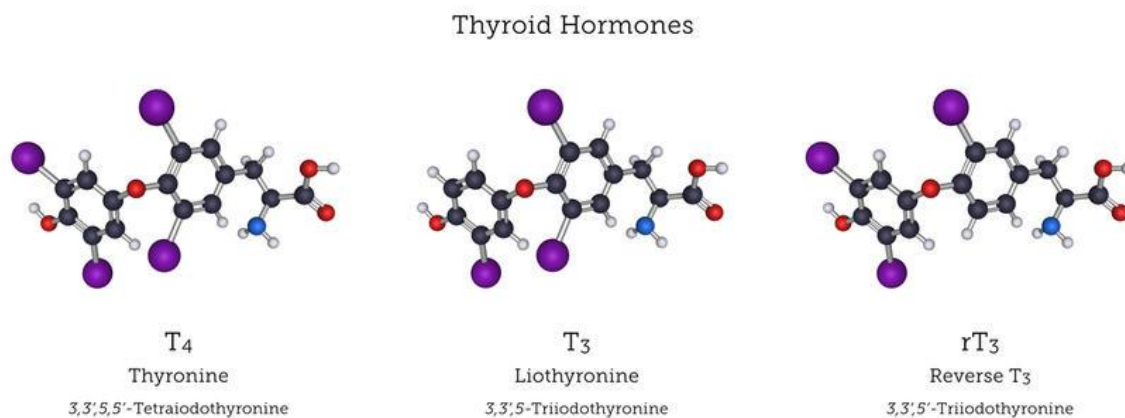
Hypothyroidism

Over active thyroid is a situation in which your thyroid organ doesn't create sufficient amount of hormones. The indication and manifestation of hypothyroidism vary broadly, depends on the inflexibility of the hormone insufficiency. The patients still obtain more weight, rise the responsiveness to cold, lack of moisture in skin, enlarged face, croakiness, gruffness, weakness of the muscle, high cholesterol in blood, muscle pain, tenderness and stiffness, joints swelling , irregular discharge , weakening hair, low heart beats , constipation, sadness.

Hyperthyroidism

Thyroid is an endocrine gland located in the base of neck and its produces different hormones. Incase thyroid gland is more effective, it's able to generate more amount thyroid hormones in the blood circulatory system. This is referred as hyperthyroidism. Hyperthyroidism shows different changes in our metabolic pathway, loss of weight, up taking of more food, Arrhythmia, sweating. Grave's disease is a result of hyperthyroidism, symptoms of hyperthyroidism such as Tremor, trembling, Changes in bowel patterns, enlargement of thyroid gland, the patient will have Fatigue, weakness of the muscle, difficult to sleep, multi-nodular goiter, single toxic goiter, thyroiditis, autoimmune thyroid disease, thyrotropin producing tumor, thyroid cancer.

Thyroid Hormone structures



Thyroxine

Protein molecule that binds with iodine. Thyroxine protein carry four molecules of iodide and Triiodothyronine carry three molecules of iodide so, the molecules called as T₄ and T₃. The iodine molecule is present only in the thyroid hormone and uptake of iodine from the food is essential for our health. Thyroid gland produces the more amount of thyroid hormone (T₄). The thyroxine hormone appears in two forms, bound T₄ and free T₄. T₄ may be reduced in the conditions, such as primary hypothyroidism, secondary hypothyroidism and tertiary hypothyroidism treatment with oral estrogen results in a marked increase in thyroxine-binding globulin. Goiter, hyperthyroidism, plummer's disease are developed, because of more amount thyroxine (T₄) in the body system.

Triiodothyronine

Triiodothyronine is a hormone, play the serious role in body development and metabolisms. Approximately twenty percentage of triiodothyronine is circulated into the bloodstream. Thyroid hormones shows the significance roles in heart function, digestive functions, controlling of muscle, brain development, maintenance of bones and monitoring the metabolites. High amount of T₃ binds to protein from the circulatory blood system termed as Total T₃. The T₃ that cannot bind to protein is termed free T₃. The common kind of T₃ test, otherwise called as the T₃ total test, measuring the two forms of T₃ from the serum sample, the serum T₃ use to determine the condition of hyperthyroidism and thyrotoxicosis. Goiter is otherwise named as thyrotoxicosis. This is an enlargement of the neck due to growth of the thyroid. The thyroid gland secretes the too much of thyroid hormones, resulting in muscle weakness (Thyrotoxic periodic paralysis). The thyroid condition which shows the different type of symptoms such as anxiety, constipation problem, irregular discharge, intolerance to the heat, loss of weight, Arrhythmia, heat beating fast, fatigue, impatience, trembling, hair lose.

Principle of a biomerieux Minividas Chemiluminescence Immunoassay

The Solid Phase Receptacle acts a stable state aspiration device for the test. Reactants for the test are prepare-to-use and pre allocate in the covered reagent strips. Most of the experiment steps are done automatically by the mini vidas immunological analyzer. In and out of cycled mechanisms of SPR test processed regularly. Free ingredients are removed through cleaning buffer and the

last reaction step is 4-Methyl-umbelliferyl phosphate cycled in and out of the SPR. Then, hydrolysis of this substrate into a luminance product by immobilized enzyme, the 4-Methyl-umbelliferone luminance is detected at 450 nm. The power of the luminance based on the absorption of alkaline phosphatase present on the SPR that alter the substrate. At the end, results are automatically measured by the analyzer. For some tests, two detection steps are performed successively. For antigen detection, the SPR is generally coated on the interior with capture antibody or sometimes with byproducts of the analyte. For antibody detection, the SPR is coated with an encapsulated antigen or antibody administered to the antigen. Depending on the test, the conjugate can be byproducts of the analyte or an antibody labeled with alkaline phosphatase. For more particular details, mention to the assay package inserts.

Principle of a biomerieux Minividas Chemiluminescence Immunoassay

Materials and Methods

The serum sample collected under the good laboratory practices. The samples were tested quickly after collection. The sample is stable for 3 to 5 days at 2°C to 4°C. If the sample unable to process within 24 hours, freeze until the test can be done. Permit the sample to reach room temperature before testing. The sample was collected by using micropipette and add 3 to 5 drops (100-150 µl) of sample into the 1st well of the strip and read the result.

To switch on the instrument, the machine under goes to installation, select the continue key and press center key button, then go to menu option, select and feed the entry of sample ID, control and standard option, bar-coding the strip, and others, To select the start button to allow for sample running up to 17-20 minutes and print out the recorded data from the instrument.

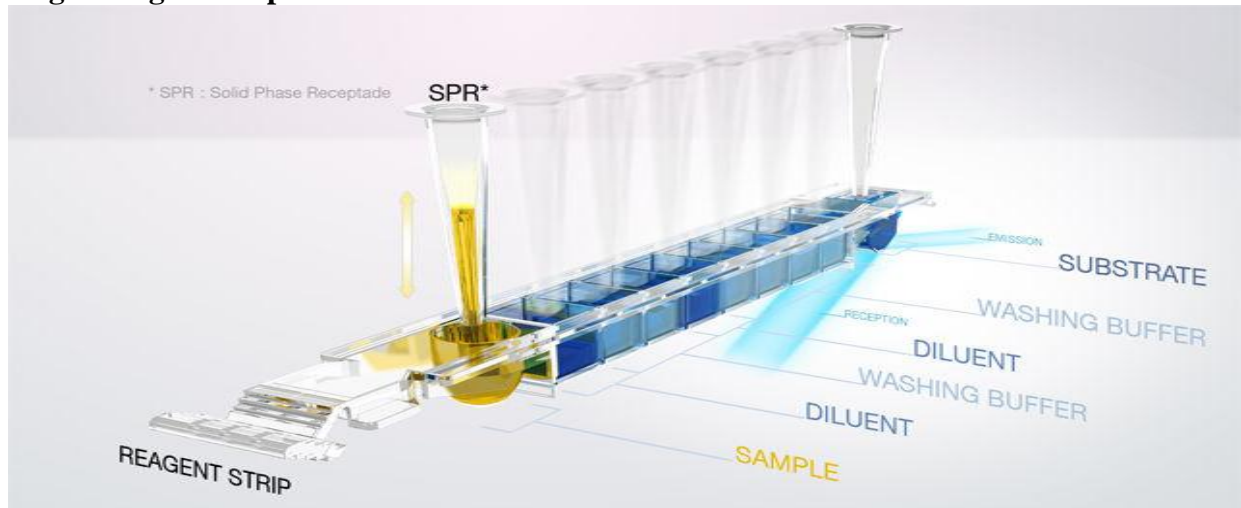
Biomerieux Minividas Chemiluminescence Immunoassay kit

The biomerieux minividas chemiluminescence Immunoassay kit contains everything required to carry out a specific assay, single or dual reagent strips, SPRs (Solid Phase Receptacle), standard/calibrator(s), controls, diluents.

Single Reagent Strip

Single reagent strip is made of polypropylene and contains ten wells. The sample is added in the 1st well. Conjugate, diluents, and wash buffer are already coated in the 2nd well in to 8th well. The luminance of the substrate is measured finally (10th well). Tags assure that the SRP is properly placed in the guided channel.

Single Reagent Strip



SPR

The polystyrene device (SPR) is able to grabbing the soluble proteins, viruses and bacteria. It's labeled with a color-coded, bar-coded dot drill in the middle. SPR vial is not re usable. The SPR is the stable state for the immunological response. SPR interior walls are coated with antibody or antigen that captures a target analyte. The target analyte from the sample binds to the SPRs interior coating (antibody, antigen, etc.). It is then bound by an enzyme-conjugated antibody or antigen, forming a complex. The stimulant change the hydrolysis state of the molecule into a luminous end product .The SPR is used to pipette samples and reagents and perform the following operations sampling, incubation, mixing, washing. The beveled tip of the SPR enables it to pierce the protective seal that covers the wells in a reagent strip. The reagent strip tray then moves in and out to allow liquids to be transferred from one well to another.

Loading reagent strips and SPR

Lift the cover of the reagent strip section, hold the strip by its handle and insert it into a test position, slide the strip into the section channel, open the SPR block door, place the SPR in the SPR block position directly over the reagent strip, Repeat these steps for other strips and SPRs to be loaded, Close the SPR block door and the reagent strip tray cover.

Minividas Hormonal Analyzer System



Results and Discussions

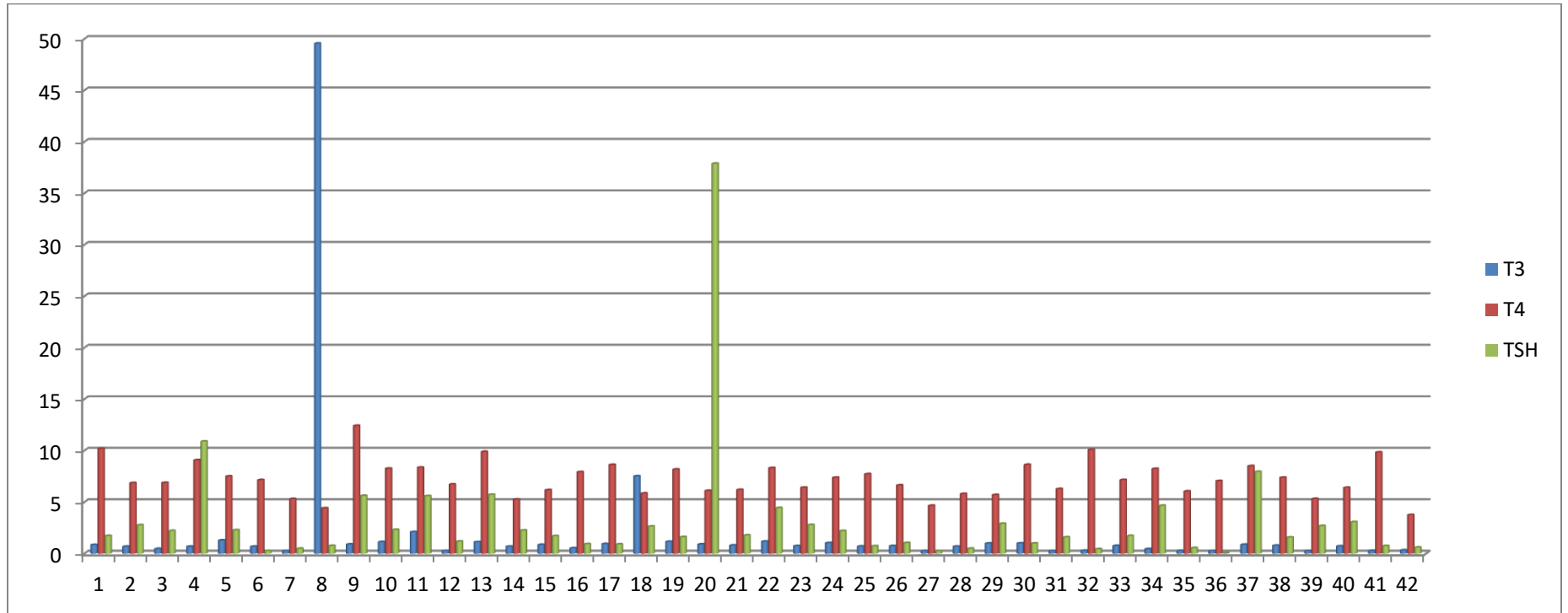
The Chemiluminescence Immunoassay of TSH, T4 and T3 were detected by using biomerieux-mini VIDAS hormonal analyzer instrument and collect the recorded sample data results observed as Subclinical thyroidism, secondary hypothyroidism, Non thyroidal illness, Goiter, asymptomatic hyperthyroidism, symptomatic hyperthyroidism. (T3 normal values 0.58-1.51mg/ml, T4 normal values 4.6-9.3 ug/dl and TSH normal values 0.25-5.0 uiu/ml).

Table 1: Chemiluminescence Immunoassay of TSH, T4 and T3 are detected by using biomerieux-mini VIDAS Hormonal Analyzer Instrument

S.No	Age (Years)	Gender	T3 mg/ml	T4 ug/ml	TSH uiu/ml
1	47	M	0.85	10.2	1.72
2	66	M	0.68	6.86	2.77
3	64	F	0.47	6.88	2.21
4	63	M	0.68	9.08	10.9
5	32	M	1.29	7.51	2.28
6	32	M	0.68	7.15	0.26
7	27	F	0.26	5.31	0.49
8	75	M	49.55	4.41	0.76
9	69	M	0.9	12.42	5.62
10	47	M	1.13	8.26	2.32
11	0.5	F	2.1	8.36	5.59
12	50	F	0.26	6.73	1.18
13	55	F	1.12	9.9	5.72
14	16	M	0.69	5.26	2.25
15	53	F	0.86	6.17	1.71
16	40	F	0.53	7.92	0.93
17	65	M	0.94	8.63	0.91
18	45	M	7.52	5.85	2.64

19	35	F	1.16	8.18	1.61
20	62	F	0.9	6.11	37.89
21	40	M	0.8	6.2	1.78
22	37	M	1.18	8.32	4.44
23	73	F	0.74	6.41	2.79
24	24	M	1.03	7.38	2.2
25	63	M	0.7	7.72	0.73
26	65	M	0.75	6.64	1.04
27	63	M	0.26	4.66	0.24
28	73	M	0.69	5.8	0.49
29	66	M	0.99	5.7	2.91
30	53	M	1.0	8.64	0.99
31	70	M	0.26	6.28	1.59
32	47	F	0.29	10.09	0.44
33	79	M	0.76	7.16	1.73
34	56	F	0.45	8.24	4.66
35	66	F	0.28	6.05	0.55
36	41	M	0.26	7.06	0.05
37	62	M	0.87	8.51	7.95
38	46	M	0.78	7.38	1.57
39	75	M	0.26	5.32	2.69
40	63	M	0.72	6.4	3.07
41	69	M	0.28	9.84	0.75
42.	52	M	0.33	3.75	0.6

Chemiluminescence Immunoassay of TSH, T4 and T3 are detected by using biomerieux-mini VIDAS Hormonal Analyzer Instrument



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